INDUCTION OF CORNEAL VASCULARIZATION WITH ALLOXAN*†

BY

CLIVE GRAYMORE AND ANDREW MCCORMICK

Department of Pathology, Institute of Ophthalmology, University of London

SEVERAL reports have appeared in the literature in recent years describing the induction of corneal vascularization by the injection of appropriate concentrations of alloxan into the anterior chamber. The problems concerned with neovascularization of the cornea, such as mechanical trauma, oedema, and chemical stimulation, have been studied in detail (Ashton, Cook, and Langham, 1951; Ashton and Cook, 1953; Ashton, 1960; Ahuja and Nema, 1966; Maurice, Zauberman, and Michaelson, 1966).

The content, nature, and distribution of the enzyme lactic acid dehydrogenase have recently been studied in the normal cornea (Graymore and McCormick, 1966; Graymore, 1966), preparatory to further investigations into the induction of corneal vascularization. Marked changes have been observed in the total content and distribution of this enzyme in the vascularizing cornea (Graymore, Ashton, and McCormick, 1968). In studying chemically-induced neovascularization, it was felt desirable to simplify the approach and standardize conditions as far as possible. Intracameral injection introduces complications which are due not only to mechanical trauma but also to the pressure changes that result from the withdrawal or administration of fluids. A further complication is that the half-life of the aqueous contents is of the order of one hour (Langham, 1953). When this factor is added to the difficulty of assessing the degree of dilution by the aqueous, it must be accepted that it is not easy to determine accuracy of dosage, in terms of both concentration and time sequence.

The following procedure has therefore been adopted in an effort to meet these difficulties.

Dutch breed rabbits of either sex, weighing between 1.5 and 2.0 kg. were anaesthetized with Nembutal and one eye was prolapsed. The other eye served as a control for all studies. A "ring" or "cup", one inch in depth, was cut from polythene tubing, internal diameter of 16 mm. and wall thickness of 2 mm., which was found to be highly suitable for the application of the alloxan. This cup was held securely in place on the exposed surface of the cornea and the globe was allowed to retract into the orbit with the lids holding it in place. Approximately 2 ml. of an aqueous solution of alloxan (2.4 g. per cent.; pH 4-5) were poured into the cup, which was allowed to remain in contact with the surface of the cornea for 30 min., the contents being changed at 5 min. intervals. Shorter periods of application were found to be unsatisfactory.

The results of this treatment were compared with those of the more usual procedure of the intracameral injection of 0.25 ml. of 2.4 g. per cent. alloxan at pH 4-5. In both cases corneal vascularization and oedema were found to occur with a similar time sequence and to a similar extent. The Figure (opposite) shows eyes from two animals treated 10 days previously by the two different procedures. The vascular ingrowth is evenly distributed round the periphery of both corneae, although this is not apparent in the plates because of the angle from which the photographs were taken.

* Received for publication January 4, 1967.

† Address for reprints: as above.

(a) Rabbit eye 10 days after intracameral injection with alloxan.

(b) Rabbit eye 10 days after surface application of alloxan.

FIGURE.—Effects of alloxan.

Essentially similar changes were observed after the continuous administration of alloxan drops directly to the surface of the cornea for 30 min. This procedure has the disadvantage, however, that the accurate control of dosage, osmolarity, and pH is difficult. Whilst this communication was being prepared, our attention was drawn to the work of Langham (1953), who referred briefly to the possibility of direct application to the corneal epithelium by continuous drip. Langham stated that "it soon became apparent that the anterior chamber was the most suitable site as it presents a simple means of bringing a given amount of alloxan into direct contact with the cornea". No further reference was made to this drip technique, so that it is difficult to comment on this statement. For the reasons given above, we felt that the "cup" procedure is by far the most simple, uncomplicated, and efficient. Langham (1953) used neutralized alloxan for his experiments. It is known that alloxan is highly unstable at neutral pH, its half life at pH 7.4, for example, being 1 min. (Patterson, Lazarow, and Levey, 1949). Since Langham used essentially similar concentrations of alloxan as those used here, it may be that his drip technique failed because of the rapid loss of activity at neutral pH, together with a low permeability of the epithelium compared with the endothelium.

Regarding the intracameral approach, it should be added that it may be followed by a severe plastic iritis which is not apparent with external application. The progress of this iritis cannot be followed because of the oedema which leads to increasing opacification of the cornea, but the early stages show a heavy fibrinous exudate within the aqueous humour without a significant cellular component, although a small hyphaema frequently develops later. The iris loses its normal lustre and shows clumping and dispersion of pigment in the superficial stroma.

Histochemical investigations show that similar patterns of change occur with all three procedures described; the detailed results will be discussed in a later paper.

Summary

A simple technique is described for inducing vascularization in the cornea of the rabbit

by the external application of alloxan directly onto the corneal surface. Reasons are given for preferring this method to the more routine procedure of intracameral injection.

The authors wish to thank Prof. Norman Ashton for his advice, interest, and encouragement, and Mr. Ralph Kissun for his continued valuable assistance. Once again it is a pleasure to record our indebtedness to the Royal National Institute for the Blind for their continued financial assistance (C.N.G.) and to the E. A. Baker Foundation for the Prevention of Blindness of the Canadian National Institute for the Blind (A.M.).

REFERENCES

- AHUJA, O. P., and NEMA, H. V. (1966). *Amer. J. Ophthalm.*, **62**, 707.
ASHTON, N. (1960). In "The Transparency of the Cornea: A.C.I.O.M.S. Symposium", eds S. Duke-Elder and E. S. Perkins, p. 131. Blackwell, Oxford.
——— and COOK, C. (1953). *Brit. J. Ophthalm.*, **37**, 193.
———, ———, and LANGHAM, M. (1951). *Ibid.*, **35**, 718.
GRAYMORE, C. N. (1966). *Exp. Eye Res.*, **5**, 325.
———, ASHTON, N., and McCORMICK, A. (1968). *Brit. J. Ophthalm.*, **52** (in press).
——— and McCORMICK, A. (1966). *Exp. Eye Res.*, **5**, 315.
MAURICE, D. M., ZAUBERMAN, H., and MICHAELSON, I. C. (1966). *Ibid.*, **5**, 168.
LANGHAM, M. (1953). *Brit. J. Ophthalm.*, **37**, 210.
PATTERSON, J. W., LAZAROW, A., and LEVEY, S. (1949). *J. biol. Chem.*, **177**, 187.