

**THE SUITABILITY OF EXPERIMENTAL CORNEAL
LESIONS FOR EVALUATING LOCAL
SULPHONAMIDE THERAPY***

BY

W. T. S. COLE, J. L. HAMILTON-PATERSON
and ARNOLD SORSBY†

LONDON

THE difficulties in assessing the value of local sulphonamide therapy in infections of the outer eye centre around the absence of satisfactory methods of inducing such infections in experimental animals. Gonococcal ophthalmia which so readily occurs in man cannot be induced in animals, even when highly virulent organisms are used and the animal is first exposed to various debilitating processes. Much the same difficulties arise with the other organisms commonly met in the purulent and muco-purulent conjunctivitides. It is said (Boros, 1940) that if the reticulo-endothelial system is first blocked by solid particles, such as those contained in solutions of Indian ink, infective lesions can readily be produced. Such experimental lesions are, however, unsuitable for pharmacological and therapeutic studies owing to the severe physiological disturbances—sometimes ending in death—that blocking of the reticulo-endothelial system produces. The one consistent experimental infection of the outer eye that can be induced in the intact animal is corneal ulceration produced by inoculation with *B. pyocyaneus*, but as has been shown elsewhere (Klein and Sorsby, 1943) such lesions are not quite suitable for determining the value of local sulphonamide therapy mainly for the reason that there is considerable doubt as to the value of the sulphonamides in general in *B. pyocyaneus* infection.

The work recorded here is largely an account of attempts to obtain experimental corneal infections suitable for the study of the value of therapeutic agents in infections of the outer eye. These attempts all failed.

1. *Experiments with pneumococcus*.—Pneumococcus types 3 and 19 are pathogenic to the rabbit, but attempts to induce corneal lesions by direct inoculation into the abraded cornea and by various additional measures were largely negative.

Pneumococcus type 19 (Lister Institute strain). Four procedures were tried out:

(a) Direct inoculation to the abraded cornea of 0.1 c.c. suspension 3×10^6 organisms; suitably exalted by passage into mice: No result in the four eyes thus treated.

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(b) Direct inoculation 24 hours after producing a burn of the cornea with solid silver nitrate: No result in four eyes thus treated.

(c) Direct inoculation as in (b) with exposure of cornea by securing the lids with sutures so that the eyes were kept wide open: No result in four eyes.

(d) Subconjunctival injection of the suspension at the limbus. Small transitory corneal opacities developed in the two eyes thus treated.

Pneumococcus type 3.

Procedures (a) and (c) indicated above were carried out in four eyes in both instances with completely negative results.

2. *Experiments with streptococcus haemolyticus*.—Lancefield A Strain was used in the same dosage and concentration as with pneumococcus, and the following procedures were employed.

(a) Direct inoculation on to the abraded cornea. One rabbit and three guinea pigs were used with negative results.

(b) Inoculation after silver nitrate burn of the cornea: Two of the rabbits used died overnight. In the surviving rabbit there was no corneal infection.

(c) Subconjunctival injection in one rabbit produced small transitory corneal opacities.

(d) Direct inoculation on to the abraded cornea after preliminary sensitization of three rabbits by means of repeated intraperitoneal injections of the living cultures of the organism. This procedure carried out when sensitization has become established (as shown by skin tests) gave slight injection of the conjunctiva with a small superficial corneal lesion which, however, healed spontaneously.

3. *Experiments with organisms kindly supplied by Dr. J. Robson*.—In view of the negative results obtained by us and the positive results recorded by Robson and Scott with staphylococcus aureus (1943 a) and pneumococcus type 19 (1943 b) an attempt was made to obtain experimental corneal lesions in the rabbit by following the technique described by these observers and using cultures of the organisms actually employed by them. We are indebted to Dr. J. Robson for his ready help in this matter. The method of inoculation employed is intracorneal injection as described by these authors: 0.1 c.c. of 100×10^6 organisms were used.

(a) *Pneumococcus type 19*.—(Robson and Scott strain). Of three rabbits inoculated, one died with broncho-pneumonia and septicaemia (as shown by a positive blood culture). In the two others a spreading corneal opacity which cleared spontaneously developed in the four eyes; in no case was a corneal ulcer formed. The animal that died had some culture injected into the anterior chamber owing to inadvertent perforation of the cornea.

EXPERIMENTS WITH PNEUMOCOCCUS TYPE 19 (Lister Strain)

Number and Animal	Type of Inoculation	(0.1 c.c. suspension 3×10^6 orgs. :—)	Result
2 rabbits	Abrasion of cornea 4 eyes inoculated	No effect
4 rabbits	Corneal burn with solid silver nitrate (crystals)	4 eyes inoculated, 4 control eyes	No effect
2 rabbits	Corneal burn with solid silver nitrate, eyelids retracted	4 eyes inoculated	No effect
1 rabbit...	Subconjunctival injection	2 eyes inoculated	Small corneal opacities

EXPERIMENTS WITH PNEUMOCOCCUS TYPE 3 (Lister Strain)

Number and Animal	Type of Inoculation	(0.1 c.c. suspension 3×10^6 orgs. :—)	Result
2 rabbits	Abrasion of cornea 4 eyes inoculated	No effect
2 rabbits	Burn of cornea with solid silver nitrate, eyelids retracted 4 eyes inoculated	No effect

EXPERIMENTS WITH HAEMOLYTIC STREPTOCOCCUS—LANCEFIELD A STRAIN

Number and Animal	Type of Inoculation	(0.1 c.c. suspension 3×10^6 orgs. :—)	Result
1 rabbit...	Abrasion of cornea 2 eyes inoculated	No effect
3 guinea pigs	Abrasion of cornea 3 eyes inoculated	No effect
3 rabbits	Burn of cornea with solid silver nitrate	6 eyes inoculated	2 rabbits died overnight.
1 rabbit...	Sub-conjunctival injection 2 eyes inoculated	Other one—no effect
3 rabbits	Abrasion of cornea after sensitization by intraperitoneal injections of living cultures 2 eyes inoculated	No effect (small corneal opacities)
			Slight local opacity with slight injection conjunctiva

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EXPERIMENTS WITH J. ROBSON STRAINS

Number and Animal	Type of Inoculation (0.1 c.c. 100×10^6 orgs. :—)	Result
3 rabbits	Intracorneal injection—six eyes ... Pneumococcus type 19...	One rabbit died with pneumo-septicaemia (perforated anterior chamber). The two others spreading corneal opacity—no ulceration
1 rabbit...	Intracorneal injection—both eyes ... Haemolytic streptococcus	Localised opacity ? abscess
1 rabbit...	Intracorneal injection—both eyes ... Staphyloc. aureus ...	Localised opacity healed in 4 days. Small opacity
1 rabbit...	Intracorneal injection—both eyes ... B. pyocyaneus ...	Mild reaction 1 eye—other eye clear

EXPERIMENTS WITH MECHANICAL MASS

Number and Animal	Type of Inoculation	Result
2 rabbits	Intracorneal injection of pneumococcal vaccine 0.1 c.c. (1000×10^6 orgs.)	Slight opacity, healed in 4 days
2 rabbits	Intracorneal injection of broth 0.1 c.c. ...	Slight opacity, healed in 4 days

(b) *Streptococcus haemolyticus*.—A severe localized opacity going on to ulcer formation developed in one rabbit. Spontaneous healing occurred after 4 days.

These results though distinctly more definite than those obtained in our investigations did not seem to us to satisfy the criteria necessary for an experimental lesion suitable for testing a therapeutic agent. Our impression was that the corneal opacity formed was not an infective lesion but essentially a pressure necrosis reaction—a reading that gained some support from the corneal opacities produced in two rabbits by the intracorneal injection of 0.1 c.c. pneumococcal vaccine (1000×10^6 organism), and in two other rabbits by 0.1 c.c. broth. These opacities though milder than those obtained by the living organisms were essentially of the same type and healed well within 4 days.

4. *Repeat experiments with B.pyocyaneus*.—In order to assess the possibility that the conflicting results obtained by Robson and Scott (1942) and Klein and Sorsby (1943) on the efficacy of local sulphonamide therapy in *B.pyocyaneus* infection of the cornea might be due to the fact that the one set of observers used sulphonamide-prone organisms and the other sulphonamide resistant strains, cultures of the organisms used by Robson and Scott were obtained by the courtesy of Dr. Robson. These organisms had, however, been in stock for some time and Dr. Robson pointed out that they might no longer be sufficiently virulent. This proved to be the case, and it was therefore impossible to use this particular strain.

Twelve strains of *B.pyocyaneus* were then tested *in vitro* against sulphonamide. The organisms were cultured on solid media containing varying concentrations (up to 500 mgm./100 ml.) of the drug. No marked variation in the behaviour of the strains could be detected, all—including the strain supplied by Dr. Robson—requiring a concentration of about 350 mgm./100ml. of sulphanilamide to inhibit their growth. In view of these findings further work was considered unnecessary.

Summary

1. Attempts to produce infective corneal lesions suitable for therapeutic tests in the rabbit by pneumococcus types 3 and 19 by direct inoculation on to the abraded cornea in the intact animal or after damaging the cornea with solid silver nitrate, and with exposure of the cornea by retracting the lids with sutures, all failed. Preliminary sensitization also failed to produce any satisfactory lesion on inoculation with streptococcus-haemolyticus.

2. The method of intracorneal inoculation with pneumococcus type 19 and staphylococcus aureus as described by Robson and Scott did not appear to give lesions suitable for therapeutic tests.

3. *In vitro* experiments with *B. pyocyaneus* to test the possibility that the conflicting results reported by Robson and Scott on the one hand and Klein and Sorsby on the other hand, might be due to varying susceptibility of different strains of the organism to sulphonilamide were negative.

4. It is concluded that there is no valid experimental evidence that local sulphonamide is effective in infections of the outer eye.

We are indebted to Dr. J. Robson for his courteous and ready co-operation.

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A CASE OF INTERSTITIAL KERATITIS AT AN EARLY AGE *

BY

PETER J. DEVLIN, D.O.M.S.

LIVERPOOL

ALTHOUGH interstitial keratitis is a common condition, the age incidence of the following case seems to make it worth recording. The age preference is for the second half of the first and the second decades of life. Only 6 per cent. of cases occur under 5 years of age (Spicer, 1924), and its appearance as early as in the case described seems to be a rarity.

E. C., a small girl, aged 15 months, was brought to hospital on April 18, 1944, with a history of having had a heavy cold for a period of 6 weeks. During this time the child's eyes had become red and sore, and it had become unable to open them.

On examination, the child was well nourished and developed for its age, though pale. Photophobia and blepharospasm were, at once, obvious. The right eye showed severe circum-corneal injection with characteristic interstitial vascularisation—the Hutchinson "salmon patch." The whole cornea was opaque from oedema and cellular infiltration.

The left eye showed a similar interstitial keratitis of less extent, but associated with visible keratic precipitates. Both pupils were markedly contracted.

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