CONTROL OF EXPERIMENTAL CORNEAL INFECTION WITH MEDICATED SEMI-SOLID CONTACT CAP AND DISK*

INFECTION WITH *Ps. PYOCYANEA* TREATED WITH STREPTOMYCIN

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MULES (1894) wrote that where "the action of micro-organisms on an abraded surface may cause or is causing destructive changes" the indications would be to remove the irritating elements and allow the natural process of repair to heal the breach of surface". He sought to achieve this by placing a gelatine wafer impregnated with iodoform on the corneal surface and keeping the eye bandaged. He reported twelve cases in all of which healing was spectacular.

Adam (1916) used a contact shell made of glycerin gelatine for the protection of the globe in cases of injuries around the orbit, and Huber (1934) used a contact shell carrying iodoform-cocaine-dionine ointment for the treatment of hypopyon ulcer.

The treatment of corneal ulcers, especially those caused by Gram-negative micro-organisms, is not satisfactory. In a series of ten corneal ulcers caused by *Ps. pyocyanea* infection reported by Bignell (1951), the eye had to be eviscerated in four cases, in three cases the residual vision was perception of light only, and in the remaining three the vision was 6/18, 6/24, and 6/36 respectively. The disastrous effect of this type of infection is emphasized when it is appreciated that treatment in this series started between two and four days after injury (usually a corneal foreign body) and consisted of the newer antibiotics, including aureomycin and streptomycin. Those cases which did better received streptomycin subconjunctivally, usually in conjunction with other treatment, either sulphonamide or local aureomycin.

Various cases have been reported in which local streptomycin has proved of value in severe corneal ulcers. Thus Couadau and Darbon (1948) used this antibiotic in a case of keratitis due to *B. faecalis alcaligenes*, Clark and Locatcher-Khorazo (1951) in corneal ulcer produced by *B. aerobacter aerogenes*, Maschler (1948) in a *Ps. pyocyanea* ring abscess, Lepri (1948) in a post-traumatic corneal infection by *Proteus Morgani*, and Sorsby, Ungar and Bailey (1952) in nine cases of corneal ulcers, two of these being caused by *Ps. pyocyanea* treated with streptomycin.

The problem of pyocyanea infections of the cornea was investigated in rabbit experiments by Grün and Reinhart (1949), who used subconjunctival injections of streptomycin, starting treatment on the second day after infection when the corneal ulcer was established. They came to the conclusion that streptomycin alone is not sufficient to cause healing, but

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that when it was supplemented with oral or local administration of sulphonamide the ulcers healed. Apparently they did not add adrenaline to the solution used for subconjunctival injection.

Recently Sorsby, Ungar, and Bailey (1952), in a series of rabbits, successfully prevented the *Ps. pyocyanea* infection by subconjunctival injections of 0.5 g. dihydro streptomycin dissolved in liq. adrenaline, 3 hrs after infection.

Present Investigations

We have aimed at ascertaining whether a semi-solid alginate cap or gelatine disk carrying the antibiotic or drug would ensure a more prolonged action than that obtainable by instillation of drops or by the use of ointments.

Experimental Technique.—Infection of the cornea was produced in the anaesthetized rabbit's eye mostly by intracorneal injection of a suspension of 24-hour-old broth culture of a freshly subcultured human pathogenic *Ps. pyocyanea*. A small wheal of about 4 mm. diameter was produced by the fluid injected into the superficial layers of the cornea. Next day in the untreated eyes an ulceration with dirty grey base was present, which led eventually to extensive scarring and shrinkage of the globe or to ectatic cornea. In a few experiments the infection was produced by scarifying an area of 4 sq. mm., placing one drop of the pyocyanea suspension over the area and rubbing it in with the scarification needle. Treatment, which was given immediately, consisted of:

- subconjunctival injection of streptomycin,
- streptomycin powder applied in an alginate cap,
- gelatine disk containing 20 per cent. streptomycin,
- glycerine gelatine disk containing 20 per cent. streptomycin.

FIG. 1.—Area of inhibition (shaded) remained almost the same. The disks were made on November 5, 1951.

- Plate test November 28, 1951.
- December 22, 1951.
- January 26, 1952.
- February 25, 1952.

The alginate cap or gelatine disk was kept in the conjunctival sac by closing the lids with a single central suture. The gelatine or glycerine gelatine disks used in the majority of these experiments, were made as follows:

A Perspex plate containing drill holes $\frac{1}{2}$ " (12 mm.) in diameter rested on a sheet of Perspex or glass, and the streptomycin gelatine solution was poured into the drill holes. After they had set, the disks were removed and stored in a wide-mouthed jar.

The gelatine-lamella-base disks proved unsuitable because if kept in an uncovered jar they dried out and became brittle, and if kept in a well-closed container they retained their consistency, but were liable to bacterial and fungus contamination. The glycerine gelatine disks kept for 4 months in a jar, showed no contamina-

tion with moulds, and remained the same when the area of inhibition for *Ps. pyocyanea* was tested by the plate method (Fig. 1). These tests were made after completion of the experiments, and the stored disks were not assayed in experimental infection.

Results

Table I shows that 0.5 g. streptomycin (in 1 ml. 0.5 per cent. amethocaine solution with 2 min. adrenaline 1: 1,000) given as a single sub-

TABLE I
TREATMENT WITH STREPTOMYCIN SUBCONJUNCTIVALLY

Rabbit No.	Mode of Infection	Treatment	Result after 24 hrs	Remarks
1	Intracorneal injection, <i>Ps. pyocyanea</i>	0.5 g. subconjunct. c. adrenaline	Clear	Remained clear
2		" "	Moderate infection	Became worse
3		" "	Clear	Remained clear
4		" "	Clear	Remained clear
5		Control	Heavily infected	
6		Control	Heavily infected	

TABLE II
TREATMENT WITH STREPTOMYCIN ADMINISTERED IN CALCIUM ALGINATE CAPS OR GELATINE OR GLYCERINE-GELATINE DISKS

Rabbit No.	Mode of Infection	Treatment	Result after 24 hrs	Remarks
1	Intracorneal injection <i>Ps. pyocyanea</i>	60 mg. in calcium alginate cap	Clear	Remained clear
2		" "	Clear	Remained clear
3		0.1 g. in gelatine disk	Clear	Remained clear
4		" "	Infected	The eyes were found open and it is suspected that the rabbits scratched off the lid suture and the gelatine came out too soon
5		" "	Infected	
6		" "	Clear	Remained clear
7		" "	Clear	Remained clear
8		" "	Clear	Remained clear
9		21-day-old gelatine disk	Infected	Progressively worse
10		0.1 g. in glycerine-gelatine disk	Clear	
11	Scarification of cornea 1 drop 24-hr culture rubbed in	" "	Clear	Remained clear
12		" "	Clear	
13		" "	Clear	
14	Intracorneal injection	Control	Heavy infection	
15		Control		
16		Control		
17	Corneal scarification plus 1 drop culture	Control		
18		Control		

conjunctival injection protects the cornea. The infection employed was a massive one such as does not occur in clinical practice, yet in three out of four eyes the cornea remained clear.

In the experiments tabulated in Table II the streptomycin was administered by alginate cap or in gelatine or glycerine gelatine disks. It is seen that in an alginate cap as little as a single dose of 0.06 g. streptomycin is capable of preventing the development of the corneal infection, while in gelatine or glycerine gelatine disks 0.1 g. gave protection against massive infection (Figs 2 and 3). From experiments with chloramphenicol (Klein and Millwood,

FIG. 2.—Untreated eye 3 days after infection with *Ps. pyocyanea*.

FIG. 3.—Protective effect of streptomycin glycerine-gelatine lamella. Infection completely controlled.

1952) it was known that the rabbit eyes do not take kindly to calcium alginate shells, and for this reason we searched for another method. The gelatine disks were well tolerated, but it was found however that at body temperature they melted and if freshly made quickly disappeared from the conjunctival sac. If however the disks were stored for longer than a week (*vide supra*) they were liable to become contaminated with fungi and lose potency, or to dry out and cause injury to the eye. In one experiment (No. 9, Table II) where a 21-day-old gelatine disk was used, the cornea became infected. However, glycerine gelatine disks (suggested by Mr. Frank Allen) have a much longer shelf life [they do not dry out even if left in an uncovered jar, and no contamination with moulds has so far been noticed] and have marked protective action (Nos 10, 11, 12, 13, Table II).

When the streptomycin-calcium-alginate cap was removed from the eye after 24 hours, and cultured, no growth of *Ps. pyocyanea* was found although other organisms were present. Streptomycin is bacteriocidal, and employed in a suitable form comes near to Mules's requirement "to remove the

irritating element, and then the natural process of repair will heal the breach of surface ”.

In order to compare the effectiveness of the medication in caps or disks with the subconjunctival method the same amount of streptomycin as was effective by the disk method was injected subconjunctivally. As Table III shows no protection was afforded to the cornea.

TABLE III
TREATMENT WITH SMALLER DOSES OF STREPTOMYCIN SUBCONJUNCTIVALLY

Rabbit No.	Mode of Infection	Treatment	Result after 24 hrs	Remarks
1	Intracorneal injection <i>Ps. pyocyanea</i>	30 mg. in sod. alginate	Heavy infection	Progressively worse
2		60 mg. in sod. alginate		
3		60 mg. c. adrenaline		
4		60 mg. c. adrenaline		
5		Control		
6		Control		

It seems that, in these series of experiments, 0.5 g. given subconjunctivally compares with 0.06 g. administered in an alginate cap and 0.1 g. administered in disk form.

Discussion

Atypical corneal ulcers, corneal abscesses, and proteolytic ulcers (Sorsby and others, 1952) are mostly caused by Gram-negative organisms, and many of them by *Ps. pyocyanea*. The infection is often preceded by the presence of a foreign body in the eye which has been removed, or by some trivial injury. They do not respond to routine treatment, and are usually resistant to penicillin, and in spite of treatment the neighbouring clear corneal portions become invaded with such rapidity that in a few days almost the whole cornea may be destroyed. Until recently these infections were regarded as almost certain losses. Lately, however, sporadic successes with streptomycin have been published.

With systemic administration of streptomycin the concentration reached in the ocular tissues is very poor (Leopold, 1950) and in the case of the cornea there is the added disadvantage that its circulation, especially in the central parts where many of these ulcers occur, is very slow. Streptomycin administered systemically is therefore ineffective. Experiments have shown that the large molecule of streptomycin penetrates the intact corneal epithelium poorly if administered locally in eyedrops, or ointment, and only slightly better by iontophoresis. When the epithelium is abraded the penetration is considerably increased and one would therefore expect local treatment to be effective in corneal ulcers. In the series of cases reported

by Bignell (1951) those treated by streptomycin eyedrops were failures, probably because the concentration in the tissues was not continuously maintained. It is known (Ridley, 1931) that eyedrops disappear from the conjunctival *cul de sac* in a matter of minutes. With this form of treatment therefore an optimum concentration for an adequate time is not obtainable.

With ointments the drug is embedded in the ointment base, and release from the vehicle is uncertain; especially in a water-in-oil type of ointment, or if the drug is suspended in a greasy vehicle without a solvent. With subconjunctival injections rapid improvements were achieved (Bignell's Cases 4, 8, 10, 1951; Clark and Locatcher-Khorazo, 1951; Sorsby and others, 1952).

In the experiments here presented streptomycin was administered locally in a calcium alginate cap, or in a gelatine or glycerine gelatine disk. This method of administration seems to be effective against experimental ocular infection with *Ps. pyocyanea*.

It is significant that one-fifth of the effective dose of streptomycin administered subconjunctivally was sufficient to control the infection when given by this method. If the same amount of streptomycin was used subconjunctivally which was effective with the glycerine-gelatine disk the former was ineffective. The present investigation supports the results of Sorsby and others (1952) and it is suggested that with subconjunctival injection a minimum dose of 0.5 g. streptomycin with added adrenaline should be regarded as the standard treatment for *pyocyanea* infection of the cornea.

Clinically there are two distinct problems, that of prevention, and that of treatment. For prevention it seems reasonable that in the first-aid posts of factories where most of these infections seem to occur, after removal of a foreign body or treatment of a superficial corneal lesion, streptomycin should be inserted into the conjunctival sac. The form of application to be used will be decided in the light of experience, and it is possible that a single application of a streptomycin ointment may be sufficient. Better still, a glycerine-gelatine-streptomycin disk with a content of 0.1 g. streptomycin should be placed in the *cul de sac* and the eye bandaged. In any case some form of streptomycin, suitable for the local treatment of the eye, should be kept in the medicine chest of the first-aid post. The glycerine-gelatine disks proved successful in laboratory experiments but their tolerance by the human eye needs further investigation. The disks should be stored in a refrigerator, and replaced every 3 months because in the presence of the small amount of water in the disks the streptomycin content may diminish.

For the treatment of an established infection with *Ps. pyocyanea* the standard method of subconjunctival streptomycin is recommended. If, however, for any reason the subconjunctival injections cannot be given, as for instance in cases of painful reaction to injection, streptomycin in glycerine-gelatine disk 3 times a day is the next choice. The eye must be kept bandaged to keep the disk in place after insertion. The experimental findings justify an extended clinical trial of this procedure.

Summary

A single application of 0.1 g. streptomycin in the form of a glycerine-gelatine disk was able to control a massive corneal infection with *Ps. pyocyanea* in rabbits.

It is suggested that streptomycin locally applied should be used as a routine prophylactic in cases of superficial injuries of the eye in industrial first-aid posts, and that streptomycin ointment or disks should be kept in store in the medicine chest.

For the treatment of a corneal ulcer due to infection with *Ps. pyocyanea* the established treatment of subconjunctival injection of a minimum dose of 0.5 g. streptomycin with added adrenaline is recommended, and glycerine-gelatine-streptomycin disks may be used as a supplement.

In cases of intolerance to subconjunctival administration the glycerine-gelatine-streptomycin disks are worth trying.

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