CORNEAL ULCERATION*

SENSITIVITY OF BACTERIA TO ANTIBIOTICS

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CORNEAL ulcers are common in all ophthalmic hospitals in India, especially in the summer. Their treatment was very unsatisfactory before the advent of antibiotics but even antibiotics were found to have their limitations in hypopyon ulcers, especially those due to *B. pyocyaneus* which always progressed to perforation of the cornea in spite of vigorous treatment. It was therefore decided to place the antibiotic therapy on a sound and scientific basis. A cultural study was made of the scrapings of corneal ulcers and conjunctival discharges. As the organisms could have come from exogenous and endogenous sources, this study was supplemented by culturing discharges from the meibomian glands, washings of the lacrimal sac and external auditory meatus, and normal conjunctival and nasal secretions. Sensitivity tests were carried out on six antibiotics in common use.

Methods of Study

Cultures.—The bacteriology of the conjunctival discharge was studied in fifty persons of different age and sex, and that of discharge from the meibomian glands in 25 normal persons.

Washings of the lacrimal sac were cultured in 25 normal persons to find if an uninfected sac also acted as a reservoir of pathogenic organisms which might infect a corneal abrasion.

The bacterial flora of the external auditory meatus were studied in 25 persons who had no history of ear discharge.

In 100 cases of corneal ulceration, in addition to detailed history-taking and routine blood and urine examinations, the following bacteriological examinations were carried out.

- (1) Smear examination and culture of the conjunctival discharge.
- (2) Smear examination and culture of the scrapings from the advancing margin of the corneal ulcer.

The specimens were taken by a platinum loop before staining the ulcers with fluorescein, as this has been held responsible for a negative culture in some cases (Juler and Young, 1945), and has also been blamed as a source of *B. pyocyaneus* (Thygeson, 1948).

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To find out whether the nose acted as a source of infective organisms, the nasal discharge was cultured in 29 cases of chronic rhinitis with ulceration of the cornea.

Tests.—The sensitivity tests were carried out with various groups of organisms isolated from twenty cases of corneal ulcer, and the results of these tests were similar in the various groups of organisms isolated from different patients. Even in the organisms isolated from cases of ear discharge the sensitivity tests gave results identical with those from cases of corneal ulcer, proving the similarity of strains in the population of the Punjab.

Technique of Sensitivity Tests

Standard stocks of each of these drugs, except aureomycin, were prepared in sterile distilled water, so that 1 ml. of each contained the following: 20 mg. sulphacetamide, 40 units penicillin, and 200 µg. of each of the other antibiotics (dihydrostreptomycin, chloramphenicol, and terramycin). These could be stored in a refrigerator for one week without deterioration. In the case of aureomycin, which deteriorates on standing, a fresh solution had to be prepared every time, by dissolving a 50-mg. tablet in 250 ml. sterile water.

Ten sterile tubes for each drug were set out in a test tube rack; 0.5 ml. infusion broth at pH 7.3–7.4 was delivered into each of the tubes except the first; 0.5 ml. standard solution of the drug was delivered into the first and second tubes; the contents of the second tube were mixed and 0.5 ml. transferred to the third tube; this procedure was repeated for the succeeding tubes. After mixing the contents of the last tube, 0.5 ml. was discarded so that all the tubes contained 0.5 ml. in different dilutions of the drug, *i.e.*, 1:1, 1:2, 1:4, and so on.

After this a 1-in-10,000 dilution of a 24-hr culture of the organism was prepared in infusion broth, and 0.5 ml. of this diluted culture was added to each tube in the series. Thus the final dilution of the culture was 1-in-20,000; that of the drugs is shown in Table I.

TABLE I
DILUTIONS OF DRUGS

Test Tube No.		1	2	3	4	5	6	7	8	9	10
Drug	Terramycin (µg./ml.) ...	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19
	Chloramphenicol (µg./ml.)	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19
	Aureomycin (µg./ml.)	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19
	Dihydrostreptomycin (µg./ml.)	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19
	Penicillin (units/ml.) ...	20	10	5	2.5	1.25	0.62	0.31	0.15	0.078	0.039
	Sulphacetamide (mg./ml.)	10	5	2.5	1.25	0.62	0.31	0.15	0.078	0.039	0.019

The rack containing the test tubes was shaken gently but thoroughly and incubated for 24 hrs at 37°C. Next day the tubes were examined for any evidence of turbidity. The concentration of the drug in the first tube showing no evidence of bacterial growth was taken to be the minimum inhibitory concentration (MIC) of the drug for that organism, as an index of the sensitivity of the micro-organism to that particular drug.

In cases of streptococcus and pneumococcus, glucose broth was used in place of ordinary infusion broth.

Results

Cultures

(1) *Normal Conjunctival Sac*.—The following results were obtained from fifty cases:

Pathogenic ...	...	<i>Pneumococci</i> ...	...	...	...	6
		<i>Strept. viridans</i> ...	...	...	...	3
		<i>Staph. pyogenes</i> ...	...	...	...	2
Non-pathogenic ...	...	<i>C. xerosis</i> ...	...	...	...	29
		Non-pathogenic staphylococcus	...	...	...	19
Negative ...	...	...	...	...	...	16

Thus 22 per cent. of the organisms were pathogenic, but in two cases more than one pathogenic organism was isolated; only 18 per cent. of the subjects had pathogenic organisms in the conjunctival sac. Detailed results are given in Table II.

TABLE II
BACTERIAL FLORA OF NORMAL CONJUNCTIVAL SAC

Age Group (yrs)	No. of Cases	Negative Growth	<i>C. xerosis</i>	Non-pathogenic Staphylococcus	<i>Staph. pyogenes</i>	<i>Strept. viridans</i>	Pneumococcus	Pathogenic Organisms	More than One Organism	
0-10 ...	7	3	4	3	1	—	—	1	4	
11-20 ...	7	3	2	3	—	1	1	2 in one case	2	
21-30 ...	7	—	6	4	—	—	2	2	5	
31-40 ...	8	1	7	5	—	—	1	1	6	
41-50 ...	7	3	4	2	1	—	—	1	2	
51-60 ...	7	3	2	1	—	2	2	4 in 3 cases	3	
Above 60	7	3	4	1	—	—	—	—	1	
Totals	No.	50	16	29	19	2	3	6	11	23
	Per cent.	100	32	58	38	4	6	12	22	

(2) *Meibomian Glands*.—The discharge from 25 normal cases gave the following results:

Pathogenic (isolated from eight cases, 32 per cent.)	<i>Strept. viridans</i> ...	...	...	...	4
	<i>Pneumococci</i> ...	...	...	...	2
	<i>Staph. pyogenes</i> ...	...	...	...	2
	<i>C. xerosis</i> ...	...	...	...	10
Non-pathogenic ...	Staphylococci	...	...	...	8
	<i>Micrococcus</i> ...	...	...	...	1
	Non-haemolytic streptococci	...	...	...	1
Negative ...	...	...	...	...	9

(3) *Lacrimal Sac*.—Washings from 25 normal persons gave the following results:

Pathogen ...	<i>Strept. viridans</i> ...	...	...	...	1
	Staphylococci ...	...	...	...	12
	<i>C. xerosis</i> ...	...	...	...	8
Non-pathogenic ...	Micrococci	...	...	...	1
	<i>N. Catarrhalis</i> ...	...	...	...	1
Negative ...	...	...	...	...	8

(4) *External Auditory Meatus*.—No pathogenic organisms were isolated from 25 normal persons, only the following being found:

Staphylococci ...	...	...	...	...	...	...	...	17
<i>C. xerosis</i> ...	...	...	...	...	...	...	...	10
<i>M. tetragenes</i> ...	...	...	...	...	...	...	...	3
Negative growth ...	...	...	...	...	...	...	...	3

(5) *Corneal Ulcer*.—The following bacteria were isolated from the conjunctival discharge and ulcer scrapings in 100 cases:

Pathogenic ...	{	<i>Staph. pyogenes</i> ...	...	...	...	...	31
		<i>B. pyocyaneus</i> ...	...	...	...	...	14
		<i>Pneumococci</i> ...	...	...	...	...	8
		<i>Strept. viridans</i> ...	...	...	...	...	8
		<i>B. proteus</i> ...	...	...	...	...	3
Non-pathogenic ...	{	<i>Strept. haemolyticus</i> ...	...	...	...	...	2
		<i>C. xerosis</i> ...	...	...	...	...	35
		Staphylococci ...	...	...	...	...	22
		<i>M. tetragenes</i> ...	...	...	...	...	3
		<i>N. catarrhalis</i> ...	...	...	...	...	1
Non-haemolytic ...		Streptococci ...	...	...	...	...	1
Negative ...		...	...	...	...	...	19

The following organisms were seen in the smears of five of the nineteen negative cases:

Gram-positive diplococci ...	...	...	...	...	...	1
Gram-positive cocci and bacilli ...	...	...	...	...	...	1
Gram-positive cocci ...	...	...	...	...	...	1
Gram-negative bacilli (<i>B. pyocyaneus</i>)? ...	...	...	...	...	...	1
Gram-positive diplococci and bacilli ...	...	...	...	...	...	1

In these hundred cases of corneal ulcer, the lacrimal sac was positive in only three, in two of which pneumococci were isolated.

(6) *Nasal Cavity*.—The following organisms were isolated from the nasal discharge in 29 cases of corneal ulcer with chronic rhinitis:

Pathogenic ...	{	<i>Staph. pyogenes</i> ...	...	...	...	...	10
		<i>Strept. viridans</i> ...	...	...	...	...	5
		<i>Pneumococci</i> ...	...	...	...	...	4
		<i>B. pyocyaneus</i> ...	...	...	...	...	1
		Friedlander's bacillus ...	...	...	...	...	1
Non-pathogenic ...	{	<i>C. xerosis</i> ...	...	...	...	...	13
		Staphylococci ...	...	...	...	...	12
		<i>M. tetragenes</i> ...	...	...	...	...	1
		<i>N. catarrhalis</i> ...	...	...	...	...	1
Negative ...		...	...	...	...	...	4

(7) *Comparison of conjunctival and nasal discharge*.—In only three of 29 patients with corneal ulcer were all the pathogenic organisms found in the conjunctival discharge also present in the nasal discharge (*Staph. pyogenes* in two; *B. pyocyaneus* in one). In seven cases, only one of the pathogens in the nose was also found in the conjunctival flora. Pathogenic organisms were isolated in the conjunctival discharge of nine of the remaining nineteen, and in the nasal discharge of another nine.

Tests.—All the Gram-positive cocci were very sensitive to all the drugs tested except sulphacetamide which was less effective; *Staph. pyogenes* was found to be resistant to sulphacetamide in the strengths used (in these tests up to 10 mg. ml.), except for a few strains whose growth was inhibited by strong concentrations only.

B. pyocyaneus and *B. proteus* were only Gram-negative organisms isolated from these ulcer cases. All the strains of *B. pyocyaneus* were found to be most susceptible to dihydrostreptomycin and next to terramycin; all were resistant to penicillin and almost all to aureomycin. *B. proteus* was found to be most sensitive to dihydrostreptomycin and resistant to penicillin and aureomycin (Table III).

TABLE III

CONCENTRATIONS OF DRUGS TO WHICH MICRO-ORGANISMS WERE SENSITIVE

Serial No.	Organism	Concentration Necessary for Total Inhibition (per ml. medium)					
		Sulphacetamide (mg.)	Penicillin (units)	Dihydrostreptomycin (μg.)	Aureomycin (μg.)	Chloramphenicol (μg.)	Terramycin (μg.)
1	<i>Staph. aureus</i>	R*	0.03	0.19	0.39	3.12	0.19
2		10	0.31	0.39	0.39	0.39	0.39
3		R	0.03	0.19	0.19	3.12	0.39
4		R	0.07	0.19	0.78	0.39	3.12
5		R	0.03	0.78	0.39	0.39	0.39
6		R	0.03	0.78	100	6.25	3.12
7		R	0.03	1.56	25	3.12	3.12
8	<i>Pneumococcus</i>	0.03	0.03	0.19	0.39	0.19	0.19
9		1.25	0.03	0.19	0.19	0.39	0.39
10		0.62	0.07	0.19	0.39	0.39	0.19
11	<i>Strept. viridans</i>	0.03	0.03	0.19	0.19	0.19	0.19
12		10	0.07	25	0.19	6.25	0.19
13		10	0.03	6.25	0.39	3.12	0.19
14	<i>Strept. haemolyticus</i>	0.01	0.03	0.19	0.19	0.39	0.19
15	<i>B. pyocyaneus</i>	5	R	1.56	R	50	100
16		0.62	R	3.12	R	R	50
17		0.31	R	25	100	R	50
18		0.15	R	3.12	R	100	50
19		0.62	R	3.12	R	R	100
20	<i>B. proteus</i>	10	R	6.25	R	12.5	50

* R—resistant to dilutions used.

Discussion

Cultures.—The commonest pathogen responsible for corneal ulceration in this part of India is *Staph. pyogenes* (31 per cent.). This finding agrees with that of Juler and Young (1945), who cultured this organism in 30.4 per cent. of their cases. Next in importance is *B. pyocyaneus*. *Pneumococcus* does not play any significant role. Only *B. pyocyaneus* produced clinically distinguishable ring ulcers in most of the cases; it was associated with trauma in nine out of fifteen cases. *Pneumococcus* failed to produce a typical ulcer serpens.

The study of the bacterial flora of the healthy normal conjunctival sac revealed that 18 per cent. of the subjects were harbouring pathogenic or-

ganisms. A break in the continuity of the corneal epithelium in such persons will lead to corneal ulceration. In the other 82 per cent. where the conjunctival sac is either sterile or is harbouring non-pathogenic organisms, the pathogenic organisms must have come from one or more of the following sources:

- (1) *Foreign Body*.—25 per cent. of our cases gave history of trauma.
- (2) *Lacrimal Sac*.—Pneumococcus is usually held responsible for dacryocystitis and culture of pneumococcus was obtained in two of our three cases who had associated infection of the lacrimal sac.
- (3) *Meibomian Glands*.—Pathogens were isolated in the discharge from the meibomian glands in 32 per cent. of normal persons (Scobee, 1942).
- (4)* *Ear*.—Browning (1927) suggested that the ear should always be examined when *B. pyocyaneus* has been isolated from the conjunctival sac.
- (5)* *Bottles*.—Thygeson (1948) thought fluorescein bottles were the source of *B. pyocyaneus* infection, and others blame penicillin bottles.
- (6) *Nasal Cavity*.—The similarity of flora in the nose and conjunctiva (above p. 000) may be due to bacteria not normally found in the nasal cavity being carried down the lacrimal passages. As regards the route of infection from the nose, we presume it to be by way of contaminated fingers and handkerchiefs.

Sensitivity Tests.—These revealed that all these drugs except sulphacetamide inhibited the growth of *Staph. pyogenes*, pneumococci, and streptococci in quite low concentrations.

Staph. pyogenes, the commonest infecting organism in corneal ulceration, was resistant to sulphacetamide in the strength used in these tests (up to 10 mg. ml.), except for a few strains which were inhibited by strong concentrations only. Similar results were obtained by us in tests carried out with sulphadiazine against thirteen strains of *Staph. pyogenes* isolated from the aural discharge. This shows that the sulphonamides are not very efficacious in corneal ulceration and should be replaced by antibiotics.

Andina and Allemann (1950) tested the sensitivity of 52 strains of *Staph. aureus haemolyticus*, 48 strains of *Strept. haemolyticus*, 62 strains of *Strept. viridans*, and eighteen strains of pneumococcus by the hole test method. Their results agree with ours, except that they found *Staph. aureus* also susceptible to sulpha-drugs.

All strains of *B. pyocyaneus* were found to be susceptible to dihydrostreptomycin, terramycin, and sulphacetamide; all were resistant to penicillin and almost all to aureomycin and chloramphenicol. Similar tests carried out by us with sixteen strains isolated from the aural discharge also support these findings. Lutz and others (1950) found that all their strains of *B. pyocyaneus* were resistant to penicillin and nearly all to streptomycin and chloramphenicol, while terramycin proved to be the most effective.

* Our observations, however, indicate that infection is mostly brought in by contaminated foreign bodies, for a history of trauma was obtained in nine out of our fifteen cases in which *B. pyocyaneus* was found; only three of them had used any eye drops before reporting to the hospital, fluorescein was not dropped into the eyes of any one of them, and none of them had discharging ears. The absence of pathogenic organisms in the normal external auditory meatus also goes against the view that the ears act as a source of infecting organisms in corneal ulcer.

B. proteus was found by Lutz and others (1950) to be most sensitive to dihydrostreptomycin and resistant to penicillin and aureomycin. Similar tests performed by us with 52 strains of *B. proteus* cultured from the aural discharge showed them all to be susceptible to dihydrostreptomycin and chloramphenicol (the latter in higher concentrations); 80 per cent. to terramycin; 40 per cent. to penicillin in the concentrations used. On the other hand, Lutz and others (1950), who also carried out these tests by the dilution method with fifty strains of *B. proteus*, came to the conclusion that chloramphenicol tested was the most effective antibiotic—only two of the fifty strains being resistant to it, whereas almost all resisted fairly high concentrations of aureomycin and terramycin. These wide variations in results may be due to differences in the strains isolated in different regions, and also perhaps to differences in technique.

Thus dihydrostreptomycin is the most effective drug in cases of corneal ulceration, and there are no strains which are resistant to it. Next comes terramycin which, though it inhibited all types of micro-organisms isolated from conjunctival discharge, required higher concentrations to check the growth of Gram-negative bacilli, and was equally effective in Gram-positive cocci. The results of *in vitro* tests are not finally conclusive, and before selecting a drug for systemic therapy the clinician must keep in mind its rate of absorption and distribution, rate of excretion, toxicity, and also its effects on the normal intestinal flora which are capable of synthesizing the vitamin B group (biotin, folic acid, riboflavin, thiamine, nicotinic acid, inositol, pantothenic acid) and vitamin K. Their effect on the rate of healing of the corneal epithelium should be considered besides their action on the infecting micro-organisms.

Summary

(1) A bacteriological study was made of fifty normal conjunctival sacs, meibomian gland secretions in 25 normal persons, washings of the lacrimal sacs in 25 normal persons, and the external auditory meatus of 25 persons.

(2) The bacteriology of corneal ulceration in one hundred cases is discussed.

(3) The results of sensitivity tests of twenty organisms carried out by the dilution method against sulphacetamide, penicillin, dihydrostreptomycin, aureomycin, chloramphenicol, and terramycin show that the strain of *Staph. pyogenes* found in this part of India is resistant to sulphacetamide. Dihydrostreptomycin and terramycin are the only two antibiotics which inhibited the growth of both Gram-positive and Gram-negative organisms isolated from the conjunctival discharge in corneal ulceration.

(4) The bacteriology of the nasal discharge in 29 patients suffering from corneal ulceration is compared with the organisms isolated from the conjunctival discharge.

(5) Various sources of infecting organisms in corneal ulcerations are discussed.

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