FUNGAL FLORA OF THE CONJUNCTIVAL SAC IN HEALTH AND DISEASE*†

INFLUENCE OF TOPICAL AND SYSTEMIC STEROIDS

BY

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ALTHOUGH the role of fungi as ocular pathogens has become more widely recognized (Maddren, 1941; Sykes, 1946; Mendelblatt, 1953; Mitsui and Hanabusa, 1955; Roberts, 1957; Haggerty and Zimmerman, 1958; Mikami and Stemmermann, 1958; Fine and Zimmerman, 1959; Chick and Conant, 1962; Currie, 1963; Ainley and Smith, 1965; Jelenkiewicz, 1965; Dominiczak, Branicka, and Lewenstam, 1965), relatively little attention has been given to the fungal flora of the healthy or diseased eye. Previous lengthy investigations conducted in Europe (Fazakas, 1935, 1953) and in the U.S.A. (Hammeke and Ellis, 1960) have shown some disparity in the frequency of occurrence of fungi in the healthy conjunctival sac; studies of smaller groups of patients (Mitsui and Hanabusa, 1955; Azevedo, 1962) are less conclusive owing to the small size and selectivity of the samples. Furthermore, some ocular mycoses may have an exogenous source, especially after surgery or trauma (Fine and Zimmerman, 1959) and an accurate knowledge of the species most likely to be encountered in the conjunctival sac is of more than academic interest. The first purpose of this paper, therefore, is to report the results of an investigation of the fungal flora of the clinically healthy conjunctivae of a large and representative sample of subjects in various age groups.

Our second purpose is to study the variations of fungal flora in corticosteroid-treated patients. It is well documented that steroids reduce tissue resistance to a wide variety of bacterial, viral, and fungal agents (Zimmerman, 1950; Kligman, Baldrige, Rebell, and Pillsbury, 1951; Selye, 1951), and it would appear that the increase of ocular mycoses within recent years may be associated with the extensive use of systemic and topical corticosteroids and of broad-spectrum antibiotics (Hogan, Thygeson, and Kimura, 1954; Suie and Havener, 1963; McLean, 1963). Consequently, we have investigated the effects of (a) topical steroids, (b) topical steroids combined with antibiotics, and (c) systemic steroids, on the conjunctival flora of various groups of patients.

The third section is concerned with the fungal flora of the eye in Sjögren's syndrome. The characteristically dry eye encountered in this syndrome (keratoconjunctivitis sicca) is associated with depletion of normal tear flow and impaired mechanical removal of foreign material, and may therefore be more susceptible to colonisation by microbial agents.

Material and Methods

Patients

(1) *Clinically Healthy Conjunctivae*

553 patients comprising 284 males and 269 females (1,106 eyes), without clinical evidence of

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external ocular inflammation, were chosen for the investigation of the fungal flora of the healthy conjunctival sac. Each decade of age was represented by at least forty patients, equally divided between the sexes (Table I). The children in the 0 to 9 year age group were obtained mainly from an orthoptic department; the 10 to 19-year-olds from an ophthalmic out-patient department and from a mass radiography centre.

TABLE I
PATIENTS WITH CLINICALLY NORMAL CONJUNCTIVAE, BY AGE GROUP AND SEX

Age Group (yrs)		0-9	10-19	20-29	30-39	40-49	50-59	60-69	70+	Total
Sex	Male	39	105	21	20	27	28	34	20	284
	Female	28	104	23	22	22	25	23	22	269
Total Patients		57	209	44	42	49	53	57	42	553

The association of conjunctival fungi with topical steroid therapy was examined in two series of patients.

(2) *Clinically Healthy Conjunctivae subjected to Topical Therapy*

Adult patients with no clinical evidence of external ocular disease were selected from an ophthalmic out-patient department and from a centre for rheumatic diseases.

(a) Cultures were taken before and after a course of betamethasone disodium phosphate (0.1 per cent. in water-miscible base) in 86 patients (26 males and 60 females; 165 eyes). The age range was 30 to 78 years (mean 62). 36 patients had various forms of cataract, 35 had refractive errors, and fifteen had glaucoma.

(b) Cultures were taken from 52 patients (17 males and 35 females; 104 eyes) before and after a course of betamethasone disodium phosphate (0.1 per cent.) and neomycin sulphate B.P. (0.5 per cent.) in water-miscible base (Betnesol-N, Glaxo) applied three times daily for 1 week. The age range was 28 to 75 years (mean 60.5). 25 of the patients in this group had various forms of cataract, three refractive errors, 23 rheumatoid arthritis, and one systemic lupus erythematosus. Altogether 138 patients (269 eyes) with clinically healthy conjunctivae were thus "treated" with the topical steroid or with the steroid/antibiotic drops for one week, the fungal flora of the conjunctival sac being determined before commencement and after the completion of treatment.

(3) *Diseased External Eyes subjected to Topical Steroid Therapy*

The second main clinical category comprised patients with various forms of external ocular disease, for which either betamethasone disodium phosphate or betamethasone disodium phosphate combined with neomycin sulphate had been prescribed.

(a) 46 patients (10 males and 36 females; 74 eyes; age range 15 to 58 years, mean 53) had been receiving the steroid preparation. Table II shows the conditions being treated, and Table III the duration of steroid treatment. Mydriatics were being instilled into nine eyes, miotics into three, and methyl cellulose drops into one eye.

TABLE II
OCULAR DISEASE

Disease under Treatment	No. of Patients	No. of Eyes
Conjunctivitis	15	25
Blepharo-Conjunctivitis	10	20
Episcleritis	5	8
Anterior Uveitis	15	20
Scleritis	1	1
Total	46	74

TABLE III
DURATION OF TREATMENT

Duration	No. of Eyes
1-4 wks	30
1-12 mths	40
1-3 yrs	4
Total	74

(b) 55 patients (10 males and 45 females; 75 eyes; age range 23 to 61 years, mean 54.5) had been receiving the steroid/antibiotic drops. The conditions under treatment are shown in Table IV and the duration of therapy in Table V. Mydriatics were being instilled into six of these eyes.

TABLE IV
OCULAR DISEASE

Disease under Treatment	No. of Patients	No. of Eyes
Conjunctivitis	16	21
Blepharo-Conjunctivitis	15	25
Episcleritis	1	1
Keratitis	15	19
Anterior Uveitis	4	4
Styes	4	5
Total	55	75

TABLE V
DURATION OF TREATMENT

Duration	No. of Eyes
1-4 wks	30
1-12 mths	38
1-3 yrs	7
Total	75

(4) *Conjunctivae of Patients receiving Systemic Steroid Therapy*

The association of conjunctival fungi with systemic steroid therapy was examined in thirty randomly chosen ward patients who were being treated in a centre for rheumatic diseases.

There were eight males and 22 females; age range 18 to 57 years (mean 52). Two of the patients had a probable recurrence of rheumatic fever and the remainder had rheumatoid arthritis. The duration of therapy varied from 1 month to more than 3 years, and the daily prednisolone intake from 5 to more than 16 mg. The total dosage of steroid ranged from 1.5 to over 9 g.

(5) *Conjunctivae of Patients with Sjögren's Syndrome*

The last clinical group chosen for the investigation of the fungal flora of the conjunctival sac consisted of 37 patients (3 males and 34 females; 74 eyes; age range 37 to 80 years, mean 55.2) with severe keratoconjunctivitis sicca attending a special "dry-eye" clinic. The clinical diagnosis was based on a history of dry, irritable, or itchy eyes, a strongly positive Schirmer II tear test (less than 5 mm.) using 10 per cent. ammonia, and the presence of marked staining of both conjunctiva and cornea with 1 per cent. rose bengal. In addition, all of these patients had rheumatoid arthritis based on American Rheumatism Association criteria (Ropes, Bennett, Cobb, Jacox, and Jessar, 1958), and they were all attending a centre for rheumatic diseases. Fourteen of the subjects were receiving systemic prednisolone (5-15 mg. daily), but none was receiving topical steroids or antibiotics. Topical treatment was discontinued for 3 to 4 days before the initial culture in order to allow as much discharge as possible to collect.

Collection of Specimens

In all cases, specimens for culture were taken from both conjunctival sacs using a stiff nickel-chrome wire loop, with which the lower fornices were vigorously scraped. In the case of the keratoconjunctivitis sicca patients, any ropy discharge was also utilized for culture. Any cultures found to be positive were repeated. Two hundred blank tubes, inoculated in the clinics under similar working conditions, were submitted to the laboratory interspersed in irregular batches with the clinical samples. Cultures were also taken at irregular intervals from the mydriatics, miotics, vital dyes, and methyl cellulose drops in current use at the ophthalmic out-patient department.

Cultivation of Yeasts and Fungi

The mycological growth medium employed throughout this investigation was 2 per cent. malt extract agar, containing 0.036 per cent. potassium tellurite as a bacterial inhibitor. The medium was dispensed as drops in cotton-wool-plugged sugar tubes. After inoculation and transmission to the laboratory, all tubes (including the blanks) were incubated at 25°C., and were examined for evidence of fungal growth at intervals of 7 days. Negative cultures were reincubated and no tube was discarded before the end of 4 weeks' incubation.

Yeasts and yeast-like fungi were identified according to the taxonomic descriptions of Lodder

and Kreger-Van Rij (1952). In particular, the specific identification of *Candida* species was based upon the results of sugar fermentation and carbohydrate assimilation tests (Lodder and Kreger-Van Rij, 1952). The identity of *Candida albicans* was confirmed by the demonstration of characteristic germ tubes in human serum, as described by Taschdjian, Burchall, and Kozinn (1960), and by the demonstration of chlamydospore formation on corn meal agar slide cultures. The identification of the filamentous fungi was based upon their gross colonial morphology on malt agar, and upon the nature and arrangement of their spores on malt agar slide cultures. The technique of slide culture was as described by Riddell (1950). In order to reduce bias during the study, the bacteriologist was not informed of the sources of the cultures.

Results

Table VI shows the frequency and generic identity of the fungal isolates from 1,106 healthy conjunctival sacs in various age groups. The overall incidence of fungi was 2.9 per cent., the lowest frequency (0.8 per cent.) being observed in the 0 to 9 year age group, and the highest (7 per cent.) in the 60 to 69-year age group. Although there were more isolates from patients over than from those under the age of 40 years, a progressive increase of incidence of isolates with increasing age was not demonstrated.

TABLE VI
ANALYSIS OF POSITIVE FUNGAL CULTURES FROM HEALTHY CONJUNCTIVAE, BY AGE GROUP

Age Group (yrs)		0-9	10-19	20-29	30-39	40-49	50-59	60-69	70+	Total
<i>Penicillium</i>	Sp.	-	-	1	1	3	1	1	-	7
<i>Aspergillus</i>	Sp.	-	1	-	-	-	3	-	-	4
<i>Rhodotorula</i>	Sp.	-	-	2	-	-	-	1	-	3
<i>Scopulariopsis</i>	Sp.	-	-	1	-	-	1	-	1	3
<i>Candida</i>	Sp.	-	2	-	-	-	-	1	-	3
<i>Isaria</i>	Sp.	1	-	-	-	-	-	1	1	3
<i>Geotrichum</i>	Sp.	-	-	-	-	-	-	3	-	3
<i>Papulospora</i>	Sp.	-	1	-	-	-	-	-	-	1
<i>Gliocladium</i>	Sp.	-	-	-	-	1	-	-	-	1
<i>Hormodendron</i>	Sp.	-	-	-	-	-	-	1	-	1
<i>Saccharomyces</i>	Sp.	-	1	-	-	-	-	-	-	1
<i>Rhizopus</i>	Sp.	-	1	-	-	-	-	-	-	1
<i>Nigrospora</i>	Sp.	-	-	-	-	-	1	-	-	1
Total	No. Per cent.	1 0.8	6 1.4	4 4.6	1 1.2	4 4	6 5.6	8 7	2 2.4	32 2.9

A comparison of the fungal flora of clinically normal external eyes before and after the topical application of a steroid or steroid/antibiotic preparation for one week is shown in Table VII (opposite). No significant increase in the frequency of isolation of fungi from the eyes of 86 patients treated with betamethasone disodium phosphate was observed, but the application of the betamethasone disodium phosphate/neomycin sulphate preparation was associated, over the same period of time, with a significant increase in incidence of fungi in the conjunctival sacs of 52 patients (χ^2 using Yates correction for continuity for small numbers = 8.2174; $P < 0.01$). On the other hand (Table VIII, opposite), the conjunctival sacs of patients with external ocular diseases being treated with the steroid or steroid/antibiotic preparation did not yield fungi any more frequently than the healthy sacs. There was no significant difference in isolation rates between patients subjected to topical steroids alone and those subjected to topical steroids combined with an antibiotic. Furthermore, repeats of the initially positive fungal cultures were uniformly negative, suggesting

TABLE VII
PATIENTS WITH CLINICALLY NORMAL EXTERNAL EYES GIVEN A TOPICAL STEROID
OR STEROID/ANTIBIOTIC PREPARATION

Treatment Group	No. of Patients	Culture			
		First		Second	
		No. Positive	Organism	No. Positive	Organism
Betamethasone Disodium Phosphate	86 (165 eyes)	2+ ^{ve}	<i>Aspergillus</i> <i>Rhodotorula</i>	3	<i>Aspergillus</i> <i>Penicillium</i> <i>Saccharomyces</i>
Betamethasone Disodium Phosphate/ Neomycin Sulphate	52 (104 eyes)	1+ ^{ve}	<i>Candida albicans</i>	11 { 3 3 3 1 1	<i>Candida albicans</i> <i>Penicillium</i> <i>Aspergillus</i> <i>Streptomyces</i> <i>Trichoderma</i>

TABLE VIII
PATIENTS WITH EXTERNAL OCULAR DISEASE RECEIVING A TOPICAL STEROID
OR STEROID/ANTIBIOTIC PREPARATION

Treatment Group	No. of Patients	Initial Culture		Repeat of Initially Positive Cultures
		No. Positive	Organism	
Betamethasone Disodium Phosphate	46 (74 eyes)	1	<i>Penicillium</i>	Negative
Betamethasone Disodium Phosphate Neomycin Sulphate	55 (75 eyes)	4	<i>Penicillium</i> <i>Aspergillus</i> <i>Scopulariopsis</i> <i>Rhodotorula</i>	Negative

that fungal contamination was transitory. Only one positive culture was obtained from the thirty patients receiving systemic steroid therapy.

Table IX (overleaf) shows the occurrence of fungi in the conjunctival sacs of 37 patients with Sjögren's syndrome after 4 days without their usual ocular toilet. Four isolates were obtained from the fourteen patients (28 eyes) receiving systemic prednisolone, and six isolates from the remaining 23 patients (46 eyes)—an overall incidence of 13.3 per cent. This is a significantly higher incidence than that in a group of patients matched for age and sex with clinically healthy conjunctival sacs ($\chi^2 = 5.258$; $P < 0.05$). Further specimens taken from these patients following resumption of their usual topical therapy did not yield fungi. Eight of the ten patients in this group, who had previously harboured conjunctival fungi, were sampled once again 4 days after ceasing ocular toilet. Seven fungal isolates were obtained, four of which were species of *Candida*.

Two of the 200 blank cultures yielded aerial contaminants.

Discussion

Previous surveys of the fungal flora of the healthy conjunctival sac have been undertaken by Fazakas (1935, 1953) in Central Europe, by Mitsui and Hanabusa (1955) in Japan, by

TABLE IX
PATIENTS WITH SJÖGREN'S SYNDROME

Treatment	No. of Patients	Initial Culture		First Repeat of Positive Cultures	Second Repeat of Positive Cultures
		No. Positive	Organism		
Systemic Steroids	14 (28 eyes)	4	<i>Penicillium</i> <i>Rhodotorula</i> <i>Rhodotorula</i> <i>Candida albicans</i>	Negative	3 Negative <i>Aspergillus</i> <i>Rhodotorula</i> / <i>Candida albicans</i> Not Repeated
No Systemic Steroids	23 (46 eyes)	6	<i>Stemphylium</i> <i>Rhodotorula</i> <i>Candida albicans</i> <i>Rhodotorula</i> <i>Tropicalis</i> <i>Penicillium</i> <i>Candida albicans</i> <i>Hormiscium</i>	Negative	4 <i>Candida albicans</i> <i>Penicillium</i> <i>Candida albicans</i> <i>Parapsilosis</i> <i>Candida albicans</i> Not Repeated

Hammeke and Ellis (1960) in the United States, and by Azevedo (1962) in Brazil. Ainley and Smith (1965), the only British workers who have so far undertaken a study of the fungal flora of the clinically normal conjunctiva, described a small series comprising 43 patients with no evidence of external ocular disease. The present investigation has utilized material from 1,106 healthy eyes, and is the largest series so far reported in Great Britain. In a study of 993 healthy eyes, Fazakas (1953) obtained 253 positive fungal cultures (25.4 per cent.). Hammeke and Ellis (1960) investigated 520 healthy eyes of adults, children, and neonates; 10.3 per cent. of 416 adults, 5 per cent. of 52 children, and 0.1 per cent. of 52 infants gave positive fungal isolates. Ainley and Smith (1965) studied 43 healthy eyes, and obtained twelve positive results (27.4 per cent.). We have obtained 32 positive cultures from 1,106 healthy eyes, a much lower incidence of 2.9 per cent.

Our clinical methods seem to be similar to those of other investigators and the lower incidence in this series may be partly explained by the cultural techniques employed. Thus, Ainley and Smith (1965) used Sabouraud's broth with subculture, after one week, to Sabouraud's agar plates, and subsequent incubation at 25°C. for up to 6 weeks. We used malt agar slants for primary isolation, and an incubation period extending to 4 weeks at 25°C. Hammeke and Ellis (1960) also employed Sabouraud's glucose agar, but the length of incubation of their cultures is not stated. In general, primary incubation in broth might be expected to yield a higher isolation rate than on solid medium.

Previous workers have indicated that variations in the frequency of certain fungi occur between various geographical regions, e.g. *Candida* species (Urrets-Zavalía, Remonda, and Ramacciotti, 1958) and *Sporotrichum* Sp. (Gordon, 1947; McGrath and Singer, 1952). Despite a low overall incidence of fungi, our results are in fairly good qualitative agreement with those of other investigators in other geographical areas with regard to the genera of fungi and yeasts most frequently found in the conjunctival sac. Thus, *Aspergillus* Species, *Rhodotorula*, *Candida*, and *Penicillium* Species appear to be common inhabitants of the healthy external eye (Fazakas, 1953; Mitsui and Hanabusa, 1955; Hammeke and Ellis, 1960; Ainley and Smith, 1965). These fungi collectively accounted for 54 per cent. of the total isolates in our series. Fazakas (1953) found that the majority of his isolates from healthy eyes were moulds, 28 per cent. of the isolates in his series of 993 eyes belonging to

the *Penicillium* group. 22 per cent. of the positive cultures in our "healthy eye" group were *Penicillium*.

In contrast to the findings of Hammeke and Ellis (1960), who reported distinct differences in the frequency of positive fungal isolates from conjunctival sacs in different age groups, we have not observed a progressive increase in incidence of fungi with increasing age, although the overall incidence of fungi in the older age groups was somewhat higher than in the very young age groups.

One may question the significance of the presence of fungi in the healthy conjunctival sac. In an attempt to provide a partial answer to this question, we repeated all of our 32 positive cultures within 4 weeks of their initial detection, but we obtained only four repeat positives. Furthermore, in none of these cases was the same species recovered. On the basis of these findings, we consider that the fungi cultivable from healthy conjunctival sacs must be regarded as transitory contaminants rather than resident commensals. Although it would appear, therefore, that little significance should normally be attached to the presence of fungi in the conjunctival sac, we nevertheless consider that it is important to have accurate knowledge of the fungal species most likely to be encountered there, even temporarily. The role of fungi as pathogens in ocular infections is becoming more widely recognized, and although an increased awareness of the possibility of ocular mycoses may be partly responsible, there is now well-documented evidence of a real increase in the incidence of mycotic infections of the eye (Haggerty and Zimmerman, 1958; Mikami and Stemmermann, 1958; Fine and Zimmerman, 1959; Chick and Conant, 1962), especially as a sequel to trauma or surgery of the eye (Fine and Zimmerman, 1959), where infection is believed to be exogenous in origin. In the latter instance, conjunctival saprophytes might assume a pathogenic role in an "opportunistic" infection, as recently discussed in the more general sphere of microbial disease by Symmers (1965).

The effect of corticosteroids in reducing resistance to a variety of bacterial, viral, and fungal infections is well recognized (Zimmerman, 1950; Kligman and others, 1951; Selye, 1951; Symmers, 1965), and it is believed that corticosteroids may permit fungi, normally regarded as harmless commensals, to behave as pathogens (Agarwal, Malik, Mohan, and Khosla, 1963; Suie and Havener, 1963). There is evidence that the extensive systemic and topical use of corticosteroids and broad-spectrum antibiotics has largely contributed to the increase of ocular mycoses (Hogan and others, 1954; Suie and Havener, 1963; McLean, 1963; Manchester and Georg, 1959; Wolter, 1962; Currie, 1963). There is also clear experimental evidence for the enhancement of the effects of fungus infection by corticosteroids (Mankowski and Littleton, 1954; Ley, 1956; Hirose, Yoshioka, Abe, Kanemitsu, and Kiya, 1957; Agarwal and others, 1963). Although such experimental conditions may have little counterpart in human ocular infections, there are frequent reports at the clinical level of ocular mycoses complicating steroid therapy, especially in relation to *Candida* species. Sykes (1946), Mendelblatt (1953), Mitsui and Hanabusa (1955), and Roberts (1957) have all reported corneal infections by *Candida albicans*. Maddren (1941) reported a case of severe angular conjunctivitis occurring in the course of extensive candidiasis in a woman, but Duke-Elder (1938) has stated that fungus infections of the conjunctiva are very rare. A case of ocular mycosis due to *Candida parapsilosis* was reported by Manchester and Georg (1959). Their patient was thought to have received corticosteroid and antibiotic drops for a long period before developing keratomycosis, the initial lesion being a superficial punctate keratitis. Currie (1963) described three cases of mycotic keratitis associated

with corneal ulceration. *Candida albicans* was implicated, and he considered that steroids had aggravated the condition. Ainley and Smith (1965) have more recently described a probable case of secondary keratomycosis due to *Candida parapsilosis* which responded to the administration of Nystatin.

It is of interest that both *Candida albicans* and *Candida parapsilosis* were recovered in our series of clinically normal conjunctivae, and that *Candida albicans* was represented in our isolates from patients receiving the topical steroid/antibiotic preparation.

Previous studies have been undertaken of the effects of corticosteroid therapy upon the incidence of fungi in the eye. Mitsui and Hanabusa (1955) obtained 42 positive cultures from 62 patients receiving topical ocular steroids (67 per cent.), while a control group of untreated patients had an incidence of 18.5 per cent. The majority of the isolates in their series were *Penicillium*, *Aspergillus*, *Candida*, *Saccharomyces*, and *Rhodotorula* species. In a second experiment, these authors selected eighteen cases initially negative for fungi by smear or culture. After topical application of hydrocortisone ointment for 3 weeks, the eyes of nine of the subjects were positive for fungi, *Penicillium* and *Rhodotorula* species predominating. Ainley and Smith (1965) failed to demonstrate any striking change in fungal flora after the application of a corticosteroid/antibiotic combination (Betnesol-N) to the eyes. Thus, of fifteen patients initially showing negative cultures, only three became positive for fungi after the administration of drops or ointment thrice daily for not less than 2 weeks. They point out that the number of patients studied was too small to give a statistically significant result.

In the present investigation, topical betamethasone treatment of patients with clinically normal external eyes and of patients with external ocular disease, did not result in any significant changes in the mycotic flora of the eye. Thus, 86 patients (165 eyes) with clinically normal external eyes received betamethasone disodium phosphate three times daily for 1 week; only two pre-treatment isolates and three post-treatment isolates were obtained. Furthermore, no generally accepted fungal pathogens were represented. 46 patients (74 eyes) with external ocular disease, who had been receiving topical betamethasone disodium phosphate for varying periods of time, yielded one fungal isolate, a saprophytic *Penicillium* species, and a further conjunctival sac scraping from the same patient was negative.

Four fungal isolates of no pathogenic significance were obtained from the eyes of 55 patients (75 eyes) with various forms of external ocular inflammation, who had been receiving the betamethasone disodium phosphate/neomycin sulphate preparation for varying periods; repeat cultures were uniformly negative. The combined steroid/antibiotic preparation, therefore, produced no significant changes in fungal flora in this group.

The effects of the topical administration of the betamethasone/neomycin preparation to the patients with clinically normal external eyes deserve comment. Thus, there was one isolate from 104 eyes before the commencement of therapy, and eleven isolates, including three strains of *Candida albicans* after its completion. It is difficult to offer a simple explanation of the comparatively large increase in ocular fungi in this particular group of subjects, but it is of interest that almost 50 per cent. of the patients in this category were hospital in-patients (23 rheumatoid arthritis, 1 systemic lupus erythematosus). When the cultural results are considered in relation to the source of the patient, ten of the eleven post-treatment isolates were derived from the 24 ward patients, whereas only one post-treatment isolate was obtained from the remaining eighteen out-patients. The higher incidence of

fungi in the ward patients might possibly reflect a high level of aerial fungal contamination of the ward environment when the specimens were collected. On the other hand, only one fungal species was recovered from thirty in-patients with rheumatoid arthritis receiving systemic steroids in the same wards as the former group.

There is no evidence from the present investigation that the neomycin component of the combined topical steroid/antibiotic preparation made any significant contribution to the alteration of fungal flora in patients so treated. Thus, there was no significant difference in the frequency of isolation of fungi from the eyes of patients with external ocular disease, whether treated with steroid/antibiotic combination or with steroid alone (Table VIII). It is, however, relevant to note that numerous workers have shown that antibiotics, especially the tetracyclines, can enhance the growth of fungi, notably *Candida albicans*, and that subjects treated with antibiotics are more often carriers of *Candida albicans* than are untreated controls (McGovern, Parrott, Emmons, Ross, Burke, and Rice, 1953; Sharp, 1954). There are relatively few reports to incriminate neomycin in this respect, though Reiersöl (1958) observed a marked increase in the incidence of faecal *Candida albicans* in patients given oral neomycin, and it is possible that local neomycin therapy might give rise to a similar increase in the conjunctiva.

The group of patients with Sjögren's syndrome yielded some interesting results. Ten primary isolates were obtained from 74 eyes. The patients were derived from a "dry-eye" clinic and they were instructed to use no local treatment, not even saline washouts, for 4 days before the first culture. Thereafter, they were allowed to return to their usual routine of thrice daily irrigations with saline and instillations of carboxymethyl-cellulose drops (0.5 per cent. solution) twice or three times daily. The second cultures were taken while the patients were receiving this irrigation regime, and the totally negative cultural results could be adequately explained on the basis of mechanical removal of foreign material from the conjunctival sacs by the irrigations. This thesis is supported by the fact that seven fungal isolates were subsequently obtained from the eyes of eight patients who consented to stop all local treatment for 4 days before the collection of further specimens. Any fungistatic effect of the carboxymethyl-cellulose drops (which contains chlorhexidine digluconate, 0.05 per cent.) was excluded by failure to demonstrate inhibition of twenty strains of *Candida albicans*, three strains of *Rhodotorula*, and two *Aspergillus* strains, in simple plate diffusion tests in agar.

Patients receiving systemic steroids and those on alternative treatment showed no significant differences in the frequency of isolation of fungi, but the results suggest that the dry eye accompanying Sjögren's syndrome is more susceptible to colonization with fungi than the healthy eye. Furthermore, patients with Sjögren's syndrome tend to belong to an ageing group, and intra-ocular surgery for cataract extraction or glaucoma may become a necessity. In view of the more frequent isolation of fungi from these patients, including the potential intra-ocular pathogens *Candida albicans* and *Candida parapsilosis*, it is considered that special pre-operative precautions are necessary, particularly since fungi are sometimes thought to be introduced as a result of surgical or accidental trauma (Fine and Zimmerman, 1959).

Summary

Studies were undertaken of the fungal flora of the healthy and diseased conjunctival sac and of the effects of steroids.

Fungi were isolated from 2.9 per cent. of 1,106 healthy conjunctival sacs, a higher incidence being observed in older age groups. Although the majority of the species isolated were non-pathogenic transient aerial contaminants, some potential intra-ocular pathogens were also represented.

The effects were studied of the topical administration of betamethasone and of a combined betamethasone/neomycin preparation to patients with clinically normal external eyes. Topical betamethasone therapy did not result in any significant changes in the fungal flora of the conjunctival sac: a significantly higher incidence of fungi was observed in the eyes of hospital in-patients receiving betamethasone/neomycin treatment.

The conjunctival sacs of out-patients with a variety of external ocular diseases being treated with betamethasone or betamethasone combined with neomycin did not yield fungi any more frequently than healthy conjunctival sacs: there was no significant difference in isolation rates between patients subjected to the topical steroid alone, and those subjected to the combined steroid and antibiotic preparation.

Fungi were obtained from 13.3 per cent. of the conjunctival sacs of 37 patients with Sjögren's syndrome. This was a significantly higher incidence than that obtained from a group of patients with healthy eyes matched for age and sex. The isolates included the potential intra-ocular pathogens *Candida albicans* and *Candida parapsilosis*, and evidence was obtained that the untreated dry eye associated with this syndrome was more susceptible to fungal colonisation than the healthy eye. The possible implications of this finding were discussed.

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