OTOMYCOSIS: A NEGLECTED DISEASE

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A SURVEY of British literature leaves the impression that otomycosis has never held a prominent place among ear diseases in this country. Dismissing the condition in a few lines, most authors suggest that it occurs infrequently and that treatment is a simple matter of applying some well-known preparation.

It is, however, apparent in most clinics that the discharging ear still presents unsolved problems. There is that small nucleus of patients who come and come again; whose treatment theme has infinite variations with but one tangible result—a steady increase in the thickness of their out-patient notes.

With these thoughts in mind the present writers decided, some four years ago, to investigate patients of this type for the presence of an active fungus infection. There were several good reasons for this. Local antibiotic therapy, while not in universal use, had attained significant proportions in the treatment of both external and middle-ear infections, and it has been recognized that antibiotic therapy elsewhere could lead to secondary infection by fungi. The very sparsity of British papers on the subject suggested that there might be much to learn about the rôle of the fungi in ear infections. Perhaps the most significant feature of the present series was the possibility of controlling clinical diagnosis by the investigations of a trained mycologist, and there were few examples of this having been done before.

From the start it was agreed that a diagnosis of otomycosis would be made only in those cases where a fungus was seen to be growing actively in a sample of debris taken from the external auditory meatus. Since fungus spores abound in the atmosphere, and might well enter the meatus only to lie dormant there, their detection by the culture of a swab taken from the meatus could not be regarded as evidence that they were playing an active part in an infective process therein. Where it appeared that the fungus was, in fact, playing an active part in infection it was planned to give treatment aimed specifically at eliminating it. Resolution of the clinical condition might then be accepted as reasonable proof that the fungus had caused it, or was a contributing factor in its maintenance.

It was, of course, understood that this proof did not necessarily satisfy Koch's postulates. No attempt to transfer the infection to animal

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hosts was to be made. This had, however, been done already by other investigators. In any case, Whalen (1938) has commented on the unreasonable restriction imposed by rigid observation of the fourth postulate.

Results of the Investigation

During the years 1956-9 180 patients were examined, of whom 83 were found to have a fungus infection (Table I). Sixty-two of these patients had infections of the external auditory meatus and 21 had infections in radical mastoidectomy or fenestration cavities.

TABLE I.

NUMBERS OF PATIENTS EXAMINED FOR OTOMYCOSIS.

Year	Total examined	Total positive
1956	7	5
1957	15	8
1958	20	11
1959	134	59

TABLE II.

NUMBERS OF DIFFERENT FUNGI ISOLATED.

1956		1957	
A. flavus	2	*A. fumigatus*	1
A. niger	2	*A. niger*	3
Candida Sp.	1	*C. albicans*	2
Yeasts	1	*C. krusei*	1
		C. parakrusei	1
1958		1959	
A. fumigatus	1	*A. fumigatus*	13
A. niger	3	*A. niger*	12
A. nidulans	1	*A. nidulans*	1
Scopulariopsis Sp.	1	*A. terreus*	3
C. albicans	4	*A. herbariorum*	1
Candida Sp.	1	*C. albicans*	12
		C. parakrusei	1
		C. guillermandi	1
		A. niger and *C. albicans* ..	1
		A. candidus Sp.	1
		Yeast Sp.	10
		S. brevicaulis	1
		Actinomycetes	2

The fungi found were predominantly species of Aspergillus or Candida (Table II). Although Aspergillus has long been recognized as a cause of otomycosis (Capps, 1938, Reeh, 1942, Johnston, 1944) there has been little reference to Candida previously. Even so, its occurrence in almost half of the cases should occasion little surprise for it is a well-known pathogen in other sites. It is, however, noteworthy that organisms such as Penicillium (Whalen, 1938) and Mucor (Johnston, 1944) do not appear in the series.

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Clinical Picture

This was not known. Descriptions in books of reference were brief and uninformative. With observation, however, patterns have become apparent, but even now they cannot be offered as anything more than impressions, and clinical diagnosis is still uncertain and inaccurate.

One symptom has occurred with some constancy. This is a sensation of irritation in the meatus which may be almost intolerable. Where the fungus

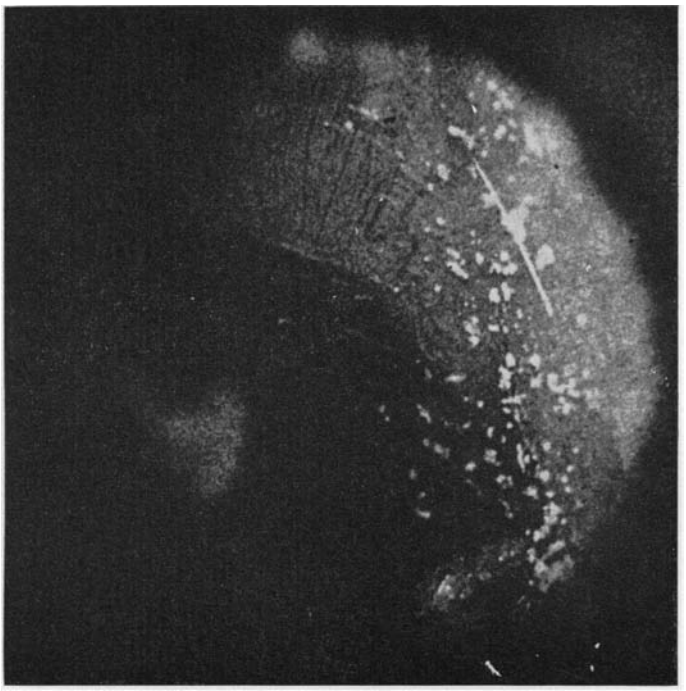


FIG. 1.
Clinical picture of infection by *A. flavus* as seen with the electric auriscope.

is causing otitis externa pain is unusual unless there is an associated pyogenic infection. Capps(1959) has, however, found that pain can be a symptom when the fungus causes infection in a radical mastoidectomy cavity and this observation has been confirmed. Discharge is scanty and is usually quite odourless. Unless some other factor causes a predisposition to it, deafness is not usual.

The meatus is rarely swollen unless self-inflicted trauma has led to a secondary pyogenic invasion. In the acute stage it is filled with a mass of grey caseous debris, quite unlike the "wet blotting paper" of classic descriptions. Removal of this debris shows a meatal lining of a distinctive

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magenta colour, covered with small glistening white masses. The tympanic membrane, although discoloured, is of normal contour.

Less acute cases may present two patterns, depending on the type of infection. Where Aspergillus is present (Fig. 1) the meatus is notably dry and the magenta colour is less evident. Clusters of conidiophores may be seen, resembling miniature pin-heads. They may be white, yellow, brown, black, or green, depending on the age and species of Aspergillus. Simpson



FIG. 2.

Clinical picture of infection by *C. albicans* as seen with the electric auriscope.

(1959) has described this picture as an asymptomatic condition among native troops in India, but it has occurred only once in a European in this series.

In Candida infections the picture more nearly resembles the acute case after cleansing (Fig. 2). The meatus is generally moist and small white aggregations are scattered evenly throughout. In the plotograph they may be seen as wavy white lines radiating from the centre of the drum.

These features are not easily seen with the naked eye, but are quite apparent when the electric magnifying auriscope is used. Previous treatment may, however, modify the clinical picture out of all recognition, particularly if oily preparations have been used. The writers have, there-

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fore been prepared to suspect a mycotic infection in any cases where there is a history of irritation or of previous antibiotic therapy, or where standard treatment, such as wicks of ichthyol in glycerine, has failed to produce a cure. In the interests of accurate diagnosis it is, of course, essential to have the confirmation of a direct mycological examination in these more doubtful cases.

Ætiology

Previous failure to clarify the clinical picture may have much to do with the reason for the surprisingly high frequency of this type of infection today, but the ætiology of the condition remains obscure. It is doubtful if the fungi are primary invaders of the external auditory meatus, although Brown (1937) held that they were, and occasionally there is a striking illustration.

CASE I.

A. B., male, aged 35, had no previous history of ear trouble. He was an enthusiastic yachtsman, and while sailing on a fresh-water lake one week-end his boat capsized, most of its equipment being dropped in about eight feet of water. He spent some time diving to retrieve this equipment, but, unable to recover it all, went back to the boat-house and searched the sail-loft for a grapnel. During this search he moved much unused equipment with a considerable disturbance of dust. Some three days later he noticed slight irritation in the ears, and as this increased over the next few days he sought advice. On examination he had an obvious infection of the ear canal with Aspergillus which was identified subsequently as *A. flavus*.

In other cases it has been possible to identify a primary source of infection elsewhere on the patient.

CASE II.

Mrs. C. D. was some four months pregnant when she presented with Candida infection in both external auditory meati; this responded satisfactorily to treatment but soon relapsed. Investigation by her obstetrician showed Candida infection of the vagina which proved resistant to treatment. The ear infection continued to relapse, but cleared up within two weeks of her delivery when the vaginal infection settled also.

The rôle of bacteria in paving the way for fungus infection remains open to question. Not all the cases in this series were subjected to bacterial investigation when the diagnosis of otomycosis was first made. Where this was done, however, a wide variety of bacterial flora was found. Of 27 cases so examined, 4 gave sterile cultures, 16 grew organisms of the pyocyaneus, proteus, or coliform types, 5 showed *Staphylococcus aureus* and 2 produced "yeasts".

The exudate occurring in bacterial ear infections seems likely to

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provide a degree of humidity favourable to fungal proliferation and it is clear that bacteria and fungi can co-exist in the same clinical condition. Antibiotic therapy then might suppress the bacteria primarily responsible, leaving the fungi free to grow. In the present series 30 cases had definite evidence of previous local antibiotic therapy, while in a further 31 cases evidence was less certain since therapy had been applied previously elsewhere and details were unavailable. Many of the Aspergillus species which were found are known to produce effective antibiotic agents in their

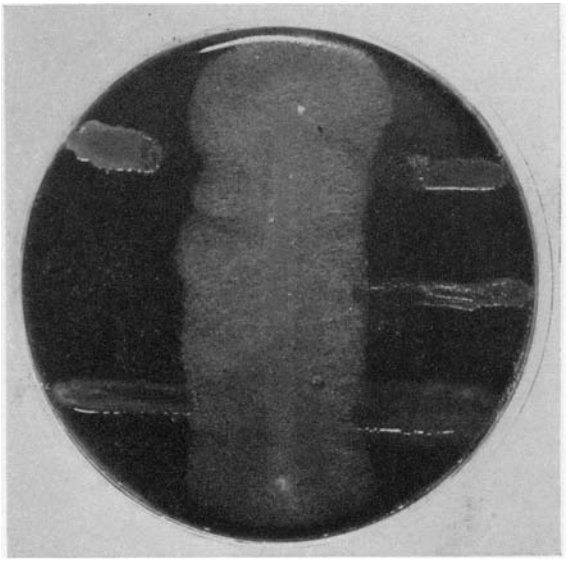


FIG. 3.
Culture plate showing the inhibiting effect of *A. fumigatus* on the growth of *Staphylococcus aureus* and *Streptococcus viridans*.

metabolism, although they may be too toxic for systemic use or have not yet been developed commercially. Fig. 3 shows the inhibiting effect of *A. fumigatus* on various known pathogenic bacteria. The failure to inhibit the growth of *Ps. aeruginosa* and *E. coli* is noteworthy.

A consideration of the metabolism of the common fungi found in the external ear can only occasion surprise that they are not invaders more frequently. Needing little more than moisture, warmth, a little protein or carbohydrate, and traces of mineral salts, their growth can be quite exuberant. Their proliferation in such unattractive sites as the space between the two lenses of a microscope objective is well known in tropical climates.

Nevertheless, examination of 32 clinically normal meati of volunteers from the hospital staff showed no evidence of actively growing fungus,

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and the subsequent culture of swabs from these meati gave only 2 positive results. As these occurred in a technician in the mycology department and a nurse in the allied department of dermatology, they could have been transient contaminants.

This does suggest that the normal secretion of the meatus may have an inhibiting effect on fungal proliferation. Senturia (1957) quotes several authorities in saying that most of the saturated and unsaturated fatty acids have some inhibitory effect on the majority of fungi. He mentions Akobjanoff *et al.* (1954) who identified nine saturated and unsaturated fatty acids in ear wax.

Mycological Aspects

It is clear from the experience gained by the authors as a result of investigating the mycology of otomycosis that the fungi most prominently associated with this condition are mainly species of the genus *Aspergillus* and various yeast-like species belonging to the genus *Candida*. They, among so many thousands of fungi whose spores and hyphae contaminate the atmosphere, are able to grow in the external auditory meatus while, evidently, the vast majority of fungi are unable to do so. They alone are able to establish themselves in this site with success and frequency in the presence of large numbers of bacteria.

A necessary prelude to an inquiry into this problem is to consider first of all what is already known of the main physiological requirements for the growth of fungi.

Main Physiological Requirements for Growth

These are, in short: adequate moisture, adequate warmth and adequate nutrition.

While such requirements vary within given limits for different species, it may be stated that, by and large, a percentage relative humidity of 70 to 80, a temperature range of 15° C. to 30° C., and a minimum supply of certain chemical elements, viz., carbon, oxygen, hydrogen, nitrogen, phosphorus, sulphur, potassium, chlorine, magnesium, as well as traces of iron, manganese and of certain vitamins, represent the conditions necessary for growth in the case of the majority of fungi. Certain fungi, however, grow, not only well at temperatures lying between 30° C. and 40° C., but actually grow and reproduce more rapidly within this temperature range than at lower temperatures, and a few find their optima for these functions at even higher temperatures.

It is also evident that certain moulds are able to grow in an environment in which the oxygen tension is low and in which the carbon dioxide concentration is relatively high; although it is well known that carbon dioxide concentration is an important factor in controlling both growth

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and reproduction in the fungi, especially when other conditions are minimal for growth. These effects of carbon dioxide concentration on fungus growth and reproduction have been studied by various authors but especially by Brown (1922).

Published data concerning carbon dioxide concentrations in the external auditory meatus appear to be lacking.

General and Selective Factors which may be Significant in enabling certain Moulds and Yeasts to become established in the External Auditory Meatus

It does not require experimental proof to show that the percentage relative humidity in the external auditory meatus is high, particularly when otitis externa is present, nor that the temperature in this site closely approximates "body temperature". Epithelial debris and serous exudate in various stages of chemical breakdown also provide a pabulum which is apparently suitable for moulds and yeasts to become established in the external auditory meatus when otitis externa is present. Such conditions should be, it might seem, suitable for many moulds and yeasts, even if not the best for particular species. Yet the fact remains that a particular group of moulds, namely certain species of Aspergillus, and a particular group of yeast-like fungi are predominantly favoured in these circumstances. To explain this, certain selective factors may be postulated. First of all, there is the factor of high temperature. All the species of Aspergillus whose active presence in the external auditory meatus was established microscopically and culturally in this series have been found subsequently to be able to grow and reproduce more rapidly at 37° C. than at 27° C., as judged by naked-eye observation within the first 24 hours following inoculation of culture slopes with a standard loopful of spore suspension. This is likewise the case with the species of Candida isolated in the series.

Another selective factor may be the ability of some of these species to produce antibiotics which enable them to establish themselves in competition with the bacteria which are almost invariably associated with them in otitis externa. A list of these antibiotics may be found in volume 1 of *Antibiotics* by Florey *et al.* (1949). From this list it may be seen that *A. fumigatus* produces several antibiotics, of which the most interesting in this connection may be fumigatin. This antibiotic is said to be active against certain strains of *Staphylococcus aureus* in glucose broth in a dilution of 1 in 50,000, and against *Escherichia coli* in a dilution of 1 in 20,000. Also certain strains of *A. niger* produce penicillin and likewise strains of *A. flavus* and *A. nidulans*. Citrinin, produced by *A. terreus*, is said to be particularly active against some Gram-positive organisms; for example, in a dilution of 1 in 24,000 it inhibits some strains of *Staphylococcus aureus*.

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A third selective factor is certainly pathogenicity. There is no lack of records of the pathogenicity of *A. fumigatus* in other sites of the body and other species of Aspergillus such as *A. nidulans*, *A. flavus* and *A. niger* have been found from time to time associated with various lesions. The pathogenicity of *C. albicans* is also not in doubt; it has been recognized for over 100 years. In recent years a voluminous literature concerning the pathogenic activities of species of Candida and in particular *C. albicans*, in man and animals has accumulated. It is best known as the agent of thrush, but it is also a secondary invader in various forms of chronic pulmonary disease, a major cause of such common diseases of the cutaneous surfaces as paronychia, perionychia and onychia, intertrigo, rashes in the napkin area especially during the neonatal period, and of vaginitis and pruritus ani in adults.

Mycological Investigation of Patients Diagnosed Clinically as Cases of Otomycosis

The need for microscopic confirmation of a clinical diagnosis of otomycosis has been emphasized already. But the mycologist is only rarely to be found on a hospital staff, and the otologist may well have to perform these investigations himself if he is to achieve accurate diagnosis. This brief summary of the microscopic and cultural features of the Aspergillus and Candida groups is included as a convenient means of reference.

Superficial Examination. Debris suspected of being fungoid in nature was first located by use of the electric auriscope. By this means it was possible, sometimes, to see quite clearly the conidiophores of species of Aspergillus projecting into the canal from the surrounding walls, roof, and floor. Minute black heads at the tips of the sporulating stalks (conidiophores) indicated that *A. niger* was present. Likewise, pale blue or green plume-like growths (columns of conidia in chains) on stalks were indicative of *A. fumigatus*. Other species could not be identified so easily by this method, since their appearances were not exclusively characteristic. Thus, the light brown conidia of *A. terreus* may be confused with the immature conidial heads of *A. niger*, and those of *A. candidus* may be mistaken for the immature conidia of most of the commoner species of Aspergillus, since pigment develops in the conidia only as they become mature.

Signs suggesting the presence of Candida species, though by no means acceptable as diagnostic, were the presence of white or cream coloured viscous deposits on the epithelium of the external meatus.

Microscopical examination was necessary in all cases in order to confirm the preliminary diagnosis.

Bacteria. Bacteria of one kind or another were nearly always present in association with fungi, but the electric auriscope was of little use in

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establishing their presence. When, however, a viscous discharge showed a greenish tinge, *Pseudomonas aeruginosa* was usually suspected, and this was often confirmed on microscopical examination after staining, as well as by culture. This, however, did not by any means exclude the presence

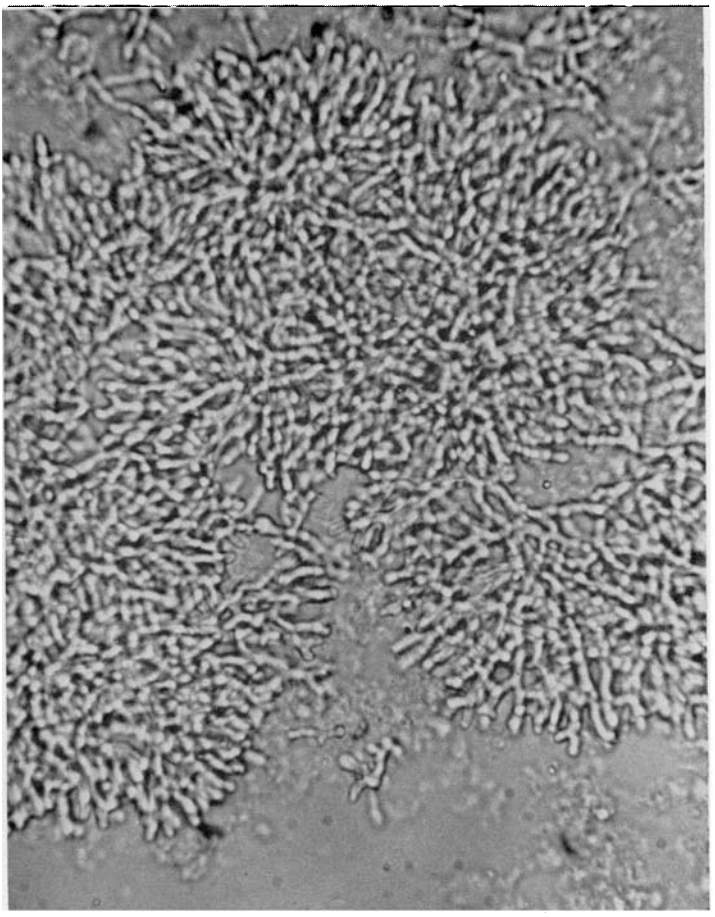


FIG. 4*a*.
Direct preparation from the ear showing discrete colonies of *A. fumigatus* mycelium 10 per cent. KOH × 514.

of yeast, or even of mould, which when present could be detected amongst these bacteria on microscopical examination.

2. *Microscopical Methods*

Moulds. When the position of the mould growth in the affected external auditory meatus was ascertained a sterile closed bacteriological wire loop was inserted into the meatus and some of the material withdrawn from it,

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placed in a drop of 10 per cent. caustic potash solution on a microscopical slide, and covered with a coverslip. This preparation was warmed slightly over a spirit lamp flame, and then examined microscopically, using a binocular microscope with 8× oculars and the 2/3 and 1/6 objectives. If

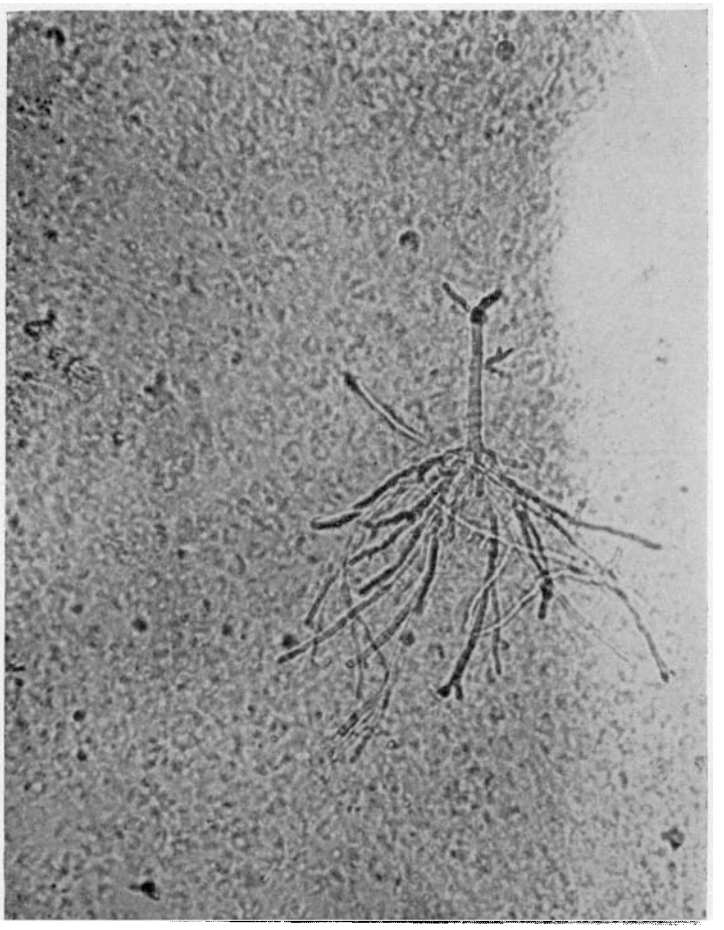


FIG. 4*b*.

Direct preparation from the ear showing minute root-like structures formed by mycelium of *A. niger*, 10 per cent. KOH × 200.

conidiophores had already been observed during the superficial examination it was usually possible to see them microscopically also. Sometimes these conidiophores were sufficiently well developed and characteristic to permit of species identification; at other times it was necessary to await the results of culture before a definite specific identification could be made. When only vegetative mycelium was present in the preparation this, in

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most cases, had not already been seen on superficial examination. In such cases, since the mycelium is not usually characteristic of a species, the only means of identification was by culture of the organism whereby

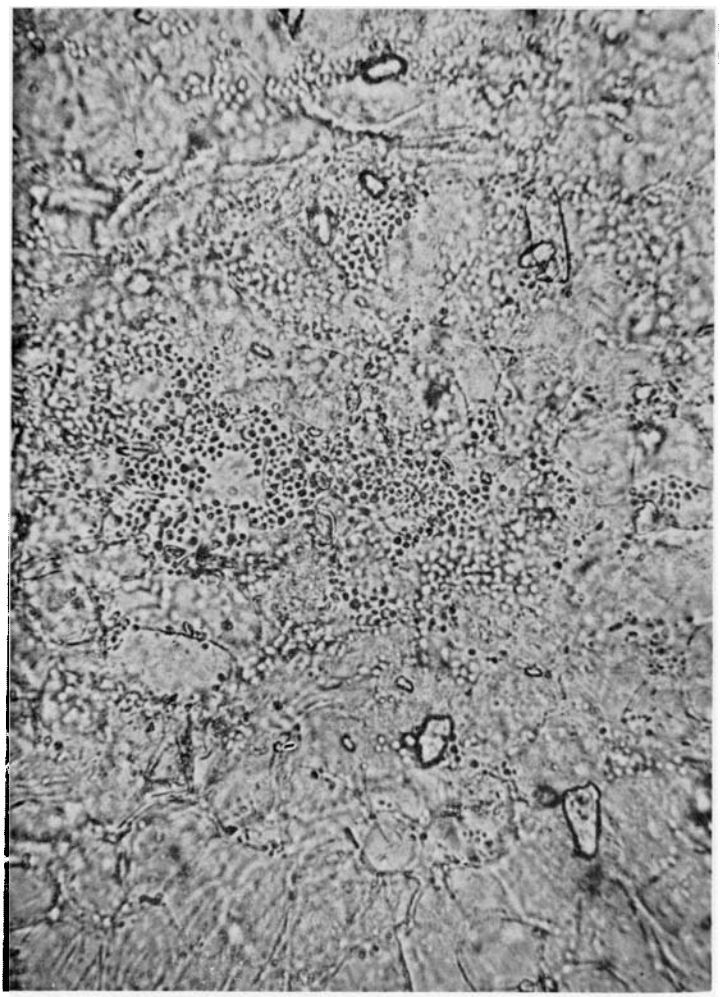


FIG. 5*a*.
Direct preparation from the ear showing budding cells of *C. albicans*, 10 per cent. KOH × 514.

the reproductive structures could be studied. Certain features, however, were present in the vegetative structures of some species so frequently that it was tempting to forecast the species when they were observed, and it is possible that, with increasing familiarity, these features might become of some significance in diagnosis. Thus, in the case of *A. fumigatus*

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in otomycosis lesions, the hyphae were nearly always relatively thick and frequently blunt and forked at the tip; occasionally discrete little colonies of this type were seen (Fig. 4*a*). Again, in the case of *A. niger*

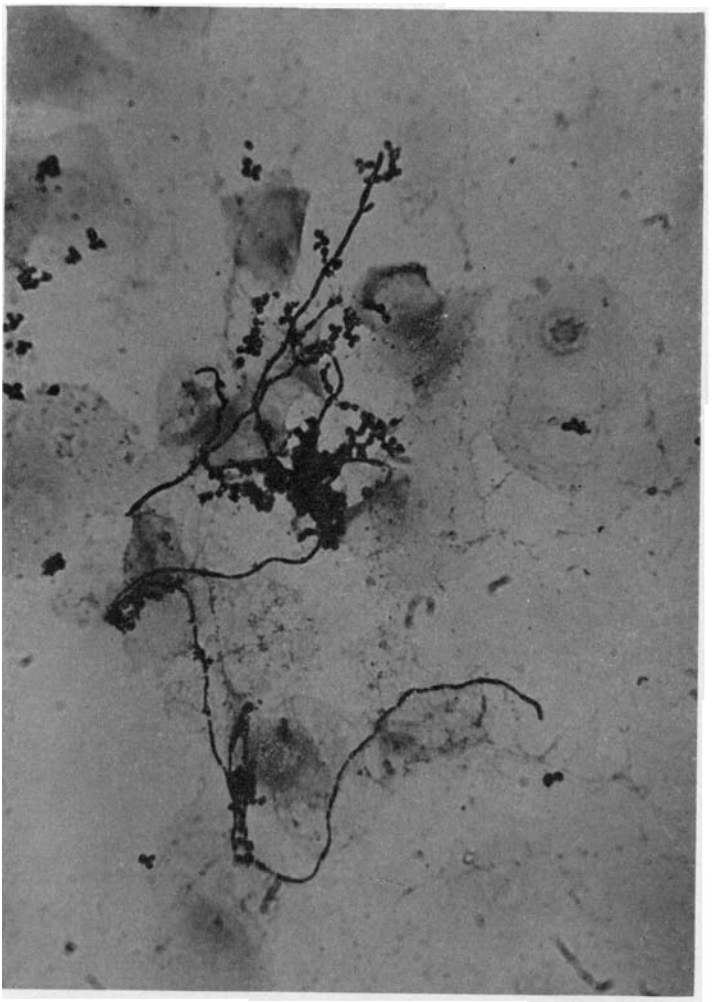


FIG. 5*b*.

Direct preparation from the ear showing mycelium and budding cells of *C. albicans*, Gram stain × 514.

a recurrent feature was a minute root-like system (Fig. 4*b*). Such vegetative growths are not seen in the mycelium of these species on standard culture media.

In addition to mounting material in 10 per cent. caustic potash solution it was sometimes desirable to mount some of it in lactophenol

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and cotton blue (Amann's medium) so that it could be preserved for future reference. This medium contains: lactic acid, 20 parts; phenol, 20 parts; glycerol, 40 parts; and distilled water 20 parts. Cotton blue dye is added as ·05 per cent. by weight. In some cases also smears of the material were fixed, and stained variously, by Gram, P.A.S. or Grocott's modification of Gomori's silver methenamine impregnation method.

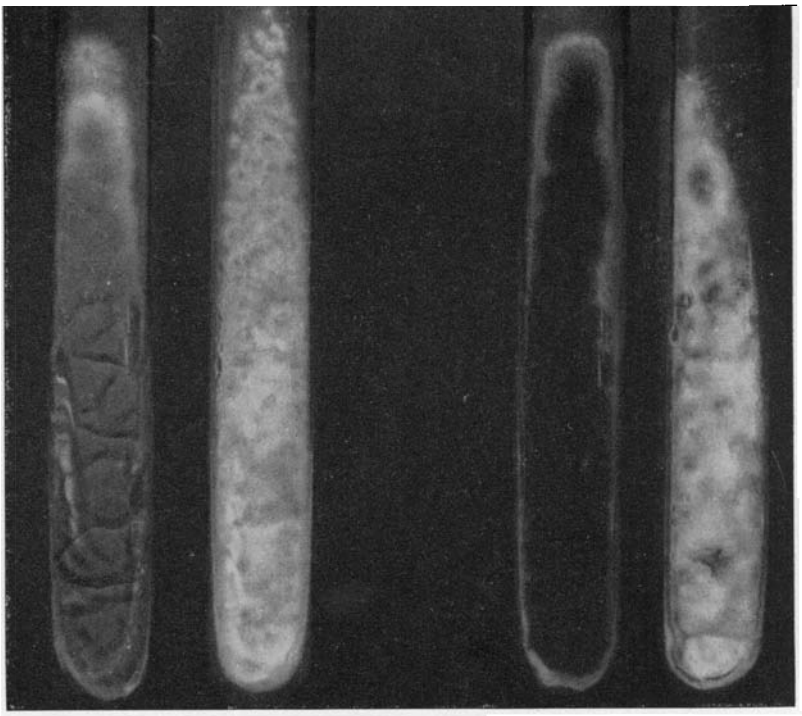


FIG. 6.

Comparison of sporulation at two different incubation temperatures after 48 hours culture. (Sporulation indicated by darkening of the surface of the culture).

Microscopical Examination of Material Containing Yeast-like Fungi.

Material suspected of containing yeast-like fungi was also mounted in 10 per cent. caustic potash solution as in the case of material suspected of containing mould. Smears were also prepared for staining by Gram or sometimes by P.A.S. These yeast-like fungi could be detected in the caustic potash solution as small ovoid budding and highly refractive cells (Fig. 5*a*). Occasionally they were associated with the pseudomycelium or true mycelium which is a different growth phase of the same organism (Fig. 5*b*). They were not always revealed by Gram stain and this could not therefore be relied upon as the sole test for their presence or absence.

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Culture of Material from Affected Ears

Material removed from the affected ears was cultured on Sabouraud glucose agar slopes in most cases. In some cases, honey-peptone agar slopes were used (the peptone incorporated in this medium was "Oxoid" bacteriological peptone). An antibiotic mixture of penicillin and streptomycin was included in the medium in such quantity as to give a final concentration of 20 units of penicillin and 40 units of streptomycin per ml., but more recently chloromycetin has been used since it can be autoclaved with the medium and is equally as effective in suppressing bacterial growth in the cultures. It is incorporated as 0·5 mg. per ml. Cultures were incubated at 37° C. in order to confirm that the fungus observed microscopically could grow at the temperature prevailing in the external auditory meatus. They were cultured at 27° C. also in case the mould was a mere contaminant, and, more important, in order to prevent the overgrowth of yeast-like organisms by fast-growing moulds such as *A. fumigatus* and *A. niger* which could well happen in cultures incubated at the higher temperature.

All the species of Aspergillus isolated in this series grew and sporulated rapidly at 37° C., while at 27° C. growth and sporulation proceeded at a slower rate. In the case of primary cultures of *A. fumigatus* incubated at 37° C. growth was generally visible to the naked eye within 24 hours, by which time sporulation also had commenced. This was often the case with *A. niger* also. Other species incubated at this temperature often produced growth also within this period. In sub-culture at this temperature growth and sporulation were even more rapid (Fig. 6).

Superficial Cultural Features of the Species Isolated from Patients' Ears.

A. fumigatus. Primary cultures incubated at 37° C. usually showed growth visible to the naked eye within 24 hours. Sporulation was indicated by a pale blue colour which spread gradually over an otherwise white superficial growth; this colour deepened and changed to a greyish blue-green with age.

A. niger. Primary cultures incubated at 37° C. produced white superficial growth visible to the naked eye usually within 24 hours. This became gradually lemon yellow in patches at the onset of sporulation and then changed gradually to dark brown when the conidia were mature.

A. terreus. Primary cultures at 37° C. revealed superficial growth usually within 24 hours but sometimes took longer to do so. This again was white at first, but became gradually tinged with a light buff colour as sporulation proceeded, and became a deeper colour as the conidia matured.

A. nidulans. Primary cultures at 37° C. produced white superficial growth visible to the naked eye, usually within 24 hours, but sometimes took longer. The onset of sporulation was indicated by the

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appearance of a pale green colour which deepened in due course. In addition to the conidiophores which were represented by this green colour, special sporing structures (perithecia) developed within a few days amongst the conidial mass. They were spherical, measuring 1-2 mm. in diameter and were ochraceous to salmon in colour.

A. flavus. Primary cultures at 37° C. produced white growth visible to the naked eye, usually within 24 hours. This changed gradually to greenish yellow as sporulation proceeded and then in due course to lime green. Some isolates produced, in addition to the conidiophores, greyish, spherical structures measuring about 1-2 mm. called sclerotia which differ from perithecia in so far as they are sterile.

A. candidus. Primary cultures produced white superficial growth within 24 hours or shortly after, at 37° C. This became more dense and white as conidiophores developed and sporulated. Older cultures did not change colour or at most, became cream-coloured. On the other hand, in these strains, the reverse was at first ochraceous, then deep red, and finally black.

A. glaucus. Primary cultures were at first white at 37° C., becoming pale green or blue-green as conidia developed. This gradually darkened and became in due course brown. In addition to the conidiophores, yellow perithecia measuring about 0·5 mm. to 1 mm. in diameter were produced.

Microscopical Features of Aspergillus spp.

General. In all species the conidiophores, which provide the chief criteria for identification purposes, are aerial, erect, usually unbranched stalks borne on the aerial mycelium. They are swollen at the tip to form the vesicle. This part of the conidiophore varies somewhat in shape according to the species and even to some extent in strains of the same species. It gives rise to a large number of short, more or less closely packed stalks which develop the conidia successively at their tips so that long chains of conidia are produced, or else, as in a number of species, form a second rank of short stalks which bear the conidia. When there are thus two ranks of short stalks, one upon the other, those of the first or proximal rank are known as metulae, while those of the second or distal rank are known as phialides. In some species the conidia form radiating chains, giving the conidial head a spherical appearance when viewed with a lens; while in other species they form chains in columns or plumes. According to the species the conidia vary in colour which usually changes with age, and are spherical or elliptical or sometimes barrel-shaped, and finally may be rough or smooth according to species. In species which produce perithecia, the spores contained within them (ascospores) are at first enclosed in fours in minute transparent sacs (asci). These ascospores are variously coloured according to the species.

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Main Microscopical Features of Species in Culture on Sabouraud Glucose Agar Medium

A. fumigatus. The conidiophores vary in length and width, even in the same culture. Typically, the proximal part of the vesicle merges very gradually into the stalk. This is due to the upper part of the stalk being almost as wide as the vesicle which surmounts it. The phialides are borne typically on the upper half of the vesicle, lying in the axis of the conidiophore, and are packed side by side in a palisade-like manner



FIG. 7.

Direct preparation from the ear showing conidiophores of *A. fumigatus* with palisade-like arrangement of phialides. Lacto-phenol and Cotton blue × 514.

(Fig. 7). In some strains the phialides may occupy a greater part of the vesicle surface and in others both types of arrangement of these phialides may be encountered in the same culture. The conidia are globose and finely roughened. They measure, according to Thom and Raper (1945) 2-3·5 μ in diameter.

A. niger. The conidiophores are long, relatively broad, erect and thick-walled. They are surmounted by a large spherical vesicle from all parts of which radiate metulae which in turn bear phialides. The conidia vary from chocolate brown to purplish brown and are globose and rough-walled. They measure, according to Thom and Raper 2·5-4 μ in diameter. The outline of the conidial head is spherical when undisturbed (Fig. 8).

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A. terreus. The conidiophores are relatively long and are surmounted by a globose or subglobose vesicle bearing metulae and phialides. The conidia are light brown, globose and are smooth-walled. They measure,



FIG. 8.

Direct preparation from the ear showing conidiophores of *A. niger* in different stages of maturity. Lacto-phenol and Cotton blue × 120.

according to Thom and Raper 1·8-2·4 μ in diameter. At maturity, they are, when undisturbed, closely packed in columns. A special and characteristic feature of the vegetative mycelium is the development of large numbers of spherical, short-stalked spores along the hyphae. In time these enlarge and

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become thick-walled, and then closely resemble the Hülle cells seen in association with the perithecia in *A. nidulans*. This feature has been observed also by Austwick (1959).



FIG. 9.

Direct preparation from the ear showing conidiophores of *A. nidulans*. Lacto-phenol and Cotton blue ×514.

A. flavus. The conidiophores in this species are relatively long. The vesicle is globose or subglobose, and the stalk is roughened owing to the presence of granules on the wall, or, in some cases, pits. The vesicles bear phialides only, or both metulae and phialides. The conidia measure,

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according to Thom and Raper, between 3 and 5μ. At maturity they form loose columns.

A. nidulans. The conidiophores are relatively short (Figs. 9 and 10*a*). They are surmounted by a distinct subglobose vesicle bearing both metulae and phialides. The stalks are coloured characteristically a light reddish brown. The conidia are globose, finely rough-walled, and measure, according to Thom and Raper 3-3·5 μ in diameter. At maturity the undisturbed conidial head is columnar. The perithecia contain dark reddish or purple ascospores. They are surrounded by numerous hyaline thick-walled cells called Hülle cells (Fig. 10*b*).

A. candidus. The conidiophores show much variation in size and structure, including the detailed structure of the metulae. The conidia of strains seen in this series were globose to subglobose and smooth-walled. According to Thom and Raper they may be elliptical or barrel-shaped in some strains and measure 2·5-3·5 μ.

Cultural Features of Yeast-like Fungi

Material suspected of containing "yeast" was cultured at 37° C. on the same media as those used for species of Aspergillus. Yeast-like colonies were usually visible within 24 hours to the naked eye. They were at first circumscribed and pellucid but in due course became more irregular in outline and opaque milk white to creamy white. Identification was carried out on potato-carrot extract agar containing bile and made up according to the formula recommended by Segretain *et al.* (1958) as well as by fermentation tests when the species was other than *C. albicans*.

Identification of C. albicans on Potato-Carrot Extract Agar plus Bile

Yeast from primary colonies was streaked in crossed parallel lines on to the surface of the potato-carrot agar medium in Petri dishes and then dragged into the medium along these lines with the point of a sterile mounted needle. The Petri dish cultures were then incubated at 20° C. Within 24 hours or sometimes after a longer period, mycelium grew out from the sides of the streaks of yeast-cells. Sooner or later, in the case of *C. albicans*, large sperical, thick-walled cells (Chlamydospores) were produced at the tips of short branches or at the tips of the main hyphae.

Treatment

Since one of the problems to be considered in this investigation was the significance of fungus invasion in the perpetuation of otorrhœa, it was desirable that therapy should be directed solely at the fungus and should have no significant effect on bacteria.

It was decided, therefore, to use "Nystatin" (Squibb & Co.), one of a series of substances developed for their antifungal properties, parti-

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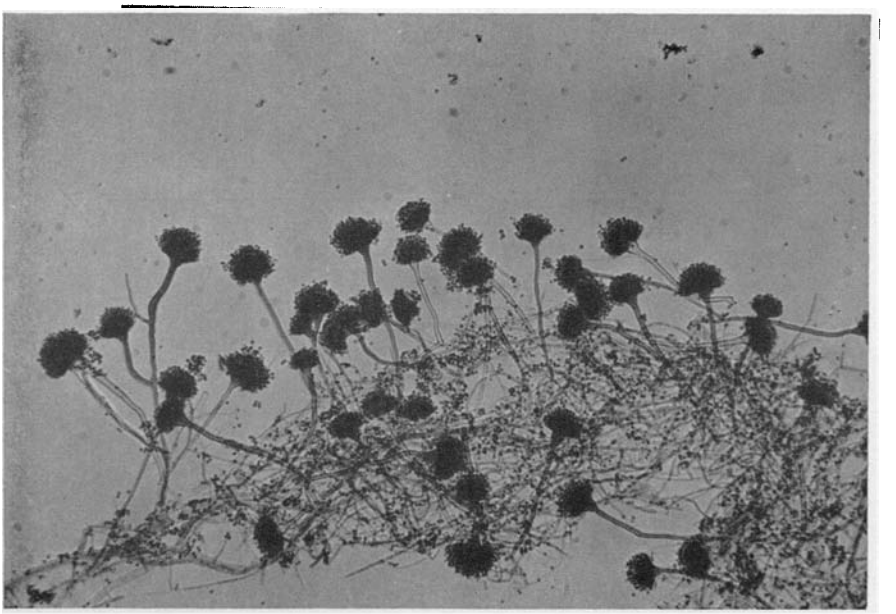


FIG. 10*a*.
Conidiophores of *A. nidulans* from culture. Lacto-phenol and Cotton blue × 200.

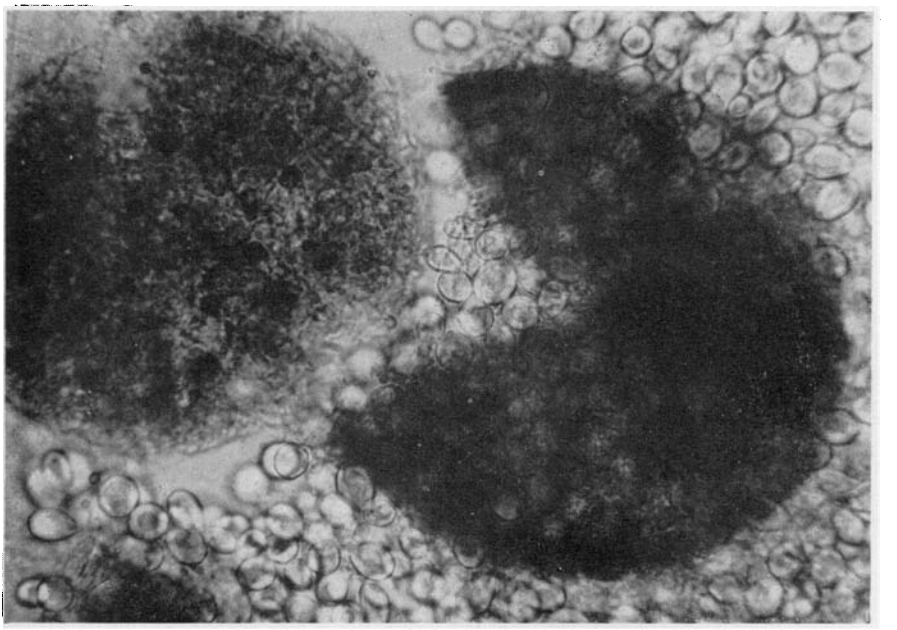


FIG. 10*b*.
Ruptured perithecium of *A. nidulans* from culture, and extruded mass including ascospores. Numerous thick-walled Hülle cells surrounding perithecium. Lacto-phenol and Cotton blue × 514.

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cularly against yeasts. The substance was said to have no significant effect on bacteria, and there had been no previous assessment of its value in otomycosis.

A routine was evolved where out-patient treatment was given for a period of three weeks. It was thought that such a period would cover adequately all the phases in the life of the invading fungi. This was necessary for there was no information about the phase in which the fungus was most susceptible to Nystatin, and it was known that antibiotics are usually only effective against bacteria in some particular phase of their life-cycle.

Since most fungi require some moisture for growth, it was decided to use a form of treatment which would eliminate moisture as far as possible. Dry mopping of the meatus formed an essential part of this, and it was obviously illogical to use any aqueous vehicle for the Nystatin. For the majority of the cases, Nystatin was applied in a powder of which boric powder formed the base (100,000 units of Nystatin per gramme of powder). Recently lactose has been used as the base without any reduction in efficiency.

The routine thus evolved consisted of attendance by the patient at the out-patient treatment clinic three times weekly for three weeks. At each attendance the meatus or radical mastoidectomy cavity was meticulously cleansed by dry mopping and the manual removal of any large pieces of debris. The epithelium of the meatus or cavity was then lightly "frosted" with the powder containing Nystatin. The patient was accepted as cured when the meatus showed a little secretion of normal wax.

Results

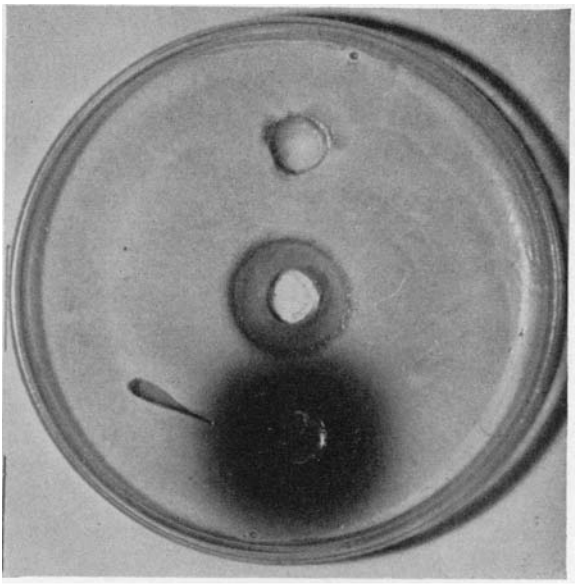
Of the 83 cases diagnosed as fungus infection, 2 were not prepared to attend for treatment, and 7 cannot be traced. A further 7 required more prolonged therapy or relapsed soon after they were thought to be cured. These relapses responded to further periods of therapy with Nystatin.

The remaining patents remained free of symptoms.

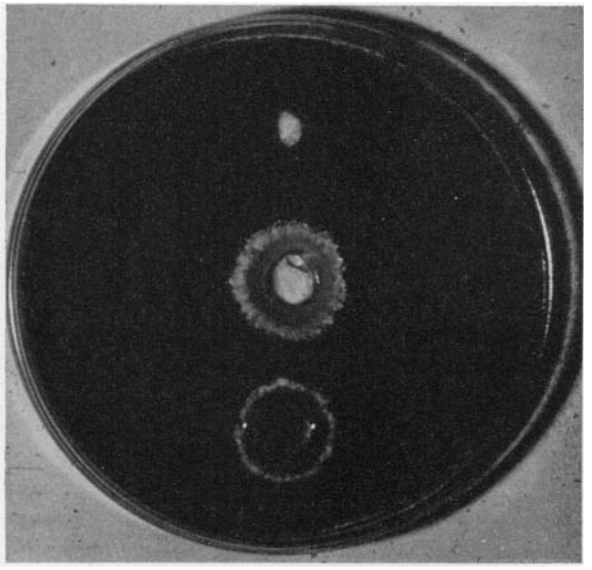
The period of therapy was uneventful in the main, but in 10 patients it was complicated by the development of an aural furunculosis. This may have been due to an associated staphylococcal infection which the fungal metabolites, being partly of the nature of antibiotics, had been holding partly in check, only to come to light with the elimination of the fungus. The development of furunculosis may, therefore, be a useful guide to the elimination of the fungus.

Unfortunately an initial bacteriological assessment was not done in all these cases. Of the 5 patients who had *Staphylococcus aureus* at an initial investigation, only one developed a furuncle while under treatment with Nystatin. It is possible, therefore, that cross-infection by the "hospital staphylococcus" may have occurred during treatment.

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C. albicans



A. niger

FIG. 11.

Comparison of the inhibiting effect of fungistatic and fungicidal agents upon the growth of *C. albicans* and *A. niger* in culture.

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Although many substances appear to be efficient fungicides, Nystatin has shown two properties not possessed by most of them. Its application does not cause pain, nor does it colour the meatus. Many of the substances used in the past could cause intense pain—tincture of iodine, thymol and metacresyl acetate (Whalen, 1938)—and it was not unknown for patients to default from treatment, possibly because they found it more intolerable than the disease. The aniline dyes—gentian violet, brilliant green—have been proved most efficient fungicides *in vitro*, but they stain the meatus to such an extent that cure must be well-nigh impossible to assess.

Some authors in the past have dismissed the matter too lightly by claiming that Industrial Spirit used locally inhibits all fungi. This may be true of concentrations greater than 70 per cent. but such concentrations are also lethal to epithelial cells. The standard B.N.F. concentration of 50 per cent. is singularly ineffective, at least *in vitro* (Fig. 11).

Conclusion

It is not claimed that this search for fungus infection has solved the otologist's perennial problem of the discharging ear, for rather more than 50 per cent. of the patients investigated gave no evidence of a growing fungus. Nevertheless, this does seem to be a field worthy of further investigation, and the writers are only too well aware of the many unsolved problems which remain. The control of such investigations may be hampered for some time by the unavailability of mycologists in hospital practice.

From the clinical aspect it cannot be stressed too much that diagnosis is still inaccurate, and the clinician must rely upon the control of his mycologist colleague. With such control, however, the new fungicidal agents can provide a valuable addition to the otologist's armamentarium.

Summary

1. During the years 1956-9, 180 patients with intractable otorrhœa, due to either otitis externa or infection of radical mastoidectomy and fenestration cavities, were investigated for the possibility of an active fungus infection.

2. Eighty-three of these patients had positive evidence of such an infection, and were treated with a specific fungicidal agent with resolution of the otorrhœa.

3. The clinical picture of fungus infection in the external ear is described, but the inaccuracy of clinical diagnosis is emphasized, and criteria for diagnosis are given.

4. The microscopic and cultural characteristics of the fungi most commonly found are described.

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5. The ætiology of fungus infection of the external ear is discussed briefly. Knowledge of this aspect of the condition is scanty.

6. Possible methods of therapy are discussed, and the value of new fungicidal agents is considered.

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BIBLIOGRAPHY

AKOBJANOFF, L., CARRUTHERS, C., and SENTURIA, B. H. (1954) *J. invest. Derm.*, **23**, 43.
AUSTWICK, P. K. C. (1959) Personal Communication.
BROWN, L. G. (1937) *Brit. med. J.*, **2**, 698.
BROWN, W. (1922) *Ann. Bot.*, **36**, 257.
CAPPS, F. C. W. (1938) *Brit. med. J.*, **1**, 958.
——— (1959) Personal Communication.
FLOREY, H. W. *et al.* (1949) "Antibiotics", Vol. 1, London.
JOHNSTON, W. H. (1944) *Ann. Otol. (St. Louis)*, **53**, 5.
REEH, M. J. (1942) *Ann. Otol. (St. Louis)*, **51**, 146.
SEGRETAIN, G., DROUHET, E., and MARIAT, F. (1958) "Diagnostic de Laboratoire en Mycologie Médicale", St. Maude.
SENTURIA, B. H. (1957) "Diseases of the External Ear", Springfield, Ill.
SIMPSON, R. R. (1959) Personal Communication.
THOM, C., and RAPER, K. B. (1945) "A Manual of the Aspergilli", London.

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