that 92 per cent. of the strains were resistant to ampicillin, despite the fact that ampicillin resistance does not usually appear to be transferable in Sh. sonnei (Scrimgeour).

SUMMARY

The minimum inhibitory concentrations of trimethoprim and sulphamethoxazole were determined for 209 strains of Shigella sonnei isolated mainly in the Greater London area. All strains were sensitive to trimethoprim at a concentration of 0.32 μg per ml, but 74 per cent. of strains were resistant to sulphamethoxazole at a concentration of 100 μg per ml.

Potentiation of trimethoprim by sulphamethoxazole was demonstrated in the case of sulphonamide-sensitive strains but there was little or no potentiation in the case of sulphonamide-resistant strains.

The possible value of the combination of trimethoprim and sulphamethoxazole in the treatment of Sonne dysentery is discussed briefly.

We thank Dr Joan R. Davies and Mr W. N. Farrant for providing many of the strains of Sh. sonnei and for performing the colicine typing.

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A RAPID AND SIMPLE METHOD FOR THE LABORATORY DIAGNOSIS OF TRICHOPTHYTON VERRUCOSUM

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Plates LV and LVI

The method of treatment of ringworm depends more on the site and type of the lesion than on the species of fungus causing the infection. For this reason the results of direct microscopy of a dermatological specimen in KOH, which can be given within half an hour, are all the clinician needs in order to decide whether or not to prescribe antifungal treatment. However, a fungus is sometimes overlooked on direct microscopy and is discovered only when cultures are made. Also, it is only by culturing and identifying the fungus that its probable source can be determined and steps be taken to prevent spread of the disease. Finally, in prognosis a knowledge of the identity of the fungus is essential. For these reasons, the rapid reporting of cultural results in dermatological mycology is very desirable.

Most laboratories use agar slopes, either in screw-capped bottles or, preferably, in plugged test-tubes, for the isolation of dermatophytes. In this laboratory petri dishes have always been used and have been found to have several advantages over slopes. Their use greatly

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speeds the obtaining of cultural findings on patients infected with *Trichophyton verrucosum*, the agent of cattle ringworm in man and animals, and that of negative results on patients free from dermatophyte infections.

*T. verrucosum* presents special difficulties in the diagnostic laboratory because of its absolute requirement for thiamine and the additional need of some strains for inositol. Its colonies on glucose peptone agar are, for this reason, rarely visible to the naked eye, and those strains that do grow produce only minute, nondescript colonies after 2–3 wk. Identifiable colonies grow slowly on nutrient agar, but this medium would not be in routine use for mycology in the average bacteriological laboratory, where most mycology is carried out, and in a mycological laboratory it is convenient to be able to identify the fungus on the same medium as that used for other species. The fungus grows fairly readily on malt agar, but even on this medium, Dr J. C. Gentles informs me, from 9 to 21 days are needed for its identification in slope culture. The numerous bacteriological laboratories that have no expert mycologist available, but carry out routine mycological investigations, rarely report the finding of *T. verrucosum*, although this fungus is prevalent everywhere in Britain except in the larger conurbations. The main reason for the failure of isolation appears to be that glucose peptone agar slope cultures are used in the mistaken expectation that *T. verrucosum* will, like other dermatophytes, grow to macroscopic proportions.

Before a slope culture is reported as negative for dermatophytes, it should be incubated for long enough to allow any fungus present to grow to macroscopically visible proportions. For safety, a period of about 2 wk is usually allowed. By the method to be described, this period, and the time taken to identify *T. verrucosum*, can be cut to 4 days.

**METHOD AND RESULTS**

A petri dish 9 cm in diameter containing glucose peptone agar with chloramphenicol and actidione (Ajello *et al.*, 1963) is used for each specimen. The specimen is cut into very small fragments, of which about 20 are used to inoculate the plate. It is important for subsequent investigation that the inocula should be placed in a regular pattern on the agar and not shaken on to it at random. The plate is then incubated for 4 days at 29°C. On the 4th day it is placed upside-down on the microscope stage and each inoculum is examined under the low-power (2/3 in.) objective. The regular arrangement of the inocula ensures that none is missed.

If no hyphae can be seen growing from any inoculum it can be safely concluded that no dermatophyte will grow and, if no hyphae were found on direct microscopy of the specimen either, the case can be reported to the clinician as mycologically negative. If direct microscopy of the clinical material has given positive results, renewed attempts to culture the fungus can be made with the minimum of delay.

If hyphae are seen growing from any inoculum, it must be decided whether or not they are those of *T. verrucosum*. Typical 4-day-old hyphae of this fungus on glucose peptone agar are shown in figs. 1–3. Most conspicuously, some hyphae in every microscopic field end in a swollen vesicle, the contents of which are often plasmolysed. The hyphae also tend to be short and thick, with frequent, wide-angled branching if the colony is fairly vigorous, but with little branching in the more feeble isolates. Radial growth in relation to the inoculum is not strongly marked. Colonies of this type can be identified with certainty as *T. verrucosum*, though on continued incubation little or no further growth occurs except in occasional isolates. The characteristic terminal, or occasionally subterminal, vesicles are not a reaction to actidione, as they occur whether or not the antibiotic is present.

In contrast, the hyphae of 4-day-old colonies of most other dermatophytes show a marked radial orientation, are usually longer than those of *T. verrucosum*, branch at a much more acute angle, and, particularly, never form vesicles (fig. 4). Most dermatophyte species, including many strains of *T. rubrum*, are macroscopically visible in 4 days. Though 4-day-old colonies of some strains of *T. rubrum* may be of no greater diameter than those of *T. verrucosum*, they consist of wavy hyphae with little branching and with strong radial orientation, and again have no vesicles (fig. 5). The terminal vesicles that are a prominent feature of
Diagnosis of *Trichophyton verrucosum*

Figs. 1–3.—Four-day-old colonies of different isolates of *Trichophyton verrucosum* growing from the inoculum into glucose peptone agar and showing variation in vigour. In fig. 2 the inoculum consisted of arthrospores shed from infected hairs. × 240.
DIAGNOSIS OF *Trichophyton verrucosum*

Fig. 4.—Four-day-old colony of *Microsporum canis* growing from the inoculum into glucose peptone agar. × 240.

Fig. 5.—Four-day-old colony of a slow-growing isolate of *T. rubrum* growing from the inoculum into glucose peptone agar. × 240.
T. tonsurans cultures do not appear until the colony is much older, so that at 4 days this fungus cannot be confused with T. verrucosum.

On a medium as selective as that used it is rare to encounter a non-dermatophyte from clinical material of human origin, though actidione-resistant contaminants not infrequently grow from animal scrapings. Many years of experience with the method described have never disclosed a non-dermatophyte that could be confused with T. verrucosum.

Plates containing young colonies of any type except T. verrucosum are replaced in the incubator until the fungi are sufficiently mature to be identified in the normal manner. This takes 5 days or more, depending on the species of fungus, exactly as in slope cultures.

A further advantage of plate cultures is that fast-growing contaminants can be cut out before they swamp all the inocula.

DISCUSSION

The method described for the identification of Trichophyton verrucosum is admittedly not mycologically perfect, in that the mature fungus is never seen. However, in diagnostic work, speed, provided it is combined with accuracy, is of great importance. For the first 2 yr in which this method was used, and in occasional instances since, the colonies on glucose peptone agar were checked by transferring them to nutrient agar, where they always grew into T. verrucosum colonies identifiable by normal methods. The method has now been in use for 16 yr, and there have been no findings incompatible with the clinical or epidemiological circumstances of any individual patient. That the plate culture method is simple and reliable has been shown by its ready adoption by technicians, pathologists and dermatologists to whom it has been demonstrated.

SUMMARY

When slope cultures are used to isolate fungi from dermatological specimens, the very slow growing fungus Trichophyton verrucosum cannot be identified in less than 2 wk. The use of petri-dish cultures allows direct examination of the inocula under the low power of the microscope after a brief period of incubation, when the distinctive appearance of T. verrucosum, which forms short, thick hyphae, many of which end in swollen vesicles, permits accurate reporting in 4 days. The same method allows rapid reporting of cultures on specimens that are microscopically negative and the removal of fast-growing contaminants before they obscure neighbouring inocula.

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REFERENCE