

TEST FOR THE IN VITRO ACTIVITY OF TOPICAL ANTIMYCOTICS

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Abstract. The in vitro antifungal activity of the topical antimycotics tolnaftate, beech tar, methyl rosaniline, benzoic acid, iodine, and sulphur solutions was tested by a new method. This involves painting with the antimycotics or immersing vinyl tape strippings from fungal lesions in them. Materials from 6 cases of tinea cruris and 15 cases of tinea corporis were included in the evaluation of the method.

Key words: Topical antifungal agents; In vitro test

Various in vitro methods (2, 3, 9, 11, 13) as well as prophylactic (6, 9) and therapeutic (6) measures for experimental infections have been used for the evaluation of topical antifungal agents.

In the test described below, treatment of fungus-infected tape strippings of the horny layer was evaluated.

MATERIAL AND METHODS

Strippings were taken with vinyl tape (Scotch brand tape 681, Minnesota Mining and Manufacturing Co., Los Angeles) from untreated cases of tinea cruris or corporis. Twelve strippings were taken from each patient.

Every second piece of tape, saturated with stratum corneum was painted—the 'painting experiment', or suspended for one week—the 'immersion experiment' (Fig. 1), in one of the following agents: 2% tolnaftate solution (Focusan, Lundbeck), 50% beech tar in alcohol (spiritus pyroleum fagi, Ph. Dan 1943), 0.5% methyl rosaniline in alcohol (spiritus methylrosanilini, Ph. Nord. 1963), 5% benzoic acid in alcohol (spiritus Mycocten, Leo), 5% iodine in alcohol (spiritus jodi 5%, Ph. Nord. 1963), and 2% sulphur in zinc oxide solution. Alternate tapes were run as untreated controls. It was ensured that the various fluids did not detach the stratum corneum from the tape.

Pieces of tape, 3 cm in length, were placed, adhesive side down, on Sabouraud's dextrose agar with penicillin (12 µg/ml), streptomycin (40 µg/ml) and actidione (500 µg/ml) added, the 6 treated tapes in one Petri dish and the control tapes in another. The plates were incubated at 26°C and examined for growth for up to 4 weeks.

The painting experiment comprised 7 cases, 3 of tinea cruris and 4 of tinea corporis. In one case the infection was due to *Trichophyton rubrum*, in 2 cases to *Epidermophyton floccosum*, and in 4 cases to *Microsporum canis*.

The immersion experiment comprised three cases of tinea cruris and 11 of tinea corporis. In one of these cases there was growth of *E. floccosum*; in 5 cases, of *T. rubrum*, in 2 cases, of *T. mentagrophytes*, and in 6 cases, of *M. canis*.

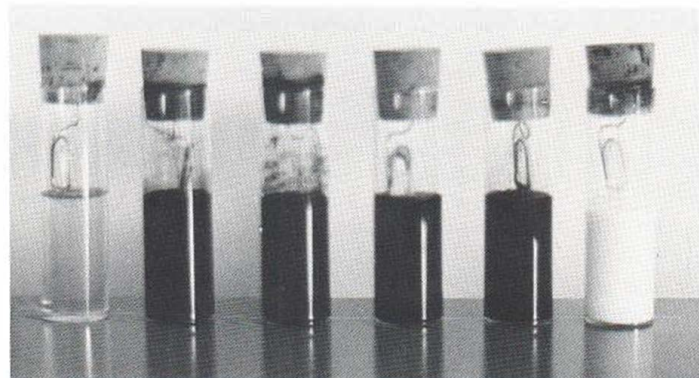


Fig. 1. Vinyl tape strippings suspended by clips in tolnaftate, beech tar, methyl rosaniline, benzoic acid, iodine, and sulphur solutions.

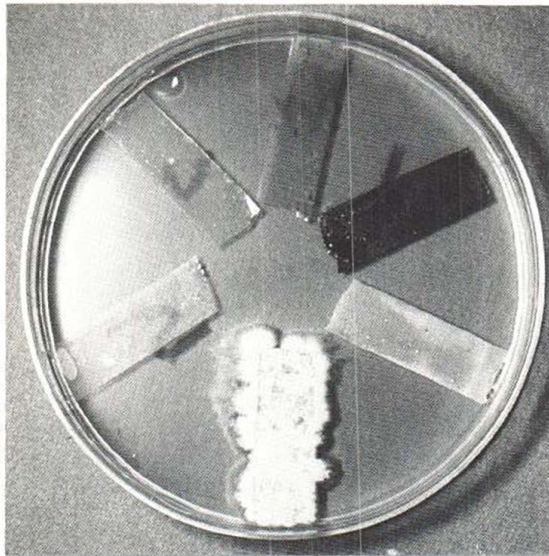


Fig. 2. Vinyl tape strippings painted with tolnaftate, beech tar, methyl rosaniline, benzoic acid, iodine, and sulphur solutions. Growth of *Microsporum canis* may be seen on the sulphur-treated tape.

RESULTS

In the painting experiments the strips treated with sulphuric lotion (Fig. 2) from 4 of the 7 cases yielded growth identified as *T. rubrum* in one case and *M. canis* in 3 cases. In one of these cases growth (*M. canis*) was also found on the tapes treated with beech tar and benzoic acid. None of the painted strips showed growth in the remaining 3 cases.

None of the tapes from the 14 cases included in the immersion experiments showed growth. Growth was found on the control tapes in 13 out of the 14 cases in which control culturing was performed. In one case of a *M. canis* infection affecting the face, it was not possible to obtain sufficient material.

DISCUSSION

By the test method described, the dermatophytes are studied in their natural growth medium, the stratum corneum. Therefore, like Knight's growth prophylactic test (9), it may be called, with some right, a modified in vivo method. However, it is so only formally, as just a single layer of the stratum corneum is used, and this one layer must be better penetrated by the drug tested than would the en-

tire mass of the stratum corneum under clinical conditions. Furthermore, the stripped horny layer is detached from the underlying skin and thereby acquires a saprophytic status which differs in several respects from the parasitic in vivo status (11).

The technique is easy and enables material from the same patient to be tested by many different substances. From the superficial stripping used in this study, it is our experience that the tape is fully saturated with keratin, but reliable results are at all events ensured when every second tape is used as untreated control, as described here.

The present test showed—like other methods of antimycotics testing (2, 3, 6, 9, 11, 13)—that the therapeutic agents used had a definite growth-inhibiting action upon fungus-infected strippings of the stratum corneum. Only the sulphur lotion failed in some cases in the painting experiment.

In analysis on the effect of antimycotic treatment the cure rate has ranged from about 30 to 100% lowest for tinea manus and pedis, highest for tinea corporis and capitis.

Factors such as liberation from the vehicle, the distribution and penetration of the substance, protein binding, immunological abnormality, as well as the pH, degree of moisture and bacterial flora of the skin appear to be of importance in explaining why antimycotic agents so commonly fail under clinical conditions (4, 7, 12, 14). Constant re-infection from fragments of skin and nails in the shoes and stockings (4, 5, 8) as well as infection from normal-looking, but infected skin surrounding the fungal infection (10) must be taken into consideration as well.

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