

out evidence of an underlying tumour reliably excludes the presence of this sign in our patient. We are also confident that the keratoses had emerged rather rapidly in conjunction with the dermatosis. The patient and her husband volunteered a remarkably similar history.

Williams reported 4 patients with seborrheic keratosis-like lesions on areas of skin affected by eczema (3). Two similar cases were presented by Barrière et al (4). In both studies it was reported that after resolution of the dermatosis the acanthomas spontaneously disappeared. We believe that our patient fits the description by Williams and by Barrière et al.

Involvement of seborrheic keratoses after control of an exfoliative erythrodermia has also been observed by Berman & Winkelmann (5). Histologic examination showed a mononuclear cell infiltration of the seborrheic keratoses. The present case confirms these findings. Several involuting seborrheic keratoses in our patient showed an inflammatory response with redness and necrosis.

The role of etretinate in the regression of the keratoses in our patient is not clear. To our knowledge, systemic retinoid therapy has not been reported to influence the course of seborrheic keratoses. On the other hand, Schumacher & Stüttgen reported an effect of topical retinoic acid in 8 patients with seborrheic keratoses (6). In our patient the seborrheic keratoses regressed further after withdrawal of etretinate therapy and they did not recur during follow-up. It is therefore uncertain whether the lesions regressed after (partial) control of the cutaneous inflammation or as a result of the etretinate therapy.

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## The 'Tape-method': A New and Simple Method for Quantitative Culture of Pityrosporum Yeasts

J. R. WIKLER, P. de HAAN and C. NIEBOER

*Department of Dermatology, Free University Hospital, Amsterdam, The Netherlands*

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A new method for quantitative culture of Pityrosporum yeasts, the 'tape-method', is presented. Samples for culture were taken from the skin by stripping with 1 cm<sup>2</sup> of tape, whereafter the tapes were placed over a drop of sterile olive oil which was pipetted on a Sabouraud medium. Plates were incubated at 37°C, and after 7 days the numbers of Pityrosporum colonies growing under the tapes were counted. With this method a difference in numbers of Pityrosporum colonies between seborrheic dermatitis and normal skin could be discerned. This difference was significant. It appeared that two successive strippings were sufficient for quantitative culture. The 'tape-method' appeared to be a reliable and inexpensive diagnostic tool. (Received November 2, 1987.)

J. Wikler, Department of Dermatology, Free University Hospital, de Boelelaan 1117, NL-1081 HV Amsterdam, The Netherlands.

The etiological significance of *Pityrosporum* yeasts in the pathogenesis of seborrheic dermatitis, pityriasis versicolor and *Pityrosporum* folliculitis has been demonstrated in several reports (1-3). Clinical trials have been made in which patients with seborrheic dermatitis, pityriasis versicolor and *Pityrosporum* folliculitis were treated successfully with antifungal agents (3-5). Some workers have demonstrated that when seborrheic dermatitis is treated with antifungal agents, clinical improvement is paralleled by a reduction in numbers of *Pityrosporum* yeasts (6). In order to count these yeasts, quantitative cultures have to be taken. The 'detergent scrub technique' has been used by some workers for this purpose, and the use of 'contact plates' for quantitative culture has also been described (6, 7). In this report we describe a new and simple method for quantitative culturing of *Pityrosporum* yeasts from the skin.

## MATERIALS AND METHODS

Samples for culture from the skin were taken by stripping, using 1 cm<sup>2</sup> of tape (Scotch brand tape 850; 3M St. Paul, Minn). The pieces of tape were subsequently placed over a drop of sterile olive oil which was pipetted on a Sabouraud's glucose-agar plate with chloramphenicol, cycloheximide and vitamin B1 added ('tape-method' for quantitative culture of *Pityrosporum* yeasts). The plates were incubated at 37°C, and after 7 days the numbers of *Pityrosporum* colonies growing under the tapes were counted with a microscope (25 ×) by using a calibrated coverglass or a magnifying glass.

### Experiment 1

Samples for culture were taken from seborrheic dermatitis of the face from 10 patients (4 females and 6 males; mean age 39 years) with controls taken from normal-looking skin of corresponding facial sites on these patients and from normal-looking facial skin from 10 healthy individuals (6 females and 4 males; mean age 37 years). Patients washed their face 4 h before cultures were taken. They were free from treatment with either antimycotics, corticosteroids (oral or topical) or tar-ointments, for at least 4 weeks before cultures were taken. Patients were treated with topical antimycotics (bifonazole or ketoconazole), twice daily for a period of one month. After treatment was discontinued, samples for culture were again taken from the same sites.

### Experiment 2

From the 10 patients with seborrheic dermatitis, samples for culture were taken four times successively, one after the other, from exactly the same spots on the face.

### Statistics

The Wilcoxon test was used for matched pairs and the *t*-test for independent pairs (the data were transformed to log number colonies). The  $\chi^2$ -test was used to compare data.

## RESULTS

The *Pityrosporum* colonies, growing under the tapes, are white-creamy in colour when seen macroscopically; under the microscope they have a crystalline appearance (Figs. 1 and 2). If the total number of *Pityrosporum* colonies is small, the colonies are of a good size and can be counted with a magnifying glass. A culture with a large number of colonies has a large number of small to very small colonies which have to be counted with a microscope (25 ×). The results of experiments 1 and 2 are shown in Figs. 3 and 4. In Fig. 3 the numbers of *Pityrosporum* colonies cultured from seborrheic dermatitis of the face, corresponding normal-looking facial skin and from normal-looking facial skin from healthy individuals are shown.

Colonies cultured from seborrheic facial dermatitis 80-700/cm<sup>2</sup>. The number of colonies cultured from normal-looking skin from patients and healthy individuals was significantly less: only a few or none were cultured ( $p < 0.01$ ). From the 10 patients treated with antimycotics, 9 improved considerably or were completely healed. After treatment only a

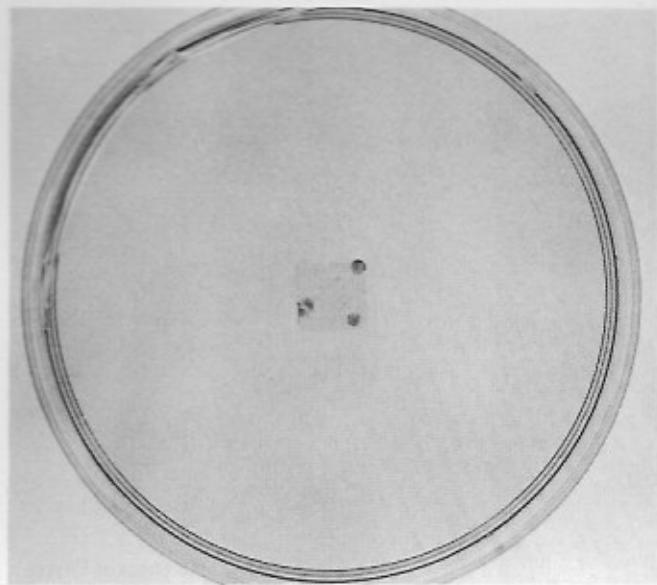


Fig. 1. *Pityrosporum* colonies growing under a tape on a Sabouraud medium.

few or no *Pityrosporum* colonies were cultured. Cultures were taken 2 days after patients discontinued the treatment.

A difference was found in the number of colonies cultured from the four successive strippings taken from seborrheic facial dermatitis; the first two strippings produced more colonies, but only if the numbers of colonies counted less than approximately 400/cm<sup>2</sup>, otherwise no difference was found (Fig. 4).

## DISCUSSION

With the 'tape-method' quantitative *Pityrosporum* cultures were taken from skin areas with seborrheic dermatitis and normal-looking skin; a quantitative distinction could be made between the former and the latter. As this difference was highly significant, the



Fig. 2. Crystalline appearance of *Pityrosporum* colonies.

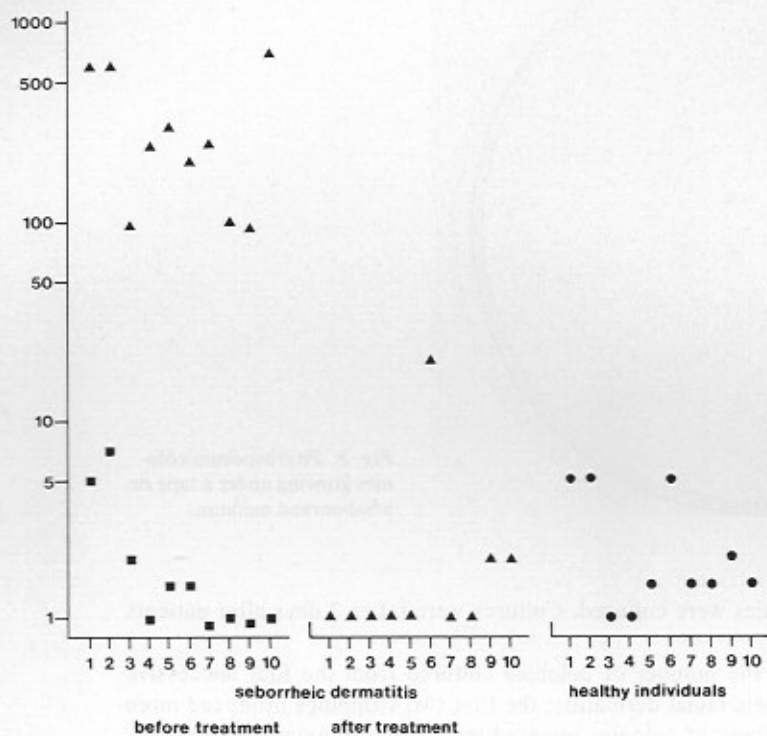
number of colonies/cm<sup>2</sup>

Fig. 3. Numbers of Pityrosporum colonies cultured from patients with seborrheic dermatitis (▲ involved, ■ not involved) and from healthy individuals (●).

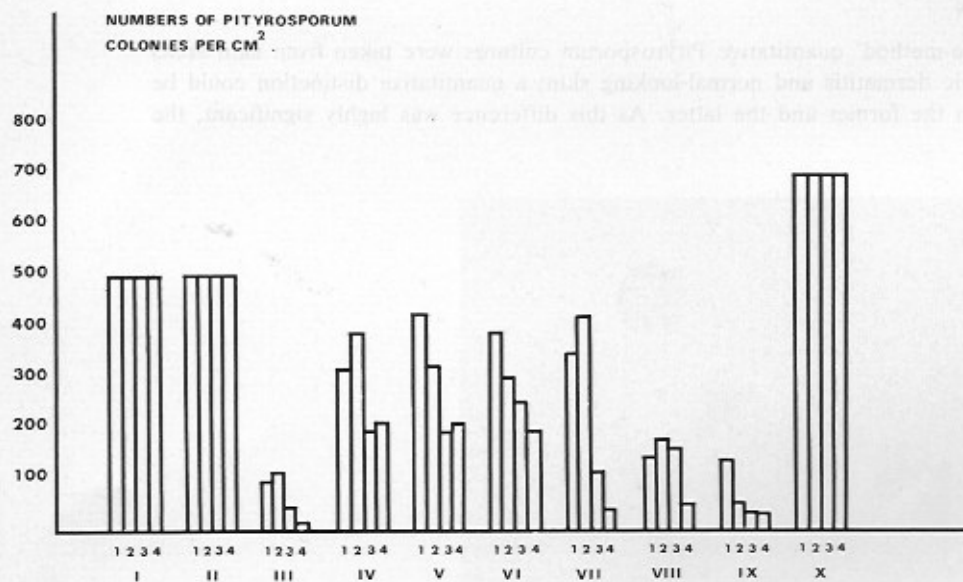


Fig. 4. Numbers of Pityrosporum colonies cultured from four successive skin strippings taken from 10 patients with seborrheic dermatitis.

'tape-method' therefore constitutes a reliable test for quantitative culturing of *Pityrosporum* yeasts.

Of the four successive strippings taken from seborrheic facial dermatitis, either the first two strippings produced the larger number of colonies, or all strippings produced the same number of colonies, depending on the total number of colonies cultured. Therefore it is sufficient to take only two strippings from the skin. The 'tape-method' has many advantages over the other techniques for quantitative culturing of *Pityrosporum* yeasts, such as the 'detergent scrub technique' or the use of 'contact plates' (6, 7): it can be used for skin areas, such as the nasolabial folds or the ear canal, which are difficult to access with the other methods, and it is a simple and inexpensive technique. With the 'tape-method' skin lesions suspected of being seborrheic dermatitis can be screened for the presence of an increased number of *Pityrosporum* yeasts, and so can be a helpful diagnostic tool. Preliminary results indicate that this method is useful in the differential diagnosis of seborrheic dermatitis versus facial psoriasis. The number of colonies is significantly lower in patients with psoriasis and equals that of 'normals'. The 'tape-method' has also proved useful for quantitative culturing of *Pityrosporum* yeasts from skin areas affected by pityriasis versicolor. With pityriasis versicolor, as with seborrheic dermatitis, dozens or hundreds of colonies can be cultured from lesions, and so by quantitative culturing the diagnosis can be confirmed.

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