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The role of the resident microflora in the pathogenesis of dandruff

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Synopsis

Current thinking implicates *P. ovale* as the cause of dandruff; the condition invariably resolves when this yeast is suppressed. However, *P. acnes* and coagulase-negative cocci are also abundant on the scalp. A contributory role for those members of the resident microflora has not been ruled out. We used a new technique to collect and weigh scales. We have determined the percentage of nucleated cells in scales, a measure of inflammation. The density of *P. acnes, P. ovale* and cocci were followed during and after treatment with antibacterial solutions and two anti-fungal shampoos, Octopirox® and Magnesium Omadine®.

The level of dandruff could not be correlated with changes in the numbers of aerobic and anaerobic bacteria. We concluded that resident bacteria probably play no role in the etiology of dandruff. Octopirox® shampoo was more efficacious than Magnesium Omadine® in reducing scaling and *P. ovale*. While *P. ovale* is necessary, Koch's postulates have not been fulfilled. The cause(s) of drandruff is (are) still unknown.

INTRODUCTION

The modern method of treating dandruff with antifungal shampoos grew out of demonstrations that the active ingredients inhibited the resident yeast, *P. ovale, in vitro* and *in vivo* (1–4). Scales, the sole manifestation of the disorder, practically disappear whenever *P. ovale* is eradicated. When treatment stops, the return of scaling parallels the restoration of *P. ovale*. A single discordant result from the University of Pennsylvania group was technically flawed in using a pharmaceutically questionable formulation of amphotericin (5). The data base strongly suggests that *P. ovale* is the determinant causal factor in dandruff, a position vividly championed by Shuster (6). Still, Koch's postulates have not been fulfilled. In one of these postulates, the pathogenicity (distinct from an adventitious form) is strictly defined by the fact that the "microbe" is found in a lesion. It

seems very unlikely that dandruff is simply an infection by a pathogenic yeast since it is recovered from normal scalps. An alternative explanation is that increased scaling is the result of an underlying inflammatory process (7), throwing up clumps of thickened, but porous, horny layer in which *P. ovale* flourishes. Mediators produced by a greatly expanded population of *P. ovale* could then percolate downward, aggravating the inflammatory process. Indeed, it is known that *P. ovale* can induce inflammation through activation of the complement (8), leading to the release of chemotactic factors. The latter would then attract neutrophils into the epidermis, disturbing keratinization. In this scenario, *P. ovale* would function in a secondary role. The response of scalp psoriasis to ketoconazole has similarily been ascribed to the pro-inflammatory activity of a large population of *P. ovale* (9). Neither we nor others have found a way to induce dandruff in non-dandruff subjects. We assume that the etiology is complex and is influenced by various constitutional factors, including heredity.

We lack a good understanding of why a minority of persons exhibit clinical dandruff when all individuals produce some scales. The difference between dandruff and non-dandruff seems to be merely quantitative, greater and larger scales (10). We undertook this study with several questions in mind: 1) Since the dandruff scalp also richly harbors *P. acnes* and cocci, can these aggravate scaling? 2) In view of improved methods of assessment, how good are the correlations between (a) weight of collected scales, (b) clinical grades, (c) *P. ovale* counts, and (d) the index of inflammation, based on the percentage of nucleated cells in scales? (3) Finally, Octopirox® (ethanolamine salt of 1, hydroxy-4-methyl-6-(2,4,4 trimethyl pentyl)-2-(1H)-pyridinone) and Magnesium Omadine® (magnesium salt of 2-pyridinethiol-1-oxide) are fungistatic agents included in popular shampoos, at least in the European countries. Can these be discriminated in regard to efficacy with the more sensitive methodology now available (10)?

MATERIALS AND METHODS

GENERAL DESIGN

Twelve white males, aged 24–43, with moderately severe dandruff served as paid volunteers. By clinical criteria none had seborrheic dermatitis. During a preparative period of three weeks, they shampooed their scalps thrice weekly with non-medicated 6.25% lauryl ether sulfate (LES) provided by us. Scale production was measured according to our recently published method (10). Basically, scales were harvested by voluminous shampooing and the wash water filtered to collect all the scales. These were dried and weighed. Scales were always harvested two days after shampooing, when scale production nears a plateau. The severity of dandruff was assessed on a 0 to 10 clinical scale. Grades 2 and below designate non-dandruff. Grade 3 is marginal dandruff. Grades 4, 5, and 6 reflect respectively mild, moderate, and severe dandruff. Our subjects were chiefly grade 5. The plan of the study is shown in Figure 1. Two groups, A and B, comprising six subjects each were compared. In the three weeks pretreatment phase the scalps were shampooed thrice weekly with 6.25 LES. In phase I, lasting four weeks, Group A received 5 ml of an ethanol:water solution (1:1), 5 ml/scalp, thrice weekly. Group B received an ethanolic solution (1:1), 5 ml/scalp, containing 1% clindamycin hydrochloride and 1% Octopirox®, thrice weekly. The latter formulation was

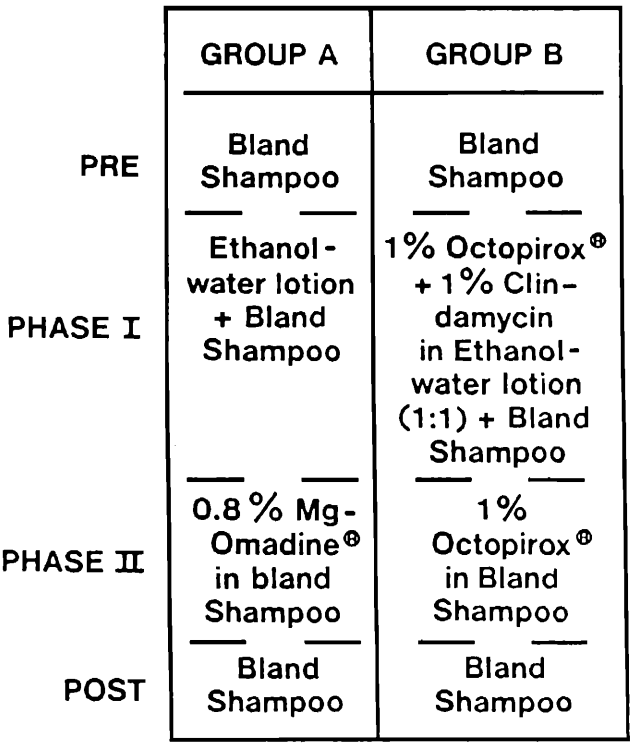


Figure 1. General design of the study. Bland shampoo = 6.25% LES in water.

both antibacterial and antifungal. The scalps continued to be shampooed as above with LES thrice weekly.

In phase II, Group A was washed thrice weekly for ten weeks with a specifically antifungal shampoo containing 0.8% Magnesium Omadine® in LES. Group B received 1% Octopirox® in the same shampoo base. In the post-treatment three-week follow-up, both groups were shampooed as usual with 6.25% lauryl ether sulfate.

QUANTIFICATION OF THE MICROFLORA

The detergent-scrub method of Williamson and Kligman (11) was used to sample the surface for microorganisms. After cutting the hair trim with the surface, a glass cylinder of 3.8 cm^2 was held firmly to the skin, and the scalp flora was dispersed in two successive 1-ml scrubs in Triton phosphate buffer.

The pooled sample was then subjected to quantitative bacteriologic analysis according to the method of McGinley *et al.* (12). The density of anaerobes (*P. acnes*) and aerobic cocci was determined after drop-plating graded dilutions of the sample. Because *P. ovale* cannot be accurately estimated by culturing, its density was determined by counting the cells in Giemsa-stained slides prepared from an aliquot of the scrub fluid.

INDEX OF INFLAMMATION (I.I.)

This is derived by establishing the percentage of corneocytes which are nucleated (parakeratotic cells). The scales are first disrupted ultrasonically in Triton phosphate buffer to produce a suspension of single corneocytes. A smear on a glass slide was stained with Giemsa, counting no less than 500 cells. Multiplying the mg of scales by the percentage of nucleated cells yields the I.I., the mg of nucleated cells produced per scalp per two days (10).

RESULTS

SCALE PRODUCTION (S.P.)

The S.P. of group B (Clindamycin 1% and Octopirox 1%) quickly decreased during phase I, reaching values of non-dandruff subjects well before the end of the four-week period (Figure 2). In phase II (Octopirox® shampoo), scale production remained quite low. In contrast, group A in phase I, using vehicle alone, showed no reduction in S.P. In phase II (0–8% Omadine shampoo), S.P. decreased slowly in Group A to a final level of 40% baseline, compared to more than a 60% decrease in Group B, using the Octopirox® shampoo. Moreover, with the latter, S.P. fell off sharply.

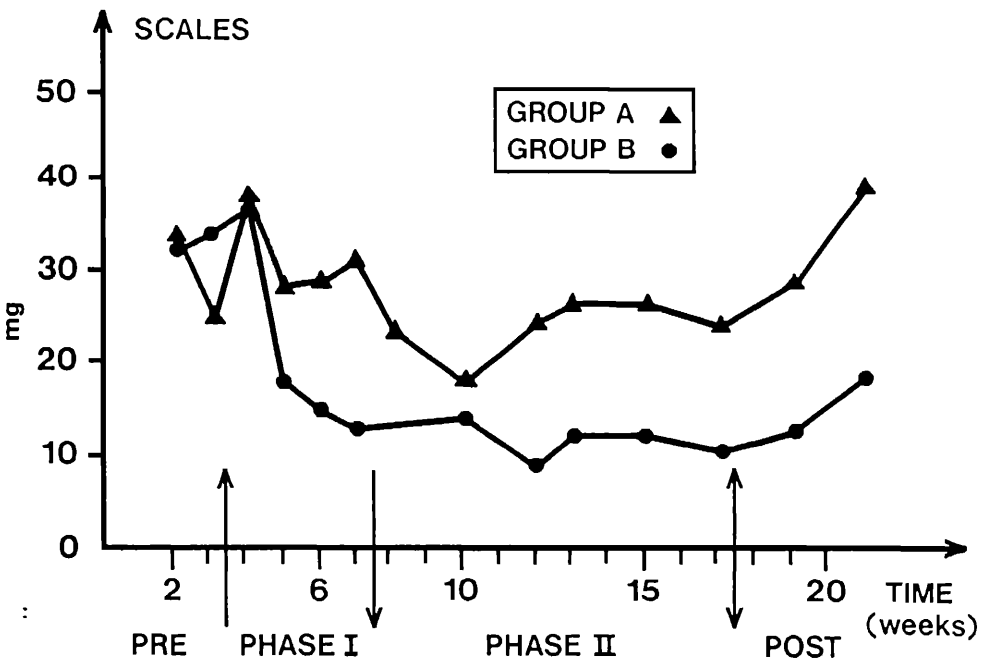


Figure 2. Variations in scale production with treatments. Group B (Octopirox® + clindamycin) shows a rapid fall in scale production, reaching a baseline in scale production (comparable to non-dandruff). Group A only responded to antifungal shampoo in phase II (Omadine® Mg).

CLINICAL GRADES

The decrease in clinical grades roughly paralleled the reductions in S.P. Virtually no scales were detectable in group B, while group A fell to the level of threshold dandruff (Figure 3). In the post-treatment phase the grades increased proportionately in both groups, but did not reach baseline levels by the end of the three-week followup.

MICROFLORA

The counts of *P. acnes* and cocci did not decline at any time in Group A (Figures 4,5). By contrast, both fell sharply in phase I of Group B, but then rather rapidly returned to baseline levels after switching to the selectively antifungal Octopirox® shampoo.

While *P. ovale* declined in both groups during antifungal shampooing, the density very quickly approached zero in Group B (Figure 6). In Group A, however, the numbers did not begin to go down for a couple of weeks, diminishing slowly thereafter and reaching a level almost comparable to Group B by the end of ten weeks. In the post-treatment phase, *P. ovale* sprang sharply upward, nearing or attaining baseline levels by three weeks.

INDEX OF INFLAMMATION (I.I.)

The downward slope of I.I. paralleled the decrease in clinical grades and S.P. (Figure

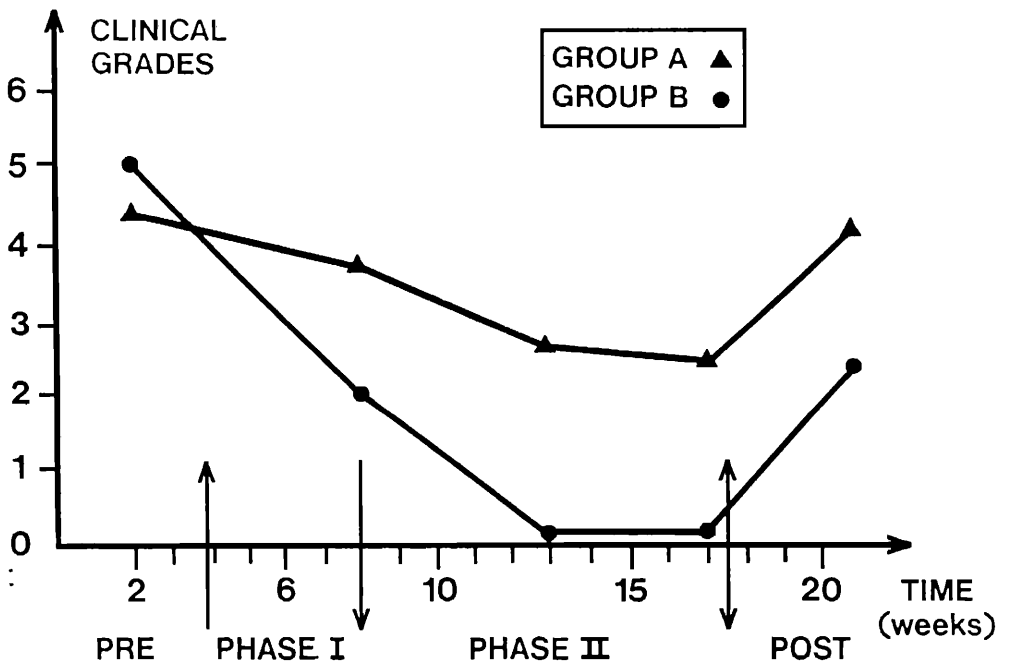
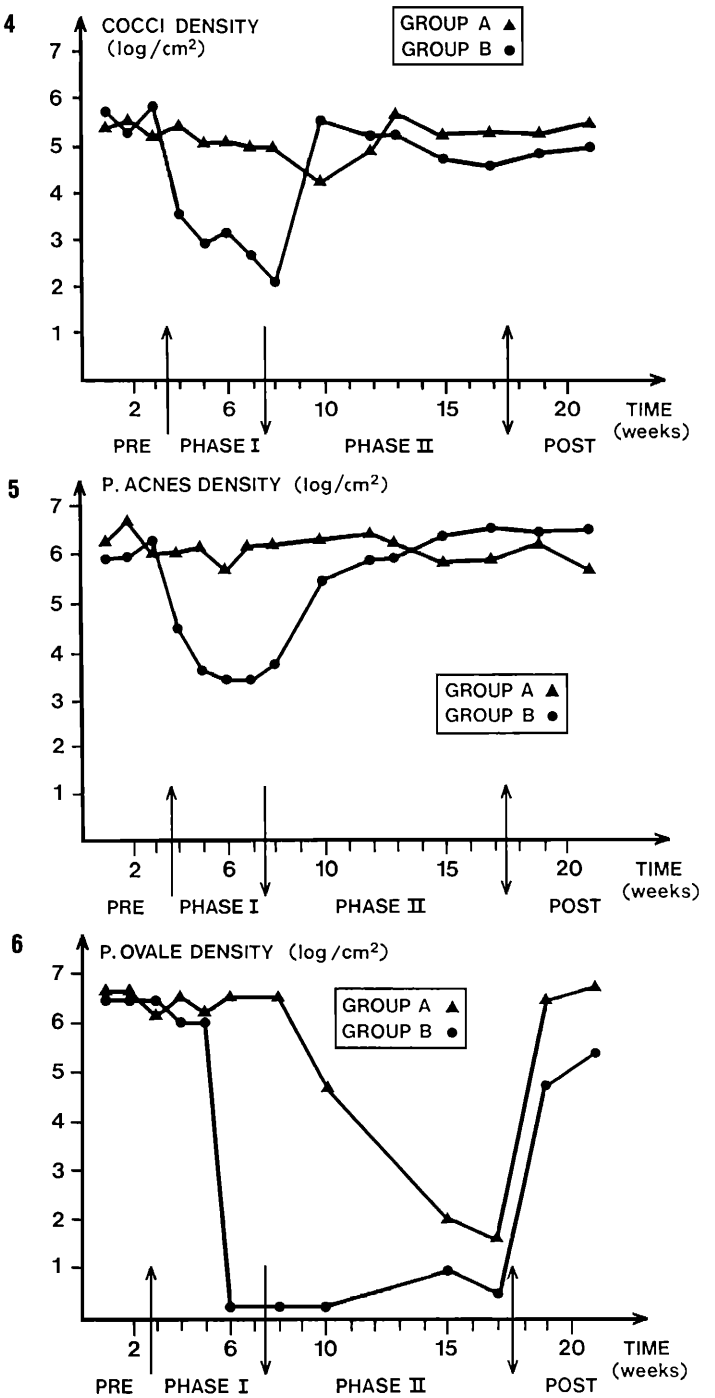


Figure 3. Clinical grades in response to treatments. Scaling diminishes slowly in Group A compared to the rapid decline in Group B. Further, after ten weeks, the latter group showed a complete absence of clinical scaling, whereas this was not achieved by Group A after 17 weeks.



Figures 4,5,6. Influence of treatments on the densities of, respectively, cocci (Figure 4), *P. acnes* (Figure 5), and *P. ovale* (Figure 6) on the scalp surface. The mixture Octopirox and clindamycin is clearly bacteriastic and fungistatic, while the vehicle has no effect. Antifungal shampoos, in phase II, appear fungistatic and do not influence bacterial population. Octopirox® more quickly supresses *P. ovale* than Omadine® Mg. Stopping the treatment restores the initial density of the scalp flora.

7). In phase I the I.I. of group A did not change, in contrast to Group B. During phase II antifungal shampooing, nucleated cells had almost completely disappeared in both groups, analogous to the final values for *P. ovale*. In the post-treatment phase, the I.I.s of both groups had increased, more so in Group A but still below the pre-treatment values.

DISCUSSION

The results exclude the bacterial flora from consideration in the pathogenesis of dandruff. The density of *P. acnes* and cocci remained at pre-treatment levels when the major parameters of dandruff, clinical grades, S.P., and I.I. were falling. These last three decreased in proportion to the decreasing population of *P. ovale*. Each of these measurements was in good correlation with the density of *P. ovale* and with each other. This result speaks for the validity of the individual measurements. At the concentrations used in a LES base, Octopirox® was superior to Magnesium Omadine® by all counts. It was notably able to cause regression of dandruff more swiftly, in agreement with previous findings (4). Those who believe *P. ovale* is the cause of dandruff will gain consid-

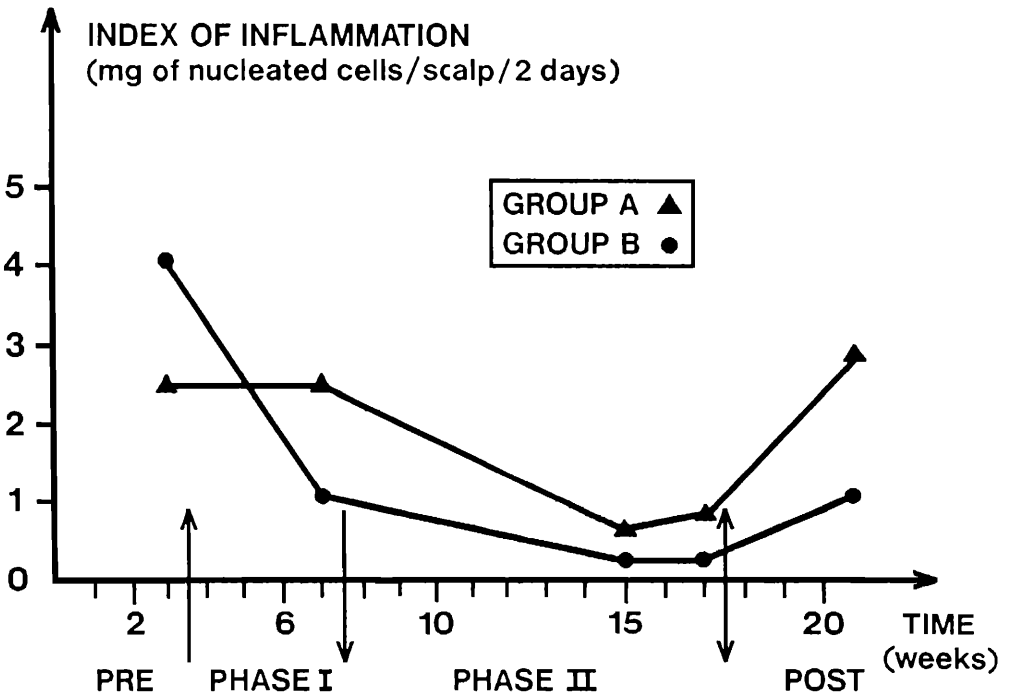


Figure 7. Influence of the treatments on the inflammatory process (in mg of nucleated cells produced per scalp in two days). The mixture of clindamycin and Octopirox® rapidly diminishes the index of inflammation, which remained very low in Group B, using antifungal treatment (Octopirox®) in phase II. The vehicle (water-ethanol) used in Group A shows no effect, while in phase II, antifungal treatment alone (Omadine® Mg) leads to a decline in the inflammatory process. Stopping the treatments shows the trend to recover the initial values.

erable support from our findings. While we do not deny its commanding importance, the evidence is not complete that dandruff is simply an infection by *P. ovale.* It is noteworthy that, in our study, Group B in Phases I and II showed threshold values of scaling despite the absence of the yeast at the skin surface. Dandruff cannot be induced by inoculating normal scalps with *P. ovale* or with dandruff scales. We have repeatedly failed in such attempts. In animals, when *P. ovale* is topically applied daily in a lipidic medium, scaling and inflammatory reactions are induced (13–15). However, all that scales is not necessarily dandruff, since scaling is a non-specific response to inflammation. In these animal models, scaling resolves when treatment stops. Besides, the condition, rapidly regresses, unlike human dandruff which persists in a stable fashion for many decades of adult life. Then, too, dandruff can be caused to regress by therapies which have no effect on *P. ovale,* [for example, topical corticosteroids and tar shampoos (16)].

In our experimental conditions, a possible cytostatic action on the epidermis is unlikely since Octopirox® acts only via an antifungal mechanism (17). Hereditary influences cannot be ruled out. In our view, dandruff is a multifactorial condition whose initiation is still a mystery. Once scaling begins, *P. ovale* has increased living room and proliferates accordingly. Its products perpetuate the process, an amplification role rather than an inductive one.

According to Shuster (6), there is no controversy. *P. ovale* is the sole cause of dandruff. We continue to be puzzled by the unsolved problems of pathogenesis, acknowledging meanwhile that controlling *P. ovale* is sensibly the major aim of anti-dandruff therapy.

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