Dandruff has an altered stratum corneum ultrastructure that is improved with zinc pyrithione shampoo

Ronald R. Warner, PhD,[a] James R. Schwartz, PhD,[b] Ying Boissy, BS,[a] and Thomas L. Dawson Jr, PhD[c] *Ross, Ohio*

Transmission electron microscopy of scalp tape strips indicates that dandruff scalp possesses abnormal stratum corneum (SC) ultrastructure that is normalized by treatment with small-particle zinc pyrithione (ZPT). Similar abnormalities occur throughout the scalp of those with dandruff, even where no flaking is present. SC abnormalities are consistent with hyperproliferation, including parakeratosis, lipid droplets within corneocytes, few desmosomes, corneocyte membrane interdigitation, and excessive disorganized intercellular lipid. Reversal of SC abnormalities would require treatment of the cause(s) of dandruff, not merely flake removal. A protocol was developed to quantify scalp structural abnormalities by scoring cells from scalp tape strips for yeast number, amount of intercellular lipid, normal intercellular lipid structures, prevalence of intracellular lipid droplets, parakeratotic corneocytes, and corneocyte interdigitation. This protocol was used to compare dandruff and normal SC to dandruff SC treated with either commercial ZPT-containing shampoo or a placebo. Treatment with commercial ZPT shampoo significantly returned SC ultrastructure to normal, suggesting control of the cause of dandruff. (J Am Acad Dermatol 2001;45:897-903.)

Dandruff and seborrheic dermatitis are chronic clinical scalp conditions affecting approximately 50% of the population, the primary symptom of which is visibly excessive scalp scaling. Seborrheic dermatitis is a more severe disorder that can include increased desquamation of facial areas other than the scalp and visible inflammation. We refer to these conditions collectively as "dandruff." Although dandruff is not a life-threatening disease, its presence can lead to loss of self-esteem and a negative social image.[1] More than 100 years ago, Malassez[2] implicated the yeast *Pityrosporum* as the cause of dandruff. Although there has been much debate regarding whether the yeast is actually a causative agent in dandruff,[3,4] there is now general agreement to that effect.[3,5-7] The yeast *Pityrosporum* has now been reclassified into 7 species of *Malassezia* on the basis of both molecular and biochemical techniques.[8-12] Although there are likely multiple causative factors in the pathogenesis of dandruff, *Malassezia* has been shown to induce dandruff or dandrufflike desquamation under the proper experimental conditions.[13-17]

To date, there have been few investigations of scalp ultrastructure associated with dandruff or seborrheic dermatitis. As noted 3 decades ago, "It is surprising that a condition as common as dandruff has not been thoroughly studied in respect to its… histopathology."[18] In spite of this admonition, to our knowledge there have been no subsequent morpholgic studies at the transmission electron microscope (TEM) level. Dandruff and seborrheic dermatitis have been characterized as a hyperproliferation of the scalp epidermis, as judged by cell turnover studies and the presence of parakeratotic nuclei in the shed flakes and the attached stratum corneum (SC).[19] A previous TEM study identified several pathologic alterations in the SC of dandruff scalp.[20] This study found frequent bacteria and fungi, a large number of electron-transparent corneocyte lipid inclusions, parakeratotic nuclei, a decrease in desmosome number, and frequent separations between corneocytes. The viable epidermis was visually unaffected.

A novel technique for noninvasively sampling the SC for TEM was recently developed.[21] The lipid orga-

From the Corporate Research Division,[a] Hair Care Product Development,[b] and the Hair and Scalp Care Technology Division,[c] the Procter & Gamble Company.

This work was funded by the Procter & Gamble Company. All authors are employees of the Procter & Gamble Company.

Accepted for publication May 21, 2001.

Reprint requests: Thomas L. Dawson Jr, PhD, The Procter & Gamble Company, 11810 E Miami River Rd, Ross, OH 45061. E-mail: Dawson.tl@pg.com.

0190-9622/2001/$35.00 + 0 **16/1/117849**
doi:10.1067/mjd.2001.117849

nization of the SC on the lower leg was investigated with new RuO_4 procedures for visualizing SC lipids,[22,23] and it was found that the lipid structure of the outer SC varies with age, skin grade, and product use.[24] Definition of "normal" SC now allows investigation of pathologic abnormalities. In this study we determined ultrastructural alterations in the scalp SC that accompany dandruff. We confirmed the presence of fungi, parakeratotic nuclei, frequent corneocyte lipid inclusions, and a decrease in desmosomes. We also describe additional abnormalities associated with dandruff, including highly convoluted corneocyte cell membranes and dramatically excessive intercellular lipids. Not all these abnormalities are present in the same individual, suggesting a diversity of responses. We evaluated the effect of dandruff treatment with zinc pyrithione (ZPT) on SC ultrastructure. Dandruff treatment with a commercial small-particle ZPT shampoo resulted in clinically significant visual flaking reduction. We also observed an improvement in SC structure and, by inference, treatment of the cause of dandruff generating subsequent improvement in the health of the living cell layers that produce it.

MATERIAL AND METHODS

Studies were performed on male and female volunteers giving informed consent.

Microscopy of tape strips

To obtain a tape strip, the hair was parted to expose the scalp, and a piece of Scotch Magic Tape #810 (3M, St Paul, Minn) was applied to the skin surface with gentle pressure and carefully removed after 30 seconds. Under stereomicroscope observation, regions of the tape having skin flakes were cut out and placed in 0.25% RuO_4 in 0.1 mmol/L cacodylate buffer, pH 7.2 to 7.4, for 1 hour at 4°C in darkness, rinsed briefly in 0.1 mmol/L cacodylate buffer, and then dehydrated through a graded acetone series before Epon embedding and overnight polymerization at 65°C. Well-stained regions were selected by light microscopy, and thin sections were obtained on an ultramicrotome, counterstained with uranyl acetate and lead citrate, and analyzed in a Philips CM12 TEM at 100 KeV.

Clinical protocol

This was a single-center, double-blinded, randomized, parallel group comparison study in 12 healthy adult male and female moderate to severe dandruff and seborrheic dermatitis sufferers, aged 18 years and older. The study was conducted at Hill Top Research in Winnipeg, Manitoba, Canada. Panelists were assessed for dandruff severity with the adherent scalp flaking scale (ASFS).[25] In this assay, a numerical grade between 0 and 10 (with 10 representing the most severe dandruff condition)was assessed by a qualified, expert grader at 8 sites on the scalp to obtain a composite dandruff score ranging from 0 to 80. All panelists used a nondandruff shampoo (Prell, Proctor & Gamble) for 3 weeks before baseline ASFS assessment. At the baseline visit, those possessing ASFS of at least 28 were randomly assigned 1 of 2 treatments: a commercial small-particle ZPT shampoo (Head and Shoulders, Proctor & Gamble), or a placebo shampoo with the same composition as the commercial antidandruff shampoo but containing no ZPT. The treatments were packed in the same 16-oz white bottle and placed in an opaque bag. This was done because the investigator, grader, and subjects were blinded to the identity of treatment assignments. Once a subject was enrolled in the study he or she was stratified by initial scalp flaking grade (28-34 and >34) and sex. Within each strata the subject was randomly assigned to 1 of the 6 treatments. This was done so that the different strata groups had approximately the same treatment distribution ratio. From this randomization 6 subjects were randomly selected from both the treatment and placebo leg. The randomization of these 12 subjects was provided in a numerically ordered list for sampling at the site to ensure blindness of the 2 treatment groups. Also at baseline, a single tape strip was obtained from the site on the scalp with the highest individual flaking grade. Panelists were instructed to shampoo their hair every other day during the course of the 3-week study (no other hair treatment was allowed during this period). At the completion of the study, dandruff severity and a tape strip were collected, as at baseline.

Assessments of ASFS and skin morphologic characteristics were ordinal variables and analyzed statistically as continuous variables. Significance of the continuous end points was examined by analysis of variance (ANOVA). Nonparametric methods were applied when appropriate to help satisfy underlying assumptions for valid analysis and inference. An analysis of covariance, with baseline values as the covariate, was applied where appropriate. The collection of ASFS and scalp tape strips was by individuals blinded to treatment groups. The blinding was carried forward for the subsequent grading of tape strips as well.

Product efficacy

Product efficacy from a visual (clinical) standpoint was determined by subtraction of the initial ASFS score from the final score. A more positive number indicates a greater improvement in the dandruff condition (less dandruff, greater product efficacy). A negative number means dandruff worsened.

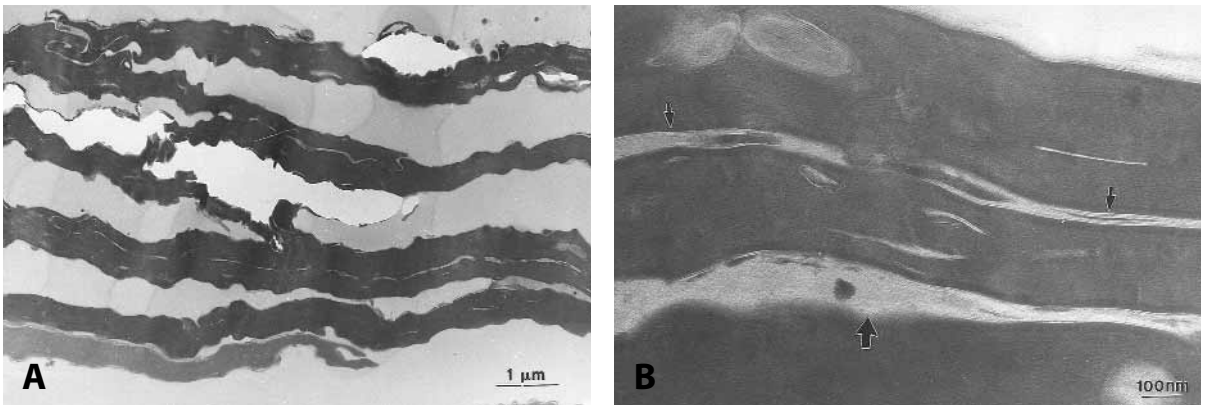

Fig 1. Normal-appearing scalp. **A,** Corneocytes are closely apposed with generally normal morphologic characteristics (large separations between some corneocytes is presumably artifact from tape stripping). **B,** Intercellular lipid ultrastructure is normal for this individual's age *(small arrows)*. Some regions have excess unstructured lipid *(large arrow).*

Product efficacy from a skin health standpoint was evaluated by observing the change in SC morphologic features from the initial and final tape strips. These changes were subjectively obvious, but to quantify the morphologic results, numerical values were assigned to the degree of improvement toward normal of 6 morphologic parameters: the number of yeast observed per microscopic field, the amount of lipid in the intercellular space relative to baseline, the prevalence of lipid inclusions within corneocytes, the amount of normal lipid structure (Landmann units[26]) in the intercellular space, the number of corneocytes containing visible nuclei, and the prevalence of abnormal corneocyte interdigitation ("scared" cells).

These 6 parameters were chosen before data collection. The numeric grading scale was –10 to +10; the scale used was –2 to +8. As with the clinical score, a greater improvement in skin morphologic features toward a normal SC structure is indicated by a more positive number. A negative number indicates a worsening of SC structure.

As before, the microscopists were blinded to treatment identity during data collection and sample analysis.

Statistical difference between groups was determined by single-factor ANOVA. Results were considered different for $P < .05$.

RESULTS

Normal scalp (no dandruff) has a relatively normal outer SC ultrastructure

The sampled site from the individual shown in Fig 1, *A* had a dandruff grade of 0. The outer SC has an appearance similar to that of other regions of the body. Many of the corneocytes are closely apposed and have normal morphologic characteristics. The large separations between corneocytes are likely artifacts of the tape-stripping process, and the intercellular spaces are in general filled with the embedding plastic, not SC material. At higher magnification (Fig 1, *B*), small regions of excess lipid can occasionally be found in the intercellular space *(large arrow),* but in general the intercellular space has a lipid ultrastructure that is normal for this individual's age *(small arrows)*. Desmosomes are normal. Also shown in the upper left and lower right corners of Fig 1, *B* are lightly staining ovoid bodies within corneocytes. These structures have previously been identified as lipid inclusions, are typically not found in normal SC at other body sites, and are only occasionally seen in this individual.

SC from a flaking area of scalp with dandruff has an abnormal (pathologic) ultrastructure

A diversity of ultrastructure was seen in the SC of dandruff-involved scalp tissue, with no one person having expression of all attributes simultaneously. The sampled sites in Fig 2 had dandruff grades of 8. Many of the corneocytes are not tightly apposed but are separated by a thick mass of intercellular lipid (Fig 2, *A* and *B*). Desmosomes are reduced in number and usually completely absent. There is infiltration by *Malassezia* (Fig 2, *A, arrows*). The intercellular lipids have at least 2 separate morphologic characteristics, a uniform, darkly staining material divided into linear, flowing structures similar to that previously described for sebum,[27] and structures that more closely resemble the "ground substance" of subepithelial compartments.[28] Some corneocytes curl upon themselves, and others have highly interdigitated membranes, presenting a "scared" structure (Fig 2, *C*). In the "scared" phenotype there was

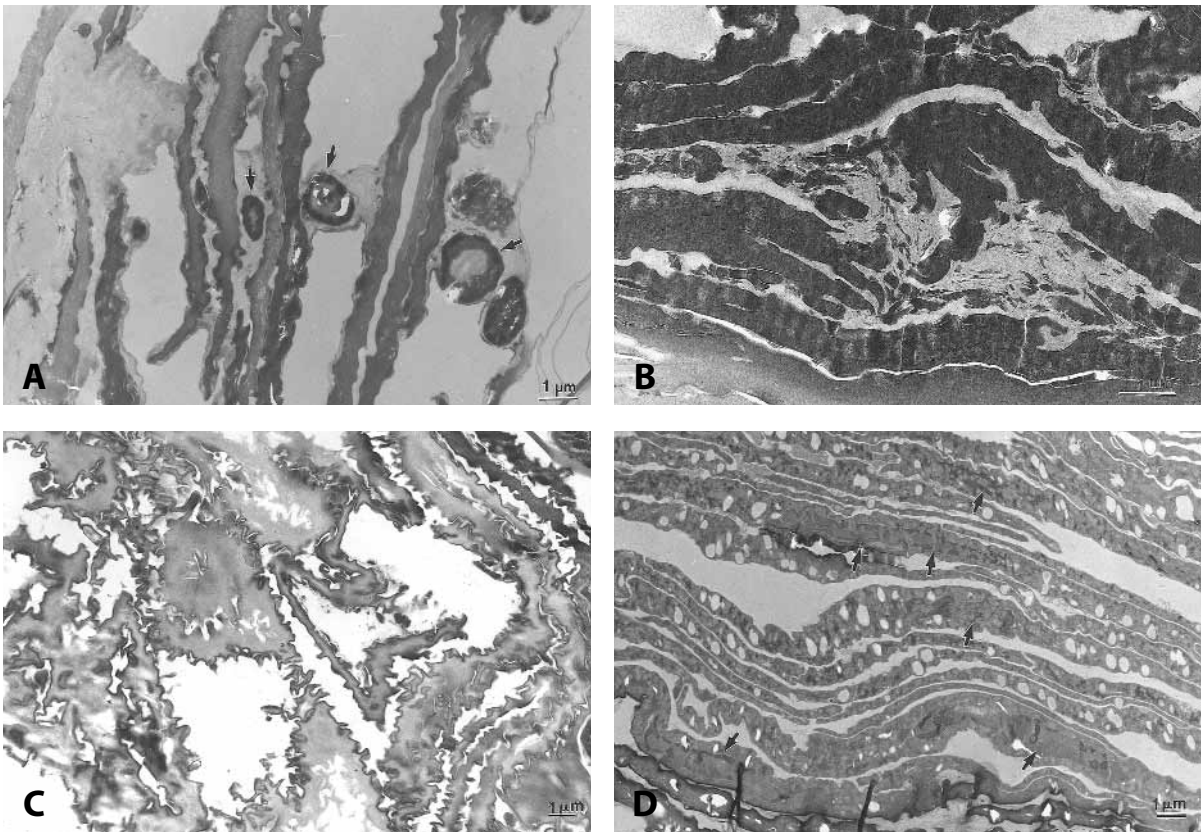

Fig 2. Microscopic physiology of dandruff. **A,** Outer SC shows corneocytes widely separated by excessive intercellular lipid. *Malassezia* are frequent and show a gradient into SC. Desmosomes are rare. **B,** Massive amount of nonlamellar intercellular lipids, few desmosomes, and curling corneocytes. **C,** Parakeratosis *(arrows)* and intracellular lipid droplets are pervasive. Desmosomes are rare. **D,** "Scared" corneocytes, exhibiting highly convoluted fingerlike membrane projections.

less intercellular sebumlike material. In the site sampled for Fig 2, *D,* the primary pathologic structures are the immense number of lipid droplets within corneocytes as well as the frequent presence of parakeratotic nuclei *(arrows).* At higher magnification there are virtually no intercellular lamellar structures of any kind and very unusual poorly staining spherical structures similar to, but generally smaller than, the lipid droplets within corneocytes.

Surprisingly, areas of dandruff scalp without obvious flakes (grade 0 or 1) had an ultrastructure similar to the pathologic findings of flaking areas. Skin samples were also collected from the lower leg of a subject with severe dandruff. These samples revealed an SC structure normal for the individual's age, with tightly packed corneocytes containing few lipids drops and having normal lamellar structures and frequent desmosomes, indicating that the dandruff pathologic condition is confined to the scalp.

Sites were sampled from areas with a dandruff grade of 5 to 6. The structural alterations of the outer SC were intermediate between the control and individuals with severe dandruff. Particularly noticeable was the intermediate level of excess intercellular material. Similar to the severe dandruff case, the uninvolved area of scalp had an ultrastructure that was very similar to the involved area.

Visual grading shows ZPT shampoo improves dandruff

For the treatment group, the average combined ASFS change was +9.8 ± 3.2. For the placebo group, the average change in combined ASFS was –1.5 ± 7.1. These results are statistically different by single-factor ANOVA ($P = .005$), and we can conclude that the 3-week treatment with a commercial ZPT shampoo resulted in improvement in visual dandruff grade.

Microscopy evaluation shows a ZPT shampoo improves scalp structure as it improves dandruff

Using the microscopy numeric grading for ultrastructural changes, the average change for the treat-

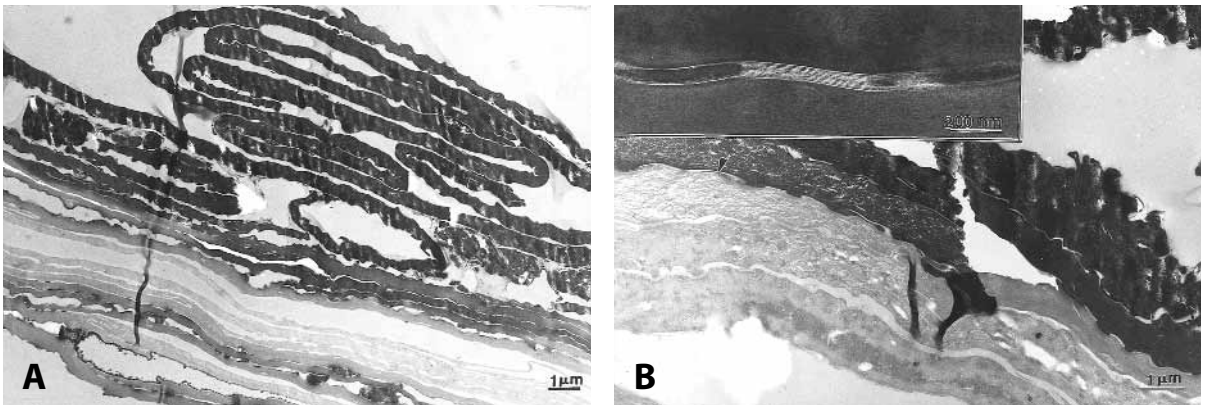

Fig 3. Posttreatment normalization of dandruff phenotype. **A,** Posttreatment morphologic features showing improved corneocyte structure (curling of the topmost corneocytes is a common artifact of tape stripping, when surface corneocytes directly contact tape). **B,** Posttreatment morphologic features, with a very normal SC of tightly apposed corneocytes, frequent desmosomes, and lamellar intercellular lipids.

ment group was +14.5 ± 8.1. For the placebo group, the average change was +3.7 ± 6.0. These results are also statistically different by single-factor ANOVA ($P = .025$) and also show that a commercial ZPT shampoo improves the scalp physiologic characteristics disrupted in dandruff.

After treatment there was considerable normalization of the SC structure toward that seen in the no-dandruff control (Fig 3). Very few lipid inclusions were present within corneocytes and no parakeratotic nuclei were found, implying a significant decrease in hyperproliferation (Fig 3, *A*). The commercial antidandruff shampoo convincingly normalized most of the intercellular space (Fig 3, *B*), and a normal lipid structure with Landmann units was common, as shown in the inset. It should also be noted that there were very few, if any, corneocytes with "scared" membrane interdigitations after small particle ZPT treatment. Control shampoo without ZPT had minor effects on the SC ultrastructure of dandruff. Incremental improvement in ASFS is common in clinical studies, most probably because of increased shampoo use mandated by the study protocol.

DISCUSSION

There have been few ultrastructural studies of dandruff. Light and electron microscopic studies of scalp biopsy specimens have described the presence of microorganisms and an apparent hyperproliferation of the scalp epidermis with parakeratotic nuclei, lipid droplets within corneocytes, a decrease in desmosomes, and frequent separations between corneocytes with corneocytes often curling upon themselves.[18-20] Using a newer fixation technology that is superior for visualizing SC lipids,[22,23] and investigating only the outer SC, we confirmed these structural abnormalities. We found that individuals with dandruff also have excess intercellular lipids with several different structural presentations. These structures include a darkly staining amorphous or rivulet structure that we believe resembles that described for sebum,[27] and a "ground substance"–like structure[28] containing numerous darkly staining particulate deposits. Corneocyte separations and corneocytes curling upon themselves are likely caused by the massive sea of intercellular lipids that was not detectable by earlier fixation protocols. We further describe corneocytes with very abnormal, highly convoluted membranes, giving the corneocytes a very spiny or "scared" appearance.

Dandruff was previously characterized as a disease of the SC induced primarily by an interference with the normal keratinization process of the epithelial cells.[18] Others have implicated disturbances of sebaceous secretions.[29] One manifestation of dandruff is the presence of lipid droplets within corneocytes. The excessive accumulation of lipid droplets within corneocytes is a typical consequence of pathologic keratinization[30] and is seen in a variety of skin disorders, including psoriasis, acne, and fungal infection.[31-33] However, the excess intercellular lipids that we observed in most individuals with dandruff has ultrastructural similarities more in common with the central chamber of the acne comedone.[34] Our observations therefore support both altered keratinization and altered sebaceous secretion in the cause of dandruff.

A diversity of ultrastructural responses was seen in different individuals with dandruff. This may be related to the previously observed focal nature of

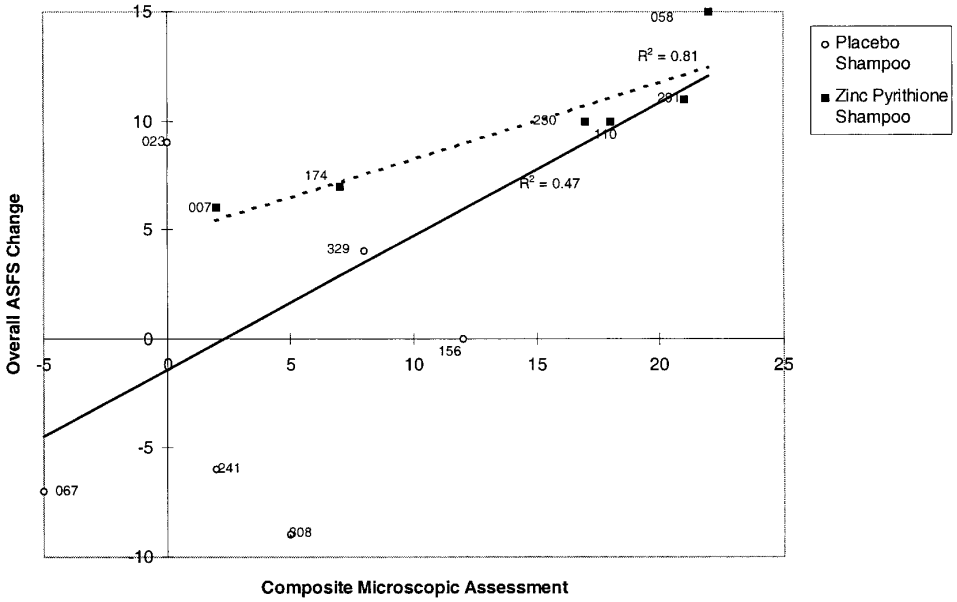

Fig 4. Correlation of microscopic phenotype with ASFS grades. There was good correlation between changes in visual (clinical ASFS) grade and changes in morphologic assessment. Placebo *(circles)* and ZPT treatments *(black squares).* Correlation of total population (*solid line,* $R^2 = 0.47$), correlation after ZPT treatment (*dashed line,* $R^2 = 0.81$).

parakeratosis in this disease. Reasons for such focal diversity are unknown, but we surmise they may be related to microbial populations. Although there is structural diversity in dandruff between individuals, structural alterations are always present. Areas of a dandruff-affected scalp have an abnormal SC structure, regardless of variations in visual grade. The diversity of SC alterations may be the result of competing disturbances of epidermal keratinization and sebaceous lipid secretion.

To quantitatively evaluate the total picture of dandruff, including all morphologic observations in an unbiased, nonweighted manner, we developed a grading system based on SC morphology. There was good correlation between changes in visual grade (clinical ASFS) and changes in morphologic assessment (Fig 4). Fig 4 includes placebo *(circles)* and ZPT treatments *(black squares)*. Although a correlation is present (*solid line*, $R^2 = 0.47$), it is confounded by wide variation in the placebo group. If these 2 groups are evaluated independently, there is a very strong correlation between the visual and morphologic changes after ZPT treatment (*dashed line*, $R^2 = 0.81$). There is no statistical correlation ($R^2 = 0.04$) between visual and morphologic changes for the placebo group. This result is not surprising given the limitations of the morphologic approach. As noted previously, the tape-stripping procedure has problems of representative sampling, such as limited sample size and unevenness with RuO_4 staining. As such, the technique is very good at visualizing large changes in morphologic characteristics, but as the changes become more subtle they are difficult to differentiate.

Whatever the presentation of ultrastructural abnormalities in dandruff, they were dramatically reduced by use of a ZPT shampoo: *Malassezia* numbers fell, excessive intercellular lipid level was decreased, parakeratosis was eliminated, corneocyte lipid inclusions were diminished, and "scared" cell morphologic characteristics were eliminated. Although the visual pathologic features of dandruff are located in the SC, this layer reflects fundamental changes occurring in the viable dermal cells that produce it. Consequently, the normalization of the SC ultrastructure by ZPT must be caused by the correction of pathology occurring in the living epidermal layers. We consequently conclude that use of a ZPT shampoo has, directly or indirectly, treated the cause of dandruff and hereby normalized epithelial keratinization and/or sebum production and improved scalp health.

REFERENCES

1. Hay RJ, Graham-Brown RA. Dandruff and seborrhoeic dermatitis: causes and management. Clin Exp Dermatol 1997;22:3-6.
2. Malassez, L. Note sur le champignon du pityriasis simple. Arch Physiologie 1874;1:451.
3. Shuster S. The aetiology of dandruff and the mode of action of therapeutic agents. Br J Dermatol 1984;111:235-42.
4. Leyden JJ, McGinley KJ, Kligman AM. Role of microorganisms in dandruff. Arch Dermatol 1976;112:333-8.

5. Pierard-Franchimont C, Hermanns JF, Degreef H, Pierard G. From axioms to new insights into dandruff. Dermatology 2000;200: 93-8.
6. Gueho E, Boekhout T, Ashbee HR, Guillot J, Van Belkum A, Faergemann J. The role of Malassezia species in the ecology of human skin and as pathogens. Med Mycol 1998;36:220-9.
7. Saint-Leger D, Kligman AM, Stoudemayer TJ. The role of resident microflora in the pathogenesis of dandruff. J Soc Cosmet Chem 1989;40:109-17.
8. Gueho E, Leclerc MC, de Hoog GS, Dupont B. Molecular taxonomy and epidemiology of Blastomyces and Histoplasma species. Mycoses 1997;40:69-81.
9. Gueho E, Midgley G, Guillot J. The genus Malassezia with description of four new species. Antonie Van Leeuwenhoek 1996;69:337-55.
10. Boekhout T, Kamp M, Gueho E. Molecular typing of Malassezia species with PFGE and RAPD. Med Mycol 1998;36:365-72.
11. Makimura K, Tamura Y, Kudo M, Uchida K, Saito H, Yamaguchi H. Species identification and strain typing of Malassezia species stock strains and clinical isolates based on the DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. J Med Microbiol 2000;49:29-35.
12. Mayser P, Haze P, Papavassilis C, Pickel M, Gruender K, Gueho E. Differentiation of Malassezia species: selectivity of cremophor EL, castor oil and ricinoleic acid for M. furfur. Br J Dermatol 1997;137:208-13.
13. Kyle RL, Engman RF. Further studies on the relation of P. ovale to seborrheic exczma. Arch Dermat Syph 1938;37:289.
14. Marples RR, Kligman AM, Lantis LR, Downing DT. The role of the aerobic microflora in the genesis of fatty acids in human surface lipids. J Invest Dermatol 1970;55:173-8.
15. Troller JA. Model system for the investigation of dandruff. J Soc Cosmet Chem 1971;22:187-98.
16. Weary PE. Comedogenic potential of the lipid extract of Pityrosporum ovale. Arch Dermatol 1970;102:84-91.
17. Van Cutsem J, Van Gerven F, Fransen J, Schrooten P, Janssen PA. The in vitro antifungal activity of ketoconazole, zinc pyrithione, and selenium sulfide against Pityrosporum and their efficacy as a shampoo in the treatment of experimental pityrosporosis in guinea pigs. J Am Acad Dermatol 1990;22:993-8.
18. Ackerman AB, Kligman AM. Some observations on dandruff. J Soc Cosmet Chem 1969;20:81-101.
19. Orfanos CE, Happle R. Hair and hair diseases. 1st ed. Berlin: Springer-Verlag; 1989.
20. McOsker DE, Hannon DP. Ultrastructural studies of dandruff-involved scalp tissue. The Toilet Goods Association 1967;47:5-8.
21. Rawlings A, Watkinson A, Rogers J, Mayo A-M, Hope J, Scott IR. Abnormalities in stratum corneum structure, lipid composition, and desmosome degradation in soap-induced winter xerosis. J Soc Cosmet Chem 1994;45:203-20.
22. Hou SY, Mitra AK, White SH, Menon GK, Ghadially R, Elias PM. Membrane structures in normal and essential fatty acid-deficient stratum corneum: characterization by ruthenium tetroxide staining and x-ray diffraction. J Invest Dermatol 1991;96: 215-23.
23. Madison KC, Swartzendruber DC, Wertz PW, Downing DT. Presence of intact intercellular lipid lamellae in the upper layers of the stratum corneum. J Invest Dermatol 1987;88:714-8.
24. Warner RR, Boissy YL. Effect of moisturizing products on the structure of lipids in the outer stratum corneum of humans. In: Maibach H, Lodén M, editors. Dry skin and moisturizers: chemistry and function. Boca Raton (FL): CRC Press; 2000. p. 351-71.
25. Van Abbe NJ. The investigation of dandruff. J Soc Cosmetic Chem 1964;15:609-30.
26. Swartzendruber DC, Wertz PW, Kitko DJ, Madison KC, Downing DT. Molecular models of the intercellular lipid lamellae in mammalian stratum corneum. J Invest Dermatol 1989;92:251-7.
27. Fartasch M. Epidermal barrier in disorders of the skin. Microsc Res Technique 1997;38:361-72.
28. Bloom W, Fawcett DW. A textbook of histology. 9th ed. Philadelphia: Saunders; 1970.
29. Spoor HJ. Dandruff updated. Am Perfume Cosmet 1966;81:81.
30. Lavker RM, Leyden JJ. Lamellar inclusions in follicular horny cells: a new aspect of abnormal keratinization. J Ultrastruct Res 1979;69:362-70.
31. Brody I. Cytoplasmic components in the psoriatic horny layers with special reference to electron-microscopic findings. In: Montagna W, Lobitz W, editors. The epidermis. New York: Academic Press; 1964. p. 551-72.
32. Knutson DD. Ultrastructural observations in acne vulgaris: the normal sebaceous follicle and acne lesions. J Invest Dermatol 1974;62:288-307.
33. Matoltsy AG, Matoltsy MN. The membrane protein of horny cells. J Invest Dermatol 1966;46:127-9.
34. Plewig G, Kligman AM. Acne: morphogenesis and treatment. New York: Springer-Verlag; 1975. p. 94-5, 108.