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A Comparative Chemical Study of Dandruff Flakes, Skin Scrapings and Callus

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Synopsis—Comparative chemical analyses of callus, skin scrapings and dandruff flakes have been performed. Compared to normal skin scrapings, dandruff shows lower moisture binding ability, lower ninhydrin-positive material content and higher sulfhydryl and pentose contents. These results suggest that dandruff is associated with an increased rate of keratin formation.

Introduction

In recent years much work has been done in analyzing epidermis and different types of pathological skin scales for various chemical components. Much of this work has been directed toward finding abnormalities in the scales from various pathological skin conditions. In most cases in the literature, results from analysis of pathological scales are compared to results from either callus or epidermis obtained from normal subjects or at post-mortem. In addition, due to different methods of analysis, etc., it is often difficult to intercompare the results obtained by different investigators.

Dandruff (even though it appears in a large percentage of the population) can in some ways be considered a pathological condition. It was

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TABLE I

Ether Extractables and Water Uptake of Dandruff, Skin Scrapings and Callus

	% Ether Extractables	% H_2O Uptake
Dandruff	39 (30–50)[a]	11 (6–21)
Skin	10 (5–19)	21 (14–39)
Callus	3	16

[a] The figures in parentheses give the range of values obtained for 15 subjects.

therefore decided to study dandruff flakes to see if any chemical abnormalities occur which might help lead to a better understanding of the disease. It was further decided to compare the chemical composition of the dandruff flakes with the composition of skin scrapings obtained from the same subject. It was hoped that the latter might serve as a better control from which to assess abnormalities. Analyses of samples of pooled callus were also included to facilitate comparison with literature values.

Materials

Dandruff was collected from 15 male subjects by brushing the hair one week after the hair was washed with a mild shampoo. During the period in which the dandruff was collected, the subjects did not apply any preparations to their hair.

Skin scrapings were obtained by gently scraping the back of the hand with a dull razor blade. Using care, almost pure stratum corneum can be obtained in this manner. Before sampling, the hands were washed and thoroughly dried, and the hairs on the back of the hands were removed with a hair clipper.

Both dandruff scales and skin scrapings were stored in capped vials in a desiccator at 4°C until analyzed. They were used directly for this study without any further treatment (such as grinding). All samples were collected during the months of May and June.

Callus was obtained from a chiropodist who did not use any topical treatment on the callus before cutting it off. The callus was ground with dry ice, and the fraction which passes through a 125 mesh and was retained by a 200 mesh sieve was used for this study. The powdered callus was also stored in a desiccator.

Experimental and Results

The first point for determination was the lipid content of the samples. Weighed samples of keratin were extracted with three 3 ml. portions of

ether, allowing one-half hour for each extraction period. The ether was removed from the samples using a capillary syringe and the amount of ether extractables present in each sample calculated. The results are presented in Table I.

As can be seen in Table I, the lipid content of dandruff flakes was, as expected, considerably higher than that of skin or callus. This mainly arises from the fact that the lipid accumulates on the scalp while it is constantly washed off the hands. Also, the concentration of sebaceous glands is considerably higher on the scalp than on the back of the hands.

The ether extracted flakes were next thoroughly dried and then allowed to equilibrate in a constant relative humidity chamber at 81% RH. The water uptake for each sample was then calculated. The data obtained are included in Table I.

While these data present averages which only suggest a lower water binding capacity for dandruff flakes *vs.* skin scrapings, a look at the individual data for every subject shows that, in all 15 subjects tested, the

TABLE II

Ninhydrin-Positives as Per Cent Isoleucine in Dandruff, Skin Scrapings, and Callus

	Ninhydrin-Positives (As Per Cent Isoleucine)		
Subject	Dandruff	Skin	Δ
1	5.8	18.3	−12.5
2	13.3	19.0	− 5.7
3	11.2	19.6	− 8.4
4	6.7	22.0	−15.3
5	6.5	23.5	−17.0
Callus	...	10.4	...

water-binding capacity of their dandruff flakes was lower than that of their skin scrapings; this suggests that there is a real lowering of the water-binding capacity of dandruff keratin *vs.* skin keratin.

Since it was known that an important part of the water-binding capacity of skin resides in its water-soluble nitrogenous components, it was next decided to investigate this fraction.

To about 5 mg. of a fat-free sample in a vial, 3 ml. of water was added. The vial was then capped and placed on a mechanical shaker for twenty hours. After centrifugation, an aliquot of the clear supernatant was taken for analysis. The ninhydrin-positive material content of this aliquot was then determined, following the procedure of Rosen (1). A calibra-

tion curve was made using isoleucine as a standard, and the results were expressed as ninhydrin-positives in terms of per cent isoleucine. The results are presented in Table II.

Again, a difference is seen between dandruff and skin, the skin having more water-soluble ninhydrin-positive material than dandruff. This is true in spite of the fact that the hands are usually washed frequently, allowing for extraction of water soluble materials. This higher content of nitrogenous extractables may explain the higher water-binding ability seen in skin scrapings.

Sulfhydryl levels in the keratin were determined by a modification of the procedure described by Flesch and Khun (2). One mg. of finely pulverized Bennett's reagent dye was dissolved in 100 ml. of amyl acetate. One ml. of water and 2 ml. of Bennett's reagent were added to a vial which contained about 5 mg. of defatted keratin sample, and the vial was capped and shaken overnight. After centrifugation, 1 ml. aliquot of the Bennett's reagent was transferred to a test tube, and 0.5 ml. of concentrated HCl was added. After mixing and allowing to stand one hour the O.D. was read at 540 mμ. Glutathione was used to prepare the standard curve. The results are presented in Table III.

As can be seen, the sulfhydryl level of dandruff of each of the five subjects tested was higher than that of their skin.

Lastly, the water-soluble pentose levels were measured. Defatted keratin samples were extracted with water in a manner analogous to that used in extraction of ninhydrin-positive material. The aqueous extracts were concentrated to smaller volumes under reduced pressure, and the pentose content was determined by the orcinol method described by McRay and Slattery (3).*

Standard curves were prepared using ribose. The results are presented in Table IV.

Here again differences exist between skin and dandruff as determined by increased orcinol-reactive material in the dandruff scales. Attempts were made to chromatograph these extracts and to stain for sugars to see if any qualitative differences occurred; however, no pentoses or hexoses were detected in the chromatograms of the extracts of dandruff, skin or callus. A similar failure to detect free sugars via chromatography of the extracts of psoriatic scales has been reported by Wheatley

* In a recent paper by Berry and Warkany (5), it has been suggested that the water extractable material from skin responsible for the positive reactions with orcinol may not be pentose but a bound organic phosphate such as uridine diphosphoglucose. Such materials when reacted with orcinol give colors similar to that obtained with pentoses. In this paper, however, orcinol positive material will be considered as pentose.

TABLE III

Sulfhydryl Levels of Dandruff, Skin Scrapings and Callus

Subject	Sulfhydryl Concentration ($mM \times 10^{-2}$ per 100 g.)		
	Dandruff	Skin	Δ
6	41.8	30.6	+11.2
7	44.5	7.9	+36.6
8	20.0	7.0	+13.0
9	53.3	14.5	+38.8
10	20.9	20.0	+0.9
Callus	...	21.2	...

TABLE IV

Pentose Content of Dandruff, Skin Scrapings and Callus

Subject	Pentose Content (mg./100 g.)		
	Dandruff	Skin	Δ
11	242	96	+146
13	210	126	+84
14	261	92	+169
15	451	312	+139
Callus	...	129	...

and Farber (4). These authors concluded that the extracts contain interfering substances which, by forming some type of combination product, prevent the detection of ribose chromatographically.

Finally, chromatograms were run on aqueous extracts of skin and dandruff and sprayed with a xylose-aniline reagent (6). This reagent locates acids of the citric acid cycle (or Krebs Cycle) and various related organic acids. Both qualitative and quantitative differences appeared, suggesting a higher concentration of these acids in dandruff scales as compared to skin scrapings.

Discussion and Conclusions

In the course of these studies several differences have been uncovered between the chemical composition of skin scrapings and dandruff flakes. Of particular interest in this study is that analyses of dandruff flakes have been compared to skin scrapings obtained from the same subject.

Thus far, all of the abnormalities seen in dandruff flakes resemble those seen in other types of exfoliative dermatitis, especially psoriasis (which has been most investigated). Therefore, a closer examination of the causes for abnormalities in psoriatic lesions may also explain the results obtained with dandruff.

It seems clear that in psoriatic skin the rate of epidermal prolifer-

ation is greatly increased. Thus Rothberg, Crounse and Lee (7) have demonstrated that radioactive amino acids administered intravenously appear in normal stratum corneum in about 27 days, indicating that normal skin has a turnover time of about 27 days. In psoriatic skin, it took only three to four days for the C^{14}-amino acid to appear in radio labeled protein in the psoriatic skin.

Recently, Van Scott and Ekel (8) measured the mitotic level in normal skin and psoriatic skin. Their evidence suggested that in psoriasis there is increased epidermal proliferation (or epidermal hyperplasia) brought about by an expansion of the germinative cell population rather than by an increase in mitotic activity of a fixed population of germinative cells. Using their data they calculated the approximate replacement time for normal and psoriatic skin and obtained values approximately the same as those found by Rothberg.

This hyperplasia of the epidermis can serve to explain most of the chemical abnormalities seen in psoriatic as well as dandruff flakes. It has been reported (9) that the sulfhydryl content of successive layers of human skin increases with increasing depth. In hyperplasia, the increased keratin turnover results in the deeper layers of the skin emerging to the surface more rapidly. This would be expected to result in incomplete crosslinking and therefore a higher sulfhydryl content in the upper layers. This higher sulfhydryl content has been reported for psoriatic scales by many investigators (9–11). The increase in sulfhydryl levels seen in dandruff flakes is considerably lower than that found in psoriatic scales. The data presented, however, clearly suggest an increased level as compared to normal skin.

Similarly, it is believed that during the keratinization process the catabolism of some of the cellular proteins results in the accumulation of free amino acids in the stratum corneum (12, 13). Again, the increased keratin turnover in hyperplasia could easily result in a deficiency in the free amino acid content of the skin and a reduction in ninhydrin-positives content. Such a change might also explain the decreased water-binding capacity of the scales. This type of decrease in moisture binding and free amino nitrogen in aqueous extracts has been found in psoriatic scales (12).

Lastly, the increased pentose content seen in hyperplastic scales (4, 14) would also be expected from too rapid keratin turnover. During keratinization, the nuclear and cytoplasmic material from epidermal cells is broken down and metabolized. In psoriasis, the nuclear material is often not completely catabolized, as evidenced by the presence of occasional nucleated cells in psoriatic flakes. This incomplete break-

down would result in a high content of pentose (or perhaps it might be best to say, high content of orcinol-positive material).

The concentration of "pentoses" in the aqueous extract of dandruff flakes was found to be considerably higher than that found in skin scrapings. The pentose content of dandruff flakes has been previously reported by Bolliger and Gross (13), and the values reported herein are in general agreement with their data.

Further evidence for this type of incomplete metabolism of cellular material is seen in the higher amounts of citric acid cycle organic acids which appear in the chromatogram of dandruff *vs.* normal skin.

Thus, the evidence collected here suggests that dandruff is a form of epidermal hyperplasia involving an increase in epidermal turnover. Whether this increase is due to increased mitotic index or an expansion of the germinative layer is unknown. The evidence collected does not support the contention that dandruff is merely normal flaking which adheres to the scalp because of large amounts of sebum, or being held by the hair. In addition, the theory relating dandruff to incomplete breakdown of the cementing substances, if true, probably is also based on too rapid an epidermal turnover.

Finally, while this study points up the nature of the scaling phenomena in dandruff, it unfortunately sheds no new light on the causative factor.

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