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Nature of Cosmetic Films on the Skin

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Synopsis—A synthetic copy of the natural moisturizing factor present in human skin has been formulated on the basis of amino acid analysis. It is shown that this synthetic material has penetrating and moisturizing properties similar to those of the natural product. The porosity for water vapor of human sebum is attributed to its content of branched-chain compounds. It is shown in *in vivo* experiments that the addition of branched-chain aliphatic compounds enhances the penetration of water vapor through oily films applied to the skin.

Introduction

In the last two decades the question of moisture control of the human skin or, to be more specific, of the stratum corneum of the epidermis has been studied extensively by a number of researchers (1–6). A major result of this work was the finding that a combination of chemical compounds in the stratum corneum of the epidermis is responsible for the moisture control of this layer of the skin. This combination of compounds is generally known as "natural moisturizing factor" (NMF). The mechanism by which the NMF is formed by the skin is still unknown. Under normal conditions the skin produces enough NMF to insure the right amount of moisture for the stratum corneum. There are, however, conditions in which not enough or an incomplete NMF combination is produced by the skin, e.g., in psoriasis (7). Extensive washing of the skin or even long contact with plain water leaches

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the NMF from the stratum corneum and leaves the skin in a dry, scaly condition (8).

One of the tasks of cosmetic preparations is to keep the skin in a normal water-balance condition. Moisturizers have been tried for this purpose, but, as has been shown by several authors, the hygroscopic materials used in cosmetics (like glycerine, glycol, or certain sugar alcohols) are not able to fulfill this task (9–12). On the other hand, attempts have been made, based on the analysis of NMF published in the literature, to reproduce parts of the NMF. Apparently these attempts have not been satisfactory because it has been recommended repeatedly to use occlusive films on the skin to keep the moisture in the skin and to regulate the moisture requirements of the epidermis in this way (13–15). The skin exhibits the physiological phenomenon of the so-called insensible perspiration which is the invisible water vapor loss from the skin into the environment. In addition, a gas exchange through the skin takes place. Carbon dioxide is constantly released through the skin, and to a certain extent oxygen is absorbed. The oxygen uptake is part of the skin cell's metabolism. These combined functions, namely insensible perspiration, the carbon dioxide output, and the oxygen uptake are called "skin-breathing." These functions will automatically be suppressed by the application of an occlusive film on the skin surface. Therefore, this procedure of regulating the moisture content of the epidermis interferes with normal physiological functions of the skin, which certainly should not be the function of cosmetic treatments.

In this connection, the question whether the sebum, which is the natural protective wax film on the skin surface, is an occlusive film or not is of utmost interest. As will be shown below, the sebum film is not occlusive but, on the contrary, is porous. Similar results have been found in the case of certain surface waxes and fats of animals. Consequently, it seems that the function of sebum is not prevention of moisture loss. As a consequence, it seems that moisturizing of the epidermis has to be done differently, possibly in the way the skin does it. At the same time it appears necessary to formulate cosmetic preparations which leave porous films on the skin surface. The porosity of these films should be similar to that of human sebum.

This research group, which has worked on the NMF problem for many years, has repeated the analysis of the NMF of human skin. Based on this latest analysis, an NMF has successfully been synthesized. The efficacy of this synthetic NMF has been studied with respect to the moisture regulation of the skin. In cooperative research by Weitzel

(16, 17), the principle in sebum which makes it porous was found to be the presence of branched-chain aliphatic compounds. The development of synthetic branched-chain compounds similar to those found in sebum was undertaken. Formulations of cosmetic bases incorporating these compounds were applied to skin, and the porosity of these films on the skin was checked.

Experimental

Collection and Analysis of Natural NMF

The NMF of the stratum corneum was extracted from the skin of 35 healthy human subjects, ranging in age from 14 to 65 years of age. Areas on the forearms and the backs of these subjects were used. Open-end cylinders with diameters from 3–5 cm were pressed on the skin with gentle pressure. The cylinders were filled with ether and left in contact with the skin with occasional shaking for 5 minutes. The ether was removed, and after the skin was dry, ethanol was added to the cylinders and left in contact with the skin for 5 minutes, with occasional shaking. After the alcohol was removed and the skin was dry, distilled water was added to the cylinders and left in contact with the skin for 15 minutes with occasional shaking. The water extracts were removed from the skin, pooled, and immediately evaporated under high vacuum at room temperature. The residue was dissolved in water and chromatographed on a Dowex 50 column. A total of 200 fractions was collected. Each fraction consisted of 5.7 ml. For the first 50 fractions 0.3 *N* HCl, for the next 50 fractions 0.5 *N* HCl, for the next 50 fractions 1.0 *N* HCl, and for the last 50 fractions 2.0 *N* HCl were used. The content of each fraction was quantitatively determined and its chemical composition determined with paper chromatograms. Figure 1 shows the results of this analysis. This analysis served as the basis for the preparation of a synthetic NMF. In the following experiments this synthetic NMF was used.*

Efficacy of Synthetic vs. Natural NMF

The efficacy of synthetic NMF (trade name, Aqualizer E-J) has been determined in 171 single tests on living human skin.

Extraction of Skin—The sebum and natural moisturizing factor were extracted from the inner forearm or back of healthy human subjects.

* The use of this product as a moisturizing material in cosmetic preparations is covered by U. S. Patent 3,231,472, English Patent 1,004,774, and French Patent 1,329,616.

Adjacent areas on the same subject were extracted. Glass cylinders, open at both ends (4 cm diameter), were attached to the skin with rubber bands. Five cc of ether was poured into each cylinder and kept in contact with the skin for 2 minutes. The ether was then removed, and this extraction was repeated five times. After the ether extraction, the same area was extracted with distilled water. Five cc of water was placed in each cylinder and left in contact with the skin for 2 minutes.

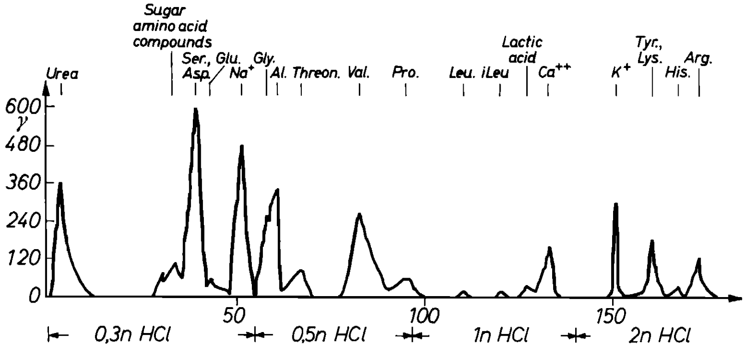

Figure 1. Analysis of NMF on Dowex 50. Column 7 × 700 mm; p = 1200 H_2O; sample weight = 320 mg; fraction volume = 5.7 ml.

The water was then removed and the skin dried with a warm air current for 2 minutes. This extraction with water and alternate drying was repeated ten times. The skin was then allowed to remain untouched for 30 minutes.

Reimpregnation—Next, a given amount of a 5% test solution (6 mg/ cm^2, on dry basis) was applied to one of the extracted areas and massaged in with a glass rod. The skin was then dried for 5 minutes with a warm air current (at 39°C) and for five additional minutes at room temperature. The application of test solution and massaging was repeated three times. After the third application of test solution, the skin area was blotted with filter paper to remove all excess test solution. This blotting was done before drying the skin for the third time.

Removal of Skin Cells—Ninety minutes after the reimpregnation, the stratum corneum of the skin was scraped off with a sharp scalpel or razor blade. To eliminate material that only clings to the surface, the outermost layer of cells scraped off was discarded. Skin cells from the adjacent extracted but not reimpregnated area and skin cells from untouched adjacent skin were also scraped off to furnish material for comparison. The three types of skin (untreated; extracted only; and reimpregnated and extracted) were collected in weighing bottles and dried in a vacuum desiccator over P_2O_5 to constant weight.

Determination of Moisture Uptake—The ability of the three types of skin scales to absorb moisture was determined by placing scrapings in a moisture chamber at 90% R.H. for 48 hours. The difference between the initial and final weight represents the moisture absorption.

Penetration—The question of penetration of Aqualizer E-J remains still unanswered. In other words, when Aqualizer E-J is applied to the skin does it only cling to the surface, or does it penetrate the stratum corneum? To answer this question, a microscopic method was developed which permits determination of the presence of Aqualizer E-J and of natural NMF by two different staining reactions. These staining reactions are called Reaction N and Reaction R. Reaction N is a ninhydrin reaction and is performed as follows:

With the aid of a needle some cell material is placed on a dry microscope slide. The stratum corneum cells are covered with a drop of ninhydrin reagent and immediately covered with a cover glass. The slide is heated for 30 seconds at 60°C and immediately observed under the microscope for blue to violet coloration of the cells. The reagent is prepared by dissolving 0.3 g of ninhydrin in 100 ml of butanol and adding 3 ml of glacial acetic acid.

Reaction R is a TTC (triphenyltetrazolium chloride) reaction and is performed as follows:

A small amount of cell material is placed on a dry microscopic slide, and two drops of the reagent are added. A cover glass is placed on top, and the slide is exposed to a temperature of 55–60°C for 3 minutes. The red coloration of the cells is observed under a microscope. The reagent is prepared by mixing one part of a 0.5% solution of TTC in distilled water with two parts of a 0.5 *N* NaOH solution. To this mixture one part of a 1% sodium lauryl sulfate solution in water is added. The solution is cloudy at first but clears on shaking. A very small precipitate settles out; the supernatant clear solution is used.

For these tests, the skin was treated in the same way as described above. The cell layers of the stratum corneum of the epidermis were scraped off, layer by layer, with a sharp scalpel. Each layer of cells was examined separately under the microscope for the presence of Aqualizer E-J or NMF to determine the penetration of Aqualizer E-J. For the evaluation of staining reactions the following scores were used:

No reaction	0
Slight reaction	1
Definite reaction	2
Strong reaction	3
Very strong reaction	4

The stronger the reaction the more Aqualizer E-J or NMF is present.

Effect of Sebum on Water Vapor Transmission

After the efficacy of the synthetic NMF was established, the effect of human sebum on the breathing of the skin was studied by measuring the insensible perspiration. For these studies the method of Gregory (18), which is basically an electrical hygrometer, was used. For the investigations described in this paper the following instrument was constructed. Two copper electrodes were melted into the bottom of a glass beaker having a diameter of 3 cm. A cotton thread, 5 cm long, saturated with KCl, is wound around these electrodes in the form of a figure eight. This saturated thread serves as a semiconductor. The open end of this glass cell is padded with rubber so that the edge of the cell may be pressed air tight against the skin. The cell is connected to a LF 39 WTM* conductance meter. Absorption of moisture changes the resistance of the cotton thread, which is measured on the instrument.

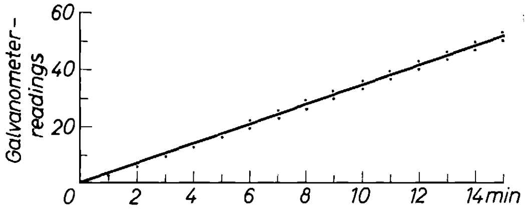

Figure 2. Sum of five measurements with a cell on a phantom during 15 minutes; temperature variations of water = ±0.2°C

Each cell is calibrated over a water surface serving as a model. As Fig. 2 shows, the deviations of one cell for five measurements during 15 minutes and at temperature changes of the water of ±0.2°C are relatively small. During these measurements it was proven that the galvanometer reading/time interval is the best usable measurement for the insensible perspiration. The resistance of the KCl saturated cotton thread changes, depending on the presence of humidity in the cell, and is related to moisture uptake or moisture loss.

The instrument also allows absolute measurements of the insensible perspiration. In this case the cell is calibrated as follows: The cell is connected with a flat cylindrical beaker having the same diameter as the cell. The beaker contains a weighed amount of water. After the galvanometer shows a certain reading, the beaker with water is removed

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from the cell and weighed. In this way different galvanometer readings can be related to the amount of water taken up by the cell, and a calibration curve can be prepared. From a calibration curve the water vapor given up into the cell by the insensible perspiration can be determined quantitatively. In the present investigation it was more important to determine the relation between the insensible perspiration and external factors than to determine its absolute amount. Accordingly the amounts of water vapor given up by the skin were not determined in absolute figures but in relative ones. As the measurement of insensible perspiration readings of the galvanometer movement in given time intervals were used; the results can be converted into absolute amounts by using a calibration curve for the cell.

Before the start of the test the subject remains in the test room for at least 30 minutes to 1 hour. Normally the forearms are used as the test area. The subject sits in a comfortable chair in front of a table and places one forearm on the table with the inner side of the forearm facing up. It is very important to make sure that the test subject is always sitting in a comfortable position during the test. For each measurement the room temperature and the relative humidity of test room are recorded. In many cases it is also important to determine the skin temperature of the adjacent skin.

First, the insensible perspiration of the untreated skin is determined during 5–15 minutes. After constant values have been obtained, the material to be tested for porosity is applied to the skin area, and then the insensible perspiration is again determined. After this determination, the material is removed by gentle wiping with cotton wool, and the insensible perspiration of the uncovered skin area is rechecked. To determine the porosity of a film on the skin surface for insensible perspiration, the insensible perspiration of the uncovered skin is taken as 100%. The insensible perspiration of the skin after application of the material to the skin is calculated as per cent of the water vapor release of the uncovered skin.

Human sebum was collected from several test subjects by extracting their skin with ether and ethanol.

Results and Discussion

Figure 1 represents the result of the analysis of the natural moisturizing factor extracted from living human skin as determined by column and paper chromatography. Based on this analysis a NMF was synthesized. The ability of stratum corneum to pick up moisture was studied on several specimens of living human skin: untreated stratum corneum; skin freed from NMF by extraction; and extracted skin reimpregnated with natural or synthetic NMF. The results (Table I) of the investigation of the moisture uptake of differently treated skin cells represent averages of 171 single measurements on a total of ten different human subjects ranging in age from 13 to 75 years. The results suggest that synthetic NMF (Aqualizer E-J) can replace natural NMF and restores the horny layer's moisture uptake completely.

The results (Table II) of the study of the penetration of synthetic NMF into the stratum corneum of the skin, using the ninhydrin (N) and TTC (R) tests, are based on the average values of 147 single measurements on a total of ten human subjects ranging in age from 13 to 75 years. Aqualizer E-J evidently penetrates deeply (Table II) into the stratum corneum of the skin. It reacts as strongly in the lowest tested horny layer of the extracted and reimpregnated skin as on the untreated skin.

If human sebum is applied to a skin area in an amount of 1 mg/cm^2, the measured insensible perspiration was 90 to 95% of that of the uncovered skin. Results of insensible prespiration measurements on skin covered with other fats are shown in Table III for comparison.

The application of some typical materials used in cosmetic preparations can decrease the insensible perspiration of the skin when applied in the amount of 1 mg/cm^2. If more of these materials were applied to the skin, the depression of the insensible perspiration would be expected to increase. On the other hand, the results of these experiments show that human sebum does not depress the insensible perspiration. What has been shown here for human sebum has been demonstrated by Weitzel and co-workers (16) for the preen gland oil of ducks. Weitzel and his group have studied the relationship between the porosity of the surface fats to their chemical composition. These researchers have found that the presence of branched-chain aliphatic compounds in surface fats is responsible for their porosity. Amongst the isolated aliphatic branched-chain compounds were acids, alcohols, esters of these, as well as esters of branched-chain compounds with straight-chain components. Recently Reinertson and Wheatley (19) and Nicolaides and Kellum (20) also found branched-chain compounds in human sebum. Table IV shows a few examples of results of determinations of insensible perspiration after application of 1 mg/cm^2 of different branched-chain compounds to the skin.

As can be seen, the films are porous and do not impede insensible

Table I
Moisture Uptake of Skin

Material	Moisture Uptake in 48 Hours at 90% R.H. (%)
Untreated skin	49.5
Extracted skin	19.2
Extracted skin reimpregnated with NMF extracted from human skin	26.0
Extracted skin reimpregnated with synthetic NMF (Aqualizer E-J)	44.5

Table II
Penetration of NMF into Stratum Corneum

	Layer of Stratum Corneum[a]							
	Second		Third		Fourth		Fifth	
Reaction	N	R	N	R	N	R	N	R
Untreated skin	1.5	0.7	2.4	1.3	2.7	1.3	2.7	2.1
Extracted skin	0.1	0.8	0.3	0.6	0.2	0.7	0.3	0.7
Extracted skin reimpregnated with skin extract	1.0	1.3	1.0	1.0	1.5	0.5	1.8	0.8
Extracted skin reimpregnated with Aqualizer E-J	2.5	1.8	2.8	2.2	2.8	2.2	3.0	2.5

[a] The first or outermost layer was discarded to eliminate material clinging to the skin surface.

Table III
Effect of Different Oils on Insensible Perspiration of the Living Human Skin

Materials[a]	Insensible Perspiration (%)
Uncovered skin	100
Skin covered with human sebum	95
Skin covered with petrolatum	37
Skin covered with Eucerin[b] (an absorption base)	61
Skin covered with 50% petrolatum and 50% lanolin	60
Skin covered with preen gland oil of ducks[c]	97

[a] Applied at the rate of 1 mg/cm^2.
[b] Beiersdorf & Co., A.G., Hamburg, Germany.
[c] Isolated from preen glands in the Biochemischen Institut, Universität Tübingen, Germany.

Table IV
Effect of Branched-Chain Aliphatic Compounds on Insensible Perspiration

Materials[a]	Invisible Water Vapor Release (%)[b]
10-Methyl stearic acid triglyceride	98
4-Methyl lauric acid triglyceride	99
2,7-Dimethyl-octadienol	100
2,7-Dimethyl-octanol	100
2,7-Dimethyl-octyl laurate	99
2,6-Dimethyl-octenyl laurate	98

[a] Applied at rate of 1 mg/cm² of skin.
[b] Normal skin value is 100.

Table V
Effect of Mixtures of Branched and Straight-Chain Aliphatic Compounds on Insensible Perspiration

Materials[a]	Branched-chain Compound Added	Compound Added (%)	Insensible Water Vapor Released from the Skin (%)
None	None	0	100
Petrolatum	None	0	38
Petrolatum	3,5-dimethylmyristic acid	10	62
Petrolatum	2,6-dimethyl-octyl palmitate	10	71
50% petrolatum plus 50% lanolin	None	0	62
50% petrolatum plus 50% lanolin	2,6-dimethyl-lauric acid	10	84
50% petrolatum plus 50% lanolin	2,6 dimethyl-octyl palmitate	10	82
50% petrolatum plus 50% lanolin	3,7-dimethyl-octyl-3,7-dimethyl octoate	10	84
50% petrolatum plus 50% lanolin	4-methyl-lauric acid triglyceride	25	94

[a] Applied at level of 1 mg/cm² of skin.

perspiration of the skin. If branched-chain compounds are added to cosmetic bases in amounts of 5–10%, the occlusiveness of these bases can be overcome. Films of these bases form porous films on the skin surface as shown by the results in Table V.* Because the complete composition of the skin's NMF was unknown and because so-called moisturizers (humectants) were apparently not effective enough, it has been recommended by several researchers to use occlusive fat films on the skin surface to prevent drying out of the stratum corneum. In this way it was hoped to help control the moisture content of the skin. As shown

* The use of branched-chain compounds in cosmetic preparations is covered by U. S. Patent 3,035,987.

in the foregoing experiments, human sebum forms a porous film on the skin surface. This porous film of sebum allows the skin to exchange moisture, carbon dioxide, and oxygen with the environment. Consequently the use of occlusive films as cosmetic products on the surface of the skin to prevent moisture loss appears to be a nonphysiological treatment because it potentially interferes with natural functions of the skin. In spite of its porosity, the sebum film serves as a protective cover for the skin. It protects the skin, among other things, against removal from the skin of NMF by excessive contact with water during swimming or bathing.

The experimental results presented here strongly suggest that synthetic NMF (based on the analysis of natural NMF) may be considered an effective moisture control. Since effective moisture regulation of the epidermis through use of a synthetic NMF seems feasible, the question of applying occlusive films to the skin surface appears less desirable.

Summary

It has been shown that the natural moisturizing factor of the human skin can be duplicated synthetically. Experiments have shown that this synthetic NMF is as effective as the natural moisturizing factor of the human skin. The use of synthetic NMF makes unnecessary use of occlusive films on the skin surface to prevent moisture loss.

It has been shown that the human sebum forms porous films on the skin surface and that this property depends on the content of branched-chain compounds in human sebum. The addition of aliphatic branched-chain compounds to cosmetic bases makes possible formulation of products forming porous films similar to those of human sebum on the skin.

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