# Fatty Acid Composition of the Living Layer and Stratum Corneum Lipids of Human Sole Skin Epidermis

M.N.A. ANSARI, N. NICOLAIDES and H.C. FU[1], University of Southern California School of Medicine, Los Angeles, California 90033


## ABSTRACT

Lipids from the living layer and stratum corneum of human sole epidermis were extracted, saponified and the fatty acids analyzed. The proportion of fatty acids to unsaponifiables (mainly cholesterol), was higher in the living layer than in the stratum corneum. Fatty acids of the living layer and stratum corneum of human sole epidermis comprise saturates, monoenes, dienes, traces of polyenes and α-hydroxy fatty acids. Homologs of monoenoic and dienoic fatty acids for both living layer and stratum corneum lipids have a similar distribution. $C_{16}$ and $C_{18}$ were major components for each type of acids. There appeared to be two clusters, especially for saturates of both living layer and stratum corneum acids. One of these clusters ranged from $C_{12}$ to $C_{20}$ with $C_{16}$ or $C_{18}$ as a maximum and the other ranged from $C_{21}$ to $C_{30}$ with $C_{24}$ as a maximum. The proportion of saturated acids with chain length $C_{20}$ and above was much higher in the stratum corneum than in the living layer. Position isomers of the monoenoic fatty acids for both the living layer and stratum corneum show a predominance of ω9 acids, due to the overwhelmingly large amount of oleic acid. Linoleic acid was by far the major component of the dienoic acids. Homolog distribution of α-hydroxy fatty acids for the living layer was similar to that of the stratum corneum and again two clusters of acids below and above $C_{20}$ with maxima at $C_{16}$ and $C_{24}$ were noticeable. Comparison of epidermal acids with those of sebaceous glands showed that each tissue can synthesize the same kind of acids but in widely different amounts. Oxidation of palmitate and stearate could supply the necessary energy for the late stages of keratinization.


## INTRODUCTION

In a process called keratinization, epidermal cells of human and animal skin undergo a degradation with the concomitant formation of a tough, protective protein called keratin. A major function of the lipids of these cells is to serve as membrane constituents. These lipids are synthesized by cells of the inner part of the epidermis (living layer), and are probably modified as the cells proceed outward into the stratum corneum (dead layer). Ultimately the end products of this process contribute to skin surface lipids.

Epidermal cells can also differentiate to form sebaceous glands. These glands produce sebum, an oily product made up of lipids with

[1]Present address: Calbiochem, Inc., 1840 N. Soto St., Los Angeles, Calif. 90032

TABLE I

Lipid Yield From Parts of Human Sole Epidermis

| Sample[a] | Fraction | Living layer | Stratum corneum |
|---|---|---|---|
| 1 | Crude lipid[b] | 100% (16 mg) | 100% ( 74 mg) |
| | Recovered lipid[c] | 71% | 80.5% |
| | Fatty acids | 50% | 33.9% |
| | Unsaponifiables | 50% | 66.1% |
| 2 | Crude lipid[b] | 100% (62 mg) | 100% (143 mg) |
| | Recovered lipid[c] | 65.5% | 82.6% |
| | Fatty acids | 44.3% | 29.2% |
| | Unsaponifiables | 55.7% | 70.8% |

[a]For identification of samples see Figure 1, footnote a.

[b]Total yield from $CHCl_3$/MeOH extracts, see Figure 1, footnote c.

[c]Total weight of fatty acids plus unsaponifiables divided by weight of crude lipid.

TABLE II

The Unsubstituted Fatty Acids of Living Layer and Stratum Corneum Lipids of Human Sole Epidermis[a]

| | Sample 1 | | | | | | Sample 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Living layer | | | Stratum corneum | | | Living layer | | | Stratum corneum | | |
| Carbon number[b] | Saturated, % | Monoene, % | Diene, % | Saturated, % | Monoene, % | Diene, % | Saturated, % | Monoene, % | Diene, % | Saturated, % | Monoene, % | Diene, % |
| 12 | Trace | | | .2 | | | .1 | | | .2 | | |
| 13 | .1 | | | Trace | | | Trace | | | .1 | | |
| 14 | 1.4 | .2 | | 1.1 | .1 | | 2.5 | .4 | | 1.9 | .5 | |
| 14.7 | .5 | ND[c] | | .5 | ND | | .2 | ND | | .7 | ND | |
| 15 | 3.7 | .4 | | 2.8 | Trace | | 4.8 | .3 | | 3.3 | .1 | |
| 15.6 | .9 | ND | | .4 | ND | | .2 | ND | | .4 | ND | |
| 16 | 40.6 | 6.3 | .4 | 24.6 | 7.8 | .1 | 48.6 | 7.3 | .3 | 25.1 | 7.7 | .2 |
| 16.5 | 1.1 | ND | | .8 | ND | | .7 | ND | | 1.0 | ND | |
| 16.7 | .5 | ND | | .3 | ND | | .5 | ND | | .5 | ND | |
| 17 | 3.7 | 1.6 | .1 | 3.6 | 1.2 | .1 | 3.4 | 1.2 | .1 | 4.0 | 1.8 | .3 |
| 17.6 | ND | ND | | Trace | ND | | ND | ND | | Trace | ND | |
| 18 | 34.8 | 86.0 | 96.7 | 18.6 | 83.6 | 97.6 | 33.9 | 86.2 | 96.7 | 19.3 | 80.0 | 96.1 |
| 18.7 | .5 | ND | | .1 | ND | | .2 | ND | | .2 | ND | |
| 19 | .4 | .4 | .1 | 2.0 | .2 | .1 | .6 | .4 | .1 | 2.8 | .5 | .2 |
| 20 | 1.2 | 2.4 | 2.5 | 3.9 | 2.4 | 2.0 | 1.0 | 2.5 | 2.7 | 4.8 | 2.9 | 3.1 |
| 21 | .3 | .3 | Trace | 1.8 | Trace | Trace | .1 | .1 | Trace | 2.3 | .1 | .1 |
| 22 | 2.9 | 1.0 | .1 | 7.5 | 1.4 | .1 | 1.2 | 1.0 | .1 | 7.8 | 1.8 | .2 |
| 23 | 1.0 | .1 | | 5.6 | .1 | | .2 | Trace | | 5.9 | .2 | |
| 24 | 3.7 | .7 | .1 | 14.0 | 2.0 | Trace | 1.0 | .5 | Trace | 12.1 | 2.9 | Trace |
| 25 | .6 | .1 | | 3.9 | .1 | | .1 | Trace | | 3.2 | .2 | |
| 26 | 1.4 | .2 | | 4.7 | .8 | Trace | .4 | .1 | Trace | 2.8 | 1.0 | Trace |
| 27 | .3 | .1 | | 1.2 | Trace | | .1 | Trace | | .6 | .1 | |
| 28 | .8 | .2 | | 1.6 | .2 | | .2 | Trace | | .7 | .5 | |
| 29 | .1 | Trace | | .6 | Trace | | Trace | Trace | | .1 | Trace | |
| 30 | .1 | Trace | | .2 | .1 | | Trace | Trace | | .2 | .4 | |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| $C_{20}$ and below | 89.8 | 97.3 | 99.8 | 58.9 | 95.3 | 99.9 | 96.7 | 98.3 | 99.9 | 64.3 | 93.5 | 99.7 |
| Above $C_{20}$ | 11.2 | 2.7 | .2 | 41.1 | 4.7 | .1 | 3.3 | 1.7 | .1 | 35.7 | 6.5 | .3 |

[a]For description of Sample 1 and 2 see Figure 1.

[b]Determined by the method of Woodford and Van Ghent (10).

[c]Not detected.

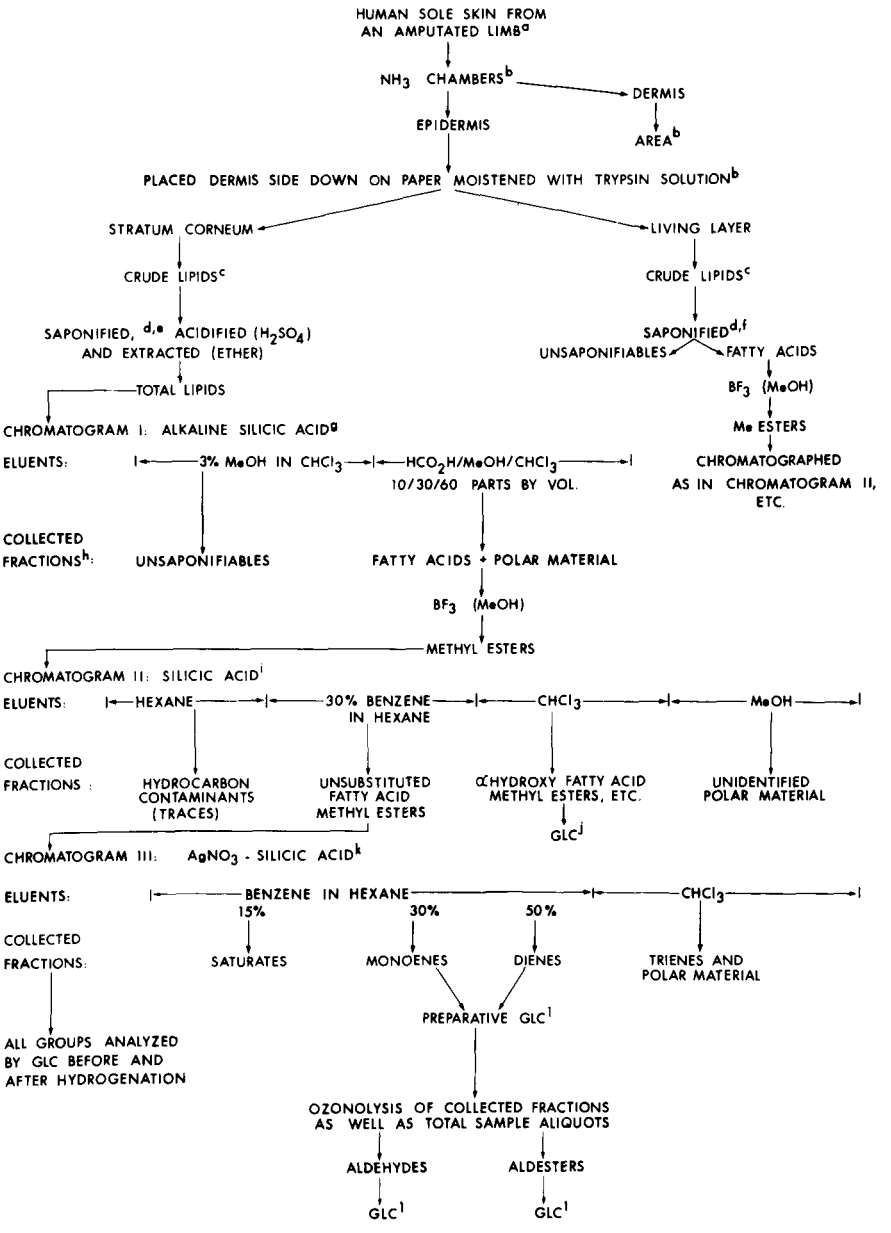



FIG. 1. Isolation and analysis of fatty acids from the living layer and stratum corneum of human sole epidermis.

[a]One sample (called Sample 1 in the Tables) from a patient with osteosarcoma of the right tibia, another (called Sample 2) from a patient with a tumor of the hip. Skin was removed from the bottom of the foot with care not to contaminate it with subcutaneous fat from the knife, wrapped in aluminum foil, then stored at -20 C until work up. Although the samples were primarily from the sole of the foot and are designated as sole skin throughout this paper, the samples included skin from the heel as well.

[b]Separation of epidermis from dermis after exposure to $NH_3$ fumes, determination of area, separation of living layer from stratum corneum by exposure of epidermis to trypsin, and extraction of lipids with $CHCl_3$/MeOH, 2/1 by vol were as previously described (3,4).

[c]The crude lipids were unwashed and therefore contained non-lipid material. Yields are in Table I.

[d]Saponifications were as in reference 5.

[e]For stratum corneum the unsaponifiable matter could not be separated from fatty acids by extraction with ether from the alkaline saponification mixture because of excessive emulsions, hence, the necessity of Chromatogram I.

[f]For living epidermis the unsaponifiable matter could be separated from fatty acids by ether extraction. Fatty acids were extracted after acidification in the normal manner. They contained small amounts of polar material removed by subsequent chromatography.

[g]As described in references 5 and 6.

[h]Completeness of separation of unsaponifiables from fatty acids verified by TLC. Plates of silica gel plain/$MgSiO_3$ 9/1 (Ref. 5) spotted then developed to $R_f$ = 0.5 with ether/$HA_c$, 99/1 then dried 10 min and redeveloped to $R_f$ = 1.0 with hexane/ether 95/5.

[i]Column 1 x 14 cm packed with 4.5 g silicic acid (Unisil 100-200 mesh Clarkson Chem. Co., Williamsport, Pa.). Loading factor 7 mg (or less) methyl esters per gram of Unisil.

[j]Besides TLC data these acids were identified as $\alpha$ hydroxy fatty acids by GLC as in reference 7.

[k]The adsorbent was made from 100 g of Unisil (100-200 mesh) added to a beaker containing 7 g $AgNO_3$ (Matheson Coleman and Bell, Rutherford, N.J.), dissolved in 100 ml water. The slurry was heated with gentle mixing until it was free flowing then dried in an oven at 115 C for 24 hr, cooled and while still warm transferred to a brown bottle and tightly stoppered. Just prior to packing the column, 5 g of the adsorbent was deactivated by adding 1 ml of water and mixing thoroughly in a small brown bottle. The adsorbent was packed into a column 1 x 14 cm i.d. as a slurry in hexane and the column protected against light with aluminum foil. Loading factor was 5 mg (or less) methyl esters per gram of adsorbent.

[l]As described in references 8 and 9.

many unusual features (1). Since the stratum corneum normally soaks up sebum, it is difficult to obtain epidermis free of sebum. However, if the skin sample is taken from either the palms or soles where sebaceous glands are absent, contamination with sebum is minimized. To obtain epidermis from skin it must first be separated from dermis, and there are a number of techniques for doing this (2). Recently a technique has also become available for separating the living epidermis from the stratum corneum (2,3). Thus, if these separations are applied to sole skin, the lipids synthesized by epidermal cells can be studied. This may help not only in understanding what lipids are contributed to the skin surface film by epidermal cells, but may assist us in understanding what role the lipids may have in the keratinization process itself. In this paper we report the fatty acid composition of the living epidermis and the stratum corneum of human sole skin with these objectives in mind.

## EXPERIMENTAL PROCEDURES

Figure 1 shows how we prepared the skin samples and obtained and analyzed the fatty acids.

## RESULTS AND DISCUSSION

Table I lists the yield of lipid from the two samples of human sole epidermis. Note that the weight of fatty acids plus unsaponifiables (i.e., recovered lipid) is appreciably less than the weight of total crude lipid. It is probable that some of this loss represents non-lipid material which dissolved in the aqueous phase and some the loss of the polar moieties of the polar lipids. We do not know how these losses are distributed. The relatively large amount of unsaponifiable matter, especially in the stratum corneum, is noteworthy. Thin layer chromatography (TLC) showed that most of this was cholesterol. The proportion of fatty acids in the stratum corneum is less than that of the living layer. Thus, if both fatty acids and unsaponifiables are synthesized in the lower part of the living layer, as appears likely, these data would indicate that some of the fatty acids were metabolized by the time the cells reached the stratum corneum.

Table II gives the homolog distribution of the saturated, monoenoic and dienoic acids from the living layer and stratum corneum lipids. Although $C_{16}$ and $C_{18}$ are major components for each type of acid, there appear to be two clusters, especially for the saturates of both the living layer and the stratum corneum acids. One of these clusters ranges from $C_{12}$ to $C_{20}$ with $C_{16}$ or $C_{18}$ as maximum and the other ranges from $C_{21}$ to $C_{30}$ with $C_{24}$ as maximum. These clusters are not unlike those found in the fatty acids of brain (11). The relative amounts of homologs in each of these clusters are totaled at the bottom of Table II. Homologs of the monoenoic and dienoic fatty acids of both stratum corneum and living layer lipids have a similar distribution, but stratum corneum has a much greater proportion of the saturated long chain acids than living layer has. These data are also consistent with the idea that some fatty acids (chiefly 16:0 and 18:0) are metabolized as cells pass from the living layer to the stratum corneum.

TABLE III

Relative Amounts of the Different Fatty Acids Types in Living Layer and Stratum Corneum Lipids of Human Sole Epidermis

| Fatty Acid Type[a] | Sample 1[b] | Sample 2 | |
|---|---|---|---|
| | Stratum corneum, % | Living layer, % | Stratum corneum, % |
| Saturated | | | |
| straight even | 38.4 | 37.3 | 35.2 |
| straight odd | 11.4 | 3.9 | 10.5 |
| branched | 1.2 | .8 | 1.3 |
| sub total | 51 | 42 | 47 |
| Monoene | | | |
| straight even | 30.5 | 39.2 | 32.9 |
| straight odd | .5 | .8 | 1.1 |
| branched | Trace | Trace | Trace |
| sub total | 31 | 40 | 34 |
| Diene | | | |
| straight even | 18.0 | 18.0 | 18.9 |
| straight odd | Trace | Trace | .1 |
| sub total | 18 | 18 | 19 |
| Total | 100 | 100 | 100 |

[a]The values of this Table were computed on the basis that the total amounts of saturates, monoenes and dienes isolated comprised 100% of the fatty acids, the further subdivision into straight even, straight odd and branched being calculated from the data of Table II. In actuality these fatty acid types represent approximately 80% of the total material isolated as fatty acids in the separation of saponifiable from unsaponifiable matter. Of the remaining 20% we estimate about one-fourth to be α hydroxy fatty acids. The remaining unidentified polar matter could include oxidized polyenoic acids.

[b]An unknown portion of the living layer acids of this sample was lost.

Table III summarizes the relative amounts of the different types of fatty acids for stratum corneum and living layer lipids. Note that stratum corneum has the greatest amount of odd and branched chain acids.

Table IV lists the homolog distribution of α hydroxy fatty acids for living layer and stratum corneum lipids. Note again the two clusters of acids above and below $C_{20}$ and that the stratum corneum of each sample has the greater proportion of acids above $C_{20}$.

Table V lists the position isomers of the fatty acid monoenes of living layer and stratum corneum lipids. Note that for 16:1 and 18:1 of both the living layer and stratum corneum, the Δ9 position isomer is by far the major isomer and that there is very little difference in the distribution of position isomers for all chain lengths of both living layer and stratum corneum lipids. Note also that because of the overwhelmingly large amount of 18:1 the extension pattern, as represented by ω9, is by far the predominant one. It thus appears that at least a part (if not all) of the oleic acid found in human skin surface lipid could be derived from the lipids of epidermal cells as earlier anticipated (12).

Table VI lists the position isomers of the dienoic fatty acids 18:2 and 20:2 for both living layer and for stratum corneum lipids. Although linoleic acid (18:Δ9,12) is by far the major component and presumably derived from

TABLE IV

α-Hydroxy Fatty Acids of the Living Layer and Stratum Corneum Lipids of Human Sole Epidermis

| α-Hydroxy fatty acids (No. C atoms) | Sample 1 | | Sample 2 | |
|---|---|---|---|---|
| | Living layer, % | Stratum corneum, % | Living layer, % | Stratum corneum, % |
| 14 | .1 | .2 | .1 | .2 |
| 15 | 2.1 | 2.2 | 3.0 | 6.0 |
| 16 | 43.2 | 27.8 | 58.8 | 44.0 |
| 17 | 8.9 | 3.2 | 10.3 | 7.1 |
| 18 | 12.6 | 10.6[a] | 17.2 | 18.4[a] |
| 19 | 1.4 | .2 | 1.0 | 1.2 |
| 20 | .6 | .8 | .5 | 1.0 |
| 21 | .1 | .1 | .1 | .1 |
| 22 | 1.7 | 2.0 | 1.8 | 2.3 |
| 23 | 3.9 | 2.9 | 1.0 | 1.7 |
| 24 | 14.2 | 23.1 | 4.4 | 7.0 |
| 25 | 3.4 | 11.1 | .3 | 3.3 |
| 26 | 7.8 | 15.8 | 1.5 | 6.7 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 |
| $C_{20}$ and below | 68.9 | 45.0 | 90.9 | 78.9 |
| Above $C_{20}$ | 31.1 | 55.0 | 9.1 | 21.1 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 |

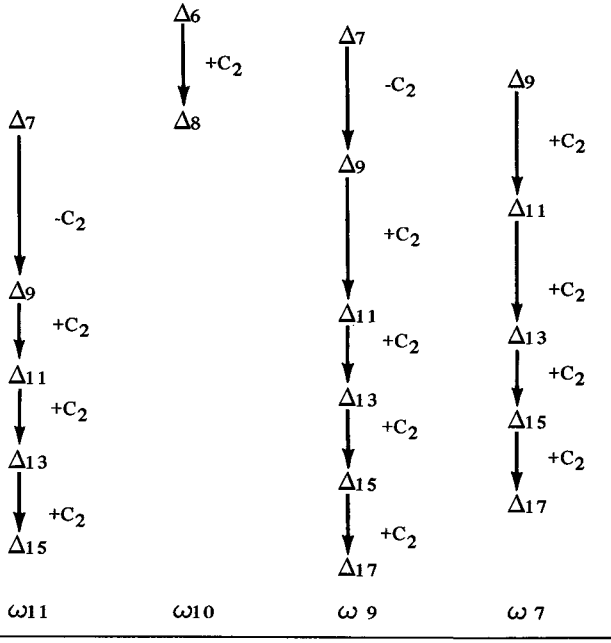
[a]Includes ~10% unsaturates.

TABLE V

Position Isomers of Fatty Acid Monoenes of Living Layer and Stratum Corneum Lipids of Human Sole Epidermis[a]

| Structures found[b] | Living layer: Per cent this chain length is of total monoenes | Living layer: Position isomer distribution, % | Stratum corneum: Per cent this chain length is of total monoenes | Stratum corneum: Position isomer distribution, % |
|---|---|---|---|---|
| 16:Δ6 | | 1 | | 1 |
| 16:Δ7 | 7.3 | 4 | 7.7 | 5 |
| 16:Δ8 | | 1 | | 1 |
| 16:Δ9 | | 94 | | 93 |
| 18:Δ7 | | 2 | | 2 |
| 18:Δ8 | | 1 | | 1 |
| 18:Δ9 | | 92 | | 93 |
| 18:Δ10 | 86.2 | 1 | 80.2 | 1 |
| 18:Δ11 | | 4 | | 3 |
| 18:Δ12 | | 1 | | Trace |
| 18:Δ13 | | Trace | | Trace |
| 20:Δ9 | | 20 | | 4 |
| 20:Δ11 | 2.5 | 70 | 2.9 | 93 |
| 20:Δ13 | | 10 | | 3 |
| 22:Δ11 | | 2 | | 2 |
| 22:Δ13 | | 90 | 1.8 | 95 |
| 22:Δ15 | | 8 | | 3 |
| 24:Δ13 | .5 | ND[c] | 2.9 | 5 |
| 24:Δ15 | | | | 85 |
| 24:Δ17 | | | | 10 |
| $C_{26}$:Δ15 | .1 | ND | 1.0 | 5 |
| $C_{26}$:Δ17 | | | | 95 |
| ω series | | | | |

Possible mode of formation




[a]Sample 2 of Table II.

[b]By reductive ozonolysis as shown in Figure 1.

[c]Not determined.

TABLE VI

Dienoic Fatty Acids of the Living Layer and Stratum Corneum Lipids of Human Sole Epidermis[a]

| Carbon number | | | | |
|---|---|---|---|---|
| Hydrogenated | Original | Deduced structures | Isomer distribution, % | Possible mode of formation |
| 18:00 | 18.52 | 18:Δ6,9 | Trace | 18:Δ9-2H → 18:Δ6,9 |
| | | 18:Δ8,11 | Trace | 18:Δ11-2H → 18:Δ8,11 |
| | | 18:Δ9,12 | 100 | Linoleic acid from diet |
| 20:00 | 20.50 | 20:Δ8,11 | 5 | 18:Δ9+$C_2$-2H → 20:Δ8,11 |
| | | 20:Δ11,14 | 95 | 18:Δ9,12+$C_2$ → 20:Δ11,14 |

[a]Both living layer and stratum corneum gave the same values.

the diet, the other isomers of the Table appear to be synthetic products of skin and can be explained by known patterns of desaturation and chain extension of monoenes. For living layer lipids traces of material were seen which corresponded to 18:3 on the basis of gas liquid chromatography (GLC) on polyester phase before and after hydrogenation.

Although Coon et al. have reported (13) that fatty acids between $C_{20}$ and $C_{30}$ occur in significant amounts in the free fatty acid fraction of barrier zone lipids of normal and psoriatic skin, the pattern of homologs found by them does not remotely resemble that found by us. Furthermore, in a later report (14) Wheatley et al. found, after extensive purification of their free fatty acid fractions, only traces of acids with chain lengths longer than $C_{20}$. They attributed the earlier findings of long chain material to contaminants and methylation artifacts.

In a study of labeled acetate incorporation into fatty acids of isolated epidermal cells grown in tissue culture, Wilkinson (15) found a distribution of the even chain homologs remarkably similar to that found by us for living layer lipids (Table II). We recalculated his results for direct comparison with ours (Table VII). These data suggest that the cells grown by this technique of tissue culture produce lipids very similar to those found in living human epidermis.

Since the epidermis is an avascular tissue it is dependent upon diffusion of nutrients from capillaries of the dermis. However, as cells leave the basal layer of the living epidermis on their way towards keratinization, available nutrients decrease. Furthermore, as keratinization proceeds, cell organelles undergo dissolution (16). Thus, the fatty acids released from the lipids of the membranes of these organelles could serve as a source of energy, and, indeed, there is other evidence that this does occur (17). The persistence of some mitochondria even in late stages of keratinization supports this concept (18). These mitochondria could perform the fatty acid oxidation and produce ATP necessary for the final stages of keratin synthesis.

Thus in summary, the data of this paper are

TABLE VII

Comparison of the Composition of Fatty Acids From Epidermal Cells Grown in Tissue Culture With Those From the Living Layer of Human Sole Epidermis

| | Fatty acids of epidermal cells grown in tissue culture[a] | | | Fatty acids of the living layer of human sole epidermis[b] | | |
|---|---|---|---|---|---|---|
| Carbon number | Saturates, % | Monoenes, % | Dienes, % | Saturates, % | Monoenes, % | Dienes, % |
| 14 | 2.4 | | | 2.2 | .3 | |
| 16 | 45.2 | 10.2 | | 51.0 | 6.9 | .4 |
| 18 | 33.6 | 84.2 | 98.1 | 39.4 | 88.4 | 96.8 |
| 20 | 2.6 | 2.8 | 1.9 | 1.3 | 2.6 | 2.6 |
| 22 | 4.4 | 2.8 | | 2.4 | 1.0 | .1 |
| 24 | 5.9 | | | 2.7 | .6 | .1 |
| 26 | 5.9 | | | 1.0 | .2 | |

[a]Data from Wilkinson (15).

[b]Data from Table II, but only the straight even numbers considered for comparison.

entirely consistent with the following interpretation. In the basal layer, blood glucose provides the primary source of energy and $C_2$ units for lipid synthesis required for the membranes of rapidly dividing cells. As keratinization proceeds in the upper portion of the living layer and the cell organelles are disrupted, the oxidation of palmitate and stearate occurs to supply the necessary ATP for the late stages of keratinization. Hence, the relative decrease of these acids in the stratum corneum as compared to those of the living layer. The longer chain fatty acids at about $C_{24}$, both straight and hydroxy (as reported here), may then be incorporated into the sphingolipids which, together with the free cholesterol, make a tight type of plasma membrane (16).

Since epidermal cells have the capability of either producing keratin or differentiating into sebaceous glands that produce sebum, it was of some interest to see whether the fatty acids of the living layer had any resemblance whatsoever to those of sebum. Sebum has significant amounts of a variety of fatty acids not ordinarily found in most living tissues (19,20), e.g., those with odd chain lengths (~10%), branched (mainly iso and anteiso, ~15%), and especially $\omega 10$ monoenes (~40%). Living layer produces ~5% odd, ~1% branched, and ~40% of monoenes predominantly $\omega 9$ but having a small trace of $\omega 10$ (Table III). Thus, in the main, living layer and sebaceous glands can make the same kinds of fatty acids, although they do so in widely differing amounts. Also, the odd and branched acids accumulate in the stratum corneum presumably because of the utilization of the biologically more valuable $C_{16}$ and $C_{18}$ acids, as noted above, leaving the less valuable odd and branched acids (as is also true in the case of sebum) to arrive at the surface lipid film. Although it has not yet been proved, it is quite conceivable that the microflora contacting the skin surface may have greater difficulty in metabolizing these acids than they would palmitic and stearic acid.

ACKNOWLEDGMENT

This work was supported by research grants AM-10010 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service; and by the Bireley Foundation, Los Angeles, California.

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[Received July 6, 1970]