Skin Lipids: Their Biochemical Uniqueness

Unlike internal organs, the skin biosynthesizes and excretes unusual fat soluble substances.

N. Nicolaides

The skin occupies a unique position in the family of tissues of the living organism; on one side it faces the atmosphere and an unpredictable environment, and on the other side it faces a cellular milieu and the circulating blood. The cells of the skin have adapted themselves to this role by developing a chemcial profile that is distinct to this organ. In this article I show that study of skin lipids as a class of chemical constituents of the skin offers a unique opportunity to investigate the functional specialization of this tissue.

Lipids, or fat soluble substances in general, are present in all cells. They subserve two major functions: as structural entities and as a means for storing energy. The major structural use of lipids is in membrane formation. Here phospholipids, sphingolipids, and the sterols play dominant roles. Their hydrophobic parts form flexible cell membranes that contain the cell's aqueous contents, thus delineating the cell from its environment, and also serve to organize the cell's internal structure into subcellular compartments or organelles. The hydrophobic property of lipids also offers the cell other structural devices. Hydrophobic substances provide relatively fixed points in the aqueous cytoplasm about which enzymatic sequences can be organized. They also provide a means to bring enzymes to the conformation necessary for them to carry out their functions. The long hydrocarbon chains of the triacyl glycerol lipids (triglycerides) offer the organism an efficient means of accomplishing energy storage, not only by providing a high yield of chemical energy from the oxidation of the many carbon and hydrogen atoms of the acyl groups, but also by providing heat to help maintain body temperature. The major portion of the organism's lipid synthesizing capability is geared to produce lipids for these two functions: structural entities and energy.

Animals do, however, produce lipids for external functions—that is, to be excreted externally, in contrast to the internal functions listed above. These lipids are synthesized in the skin, primarily in various types of sebaceous glands. The products of these glands are the "unique" lipids with which this paper is concerned. By contrasting the manner in which the excreted lipids differ structurally from those which are used internally one may gain clues to the understanding of the function of excreted lipids.

The Origin of Skin Lipids

Surface lipids can be extracted from the surface of the skin by either wiping it with fat-free cotton pledgets soaked in a solvent (hexane, ether, or acetone), or by momentarily soaking or otherwise exposing a portion of the body skin surface to solvent. Such lipids are easily obtained from human beings, and their composition has been extensively studied; consequently, they are suitable compounds with which to start in making comparisons with lipids of internal tissues. They are, however, of complex origin, and it will be profitable to consider anatomical and other elements that contribute to their composition.

Figure 1 is an artist's concept of human skin with the epidermis and its appendages separated in part from the underlying dermis. Most of the lipids of the skin surface come from the sebaceous glands, which excrete an oily, waxy material called sebum. Most of the remaining lipids come from the stratum corneum cells of the epidermis. The relative contribution of lipid from each of these sources depends upon the number of sebaceous glands present at the particular site sampled. Although sebaceous glands are found in most areas of the body, there can be as many as 900 glands per square centimeter on the scalp or face to less than 50 glands per square centimeter for the forearm. There are no sebaceous glands on either palms or soles. However, the amount of sebum on the skin surface cannot be correlated exactly with the number of glands present, since sebum flows from areas of high density to areas of low density through the stratum corneum (*1*). There are two types of sweat glands in skin: eccrine and apocrine glands. Eccrine glands are responsible for thermoregulation; the function of apocrine glands is not known. Eccrine sweat glands are widely and uniformly distributed over the body, but apocrine glands have only a limited distribution, being found chiefly in the axilla, the pubic area, the areola and nipple, and occasionally in other areas. It has not yet been established whether eccrine sweat glands, apocrine glands, hair, or microflora resident in the skin contribute substances to the surface lipids, although it is known that microflora do modify these lipids in a manner to be described. It is probable that a small amount of material originates from these sources.

Epidermal lipids free of sebum can be obtained by separating the epidermis from the dermis of palm or sole skin and extracting the lipids. The separation can be accomplished in a variety of ways. Exposure of the skin to heat, ammonia, or salt solutions will weaken the dermal-epidermal junction sufficiently to enable one to tease away the epidermis from the dermis in a manner similar to that seen in Fig. 1. One can further separate the "living layer" of the epidermis from the "dead layer"—that is, from the stratum corneum that lies above the living cells. This can be accomplished by placing epidermis, freshly separated from dermis, on filter paper moistened with trypsin solution. The cells of the living layer stick to the paper, but the stratum corneum

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remains as a sheet that can be lifted away.

Another type of sample we have found useful in these studies is vernix caseosa. This is the greasy matter that covers the newborn. It consists of fetal sebum and keratinized cells, thus it is a sample comparable to adult skin surface lipids in that it has components from both epidermis and sebum. It also has the virtue of being free from external contamination, from atmospheric oxidation, and from bacterial alteration. But fetal sebum is not identical to adult sebum, as I shall show.

Still other useful samples are the excretions of specialized types of sebaceous glands that occur in both man and animals. In man, for example, it appears that such glands exist at all points of possible entry to the body. At the edge of the eyelids there are very large types of sebaceous glands called meibomian glands; the ears have cerumenous glands; there are many large sebaceous glands at the opening of the nostrils; on the vermilion surface of the lip there are sebaceous glands that excrete directly onto the skin surface; in the buccal mucosa are sebaceous glands called Fordyce's glands; in the nipple are to be found Montgomery's glands; in the prepuce and in the anogenital areas of man and other animals there are many sebaceous glands, some of which may also serve as scent glands. Birds have a very large sebaceous gland at the base of the tail feathers called the preen gland which excretes a waxy material that is applied to their feathers in the act of preening.

Table 1. The lipid composition of various parts of adult human skin.

Component	Sebum* (%)	Epidermis† (%)	Surface lipids‡ (%)
Squalene	12	< 0.5	10
Sterol esters	< 1	10	2.5
Sterols (unesterified)	0	20	1.5
Wax esters	23	0	22
Triacyl glycerols	60§	10	25
Di- and monoacyl glycerols	0	10	10
Unesterified fatty acids	0	10	25
Glyco- and phospholipids	0	30	0
Unidentified	5	10	4

* Computed from thin-layer chromatography data of sebaceous gland lipids (*5*) and unpublished data (*12*). † Computed from Miettinen and Luukkäinen (*3*) and Nicolaides (*5*). ‡ Computed from (*5*). § Note that the sum of the tri-, di-, and monoacyl glycerols plus the unesterified fatty acids constitute 60 percent of the lipids for both sebum and surface lipids.

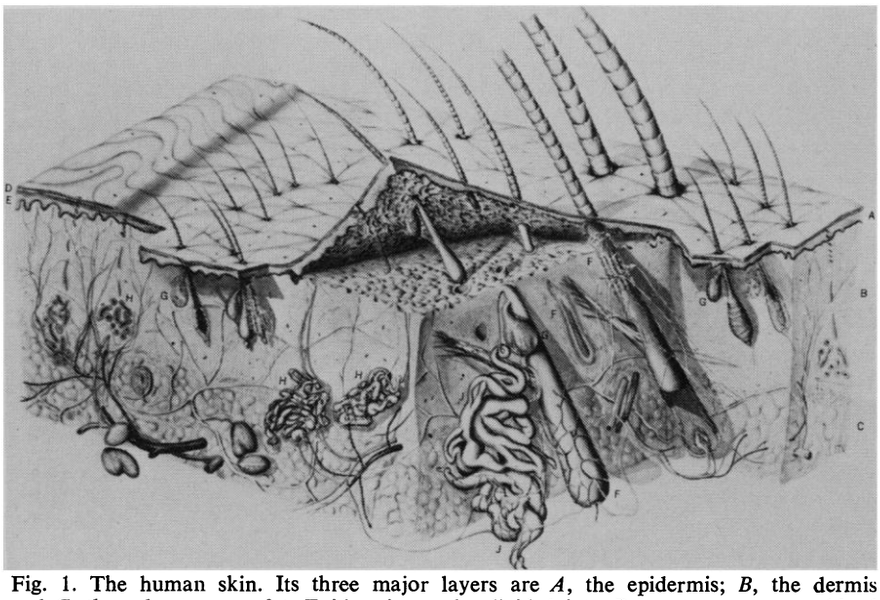


Fig. 1. The human skin. Its three major layers are *A*, the epidermis; *B*, the dermis and *C*, the subcutaneous fat. Epidermis can be divided into *D*, stratum corneum (dead layer); and *E*, living epidermis (living layer). Epidermal appendages are *F*, hair follicle; *G*, sebaceous glands; *H*, eccrine sweat glands; and *J*, apocrine sweat glands. Sebaceous glands excrete into the open space of the hair follicle from which the hair emerges. This space is open to the skin surface. [By courtesy of Dr. W. Montagna, Oregon Regional Primate Research Center, Beaverton, Oregon]

Unusual Lipids Occurring in Skin

Skin lipids manifest their uniqueness in a variety of ways. For example, during the synthesis of an internally valuable lipid, such as cholesterol, certain intermediate compounds accumulate in the skin. Squalene, a triterpenoid hydrocarbon containing 30 carbon atoms (C_{30}), is such an intermediate. Note that in human sebum, squalene is a major component (Table 1). Squalene does not normally occur in large concentrations in animal tissues except in shark liver. However, nearly all tissues synthesize squalene, but rapidly convert it to cholesterol. In adult human skin, squalene accumulates. In vernix caseosa, besides squalene, lathosterol, another intermediate, is present in relatively large amounts (*2*, *3*). In other animals, other intermediates formed during the synthesis of cholesterol accumulate. For example, lanolin, a product obtained from sheep sebum, has long been known to contain lanosterol, the first cyclized product formed from squalene on this pathway. In normal rat skin, in rat preputial gland (a large type of sebaceous gland), and in rat preputial gland tumors, many of these intermediates accumulate (*4*). This accumulation of intermediates is not confined to squalene and its cyclized products. In human skin, farnesol, a C_{15} sesquiterpene alcohol whose pyrophosphate ester is a precursor of squalene, also occurs in small amounts (*5*). A careful analysis of skin lipids might also reveal the presence of geraniol, the C_{10} monoterpene alcohol intermediate of cholesterol synthesis. It is because of this accumulation of intermediates that considerable progress was made in the early elucidation of the pathways of cholesterol synthesis (*4*); such accumulation of these intermediates does not normally occur in most internal tissues.

The skin not only accumulates unusual intermediates during the synthesis of what would be an internally valuable lipid, such as cholesterol, but it also synthesizes some unusual compounds, such as the wax esters, solely for external excretion. Wax esters serve as a means for storing energy in many aquatic animals (*6*). In the tissues of most land animals, however, they occur only in trace amounts except in the skin (*7*). Here they occur as monoesters and as diesters. In human sebum, wax monoesters are major components (Table 1). Other animals synthesize both mono- and diester waxes in significant amounts. There are at least two types of diesters (Fig. 2): type 1 is a diester of an α-

hydroxy fatty acid in which the α-hydroxy group is esterified with another fatty acid and the carboxyl group is esterified with a fatty alcohol. Type 2 is a diester of a long chain alkane diol where each OH group is esterified with a fatty acid (*8*). In addition, there are at least two kinds of alkane diols: 1,2-diols (*9*) and 2,3-diols (*10*). In human beings, diesters of 1,2-diols constitute about 3 percent of the lipids of vernix caseosa, and trace amounts occur in adult sebum. Human meibomian glands (whose ducts empty at the edge of the eyelid), excrete two types of diesters not yet characterized (*11*). In birds, the preen gland excretes diesters of alkane 2,3-diols as the major lipid, and in some birds, as the sole lipid.

The triacyl glycerols constitute 60 percent of the lipids of human sebum (Table 1). In the ducts of the sebaceous gland and on the skin surface they are hydrolyzed to a variable extent by lipases to form free (unesterified) fatty acids, mono- and diacyl glycerols, as well as free glycerol, a nonlipid (*12*). Unesterified, fatty acids do not normally occur in internal tissues because they are toxic. However, they do occur in blood, in the form of a complex with albumin. In sharp contrast, on the surface of the human skin, free fatty acids do occur, sometimes in very large amounts.

Interestingly, the triacyl glycerols and their products derived from hydrolysis occur only in the skin surface lipids of human beings. The skin surface lipids of most other animals, including some primates such as the chimpanzee and the baboon, contain little if any of these products. Animal surface lipids consist largely of mono- and diester waxes, sterol esters, and free sterols (*13*). These esters are apparently not hydrolyzed, because no free fatty acids have been found in the surface lipids of the animals examined thus far.

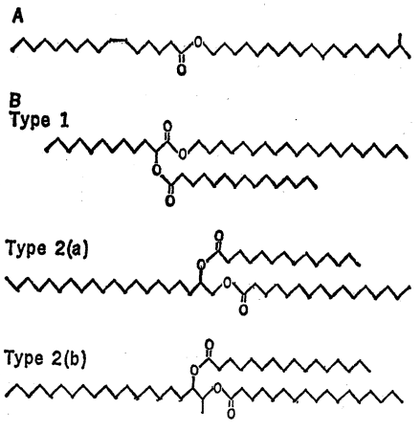


Fig. 2. Waxes occurring in the surface lipids of man and animals. (A) The monoesters are a group of long chain fatty acids esterified to a group of long chain fatty alcohols. (B) There are at least two types of diesters. Those of type 1 are α-hydroxy fatty acids esterified to another group of fatty alcohols and a third group of fatty acids. Those of type 2 may be either (a) 1,2-alkane diols esterified to two groups of fatty acids or (b), 2,3-alkane diols esterified to two groups of fatty acids.

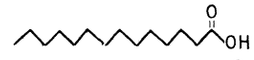
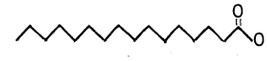
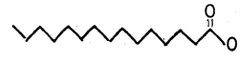
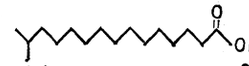
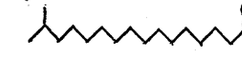
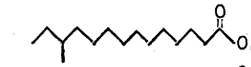
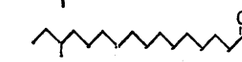
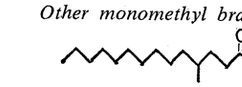
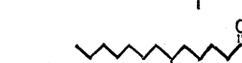
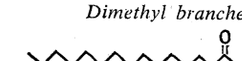
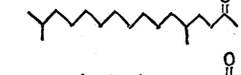
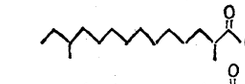
The uniqueness of skin lipids is expressed most fully in the types of fatty chains synthesized. Although human sebum consists mainly of triacyl glycerols, a commonly occurring class of internally valuable lipids, the acyl groups of sebum triacyl glycerols make them unique. This is also true of the acids and the alcohols that make up the wax esters. The uniqueness manifests itself in the number and kind of carbon skeletal types, in the extremely wide range of chain lengths, and in the unusual patterns of unsaturation. The resulting variety of acyl groups in skin lipids is exceeded in complexity by few, if any, other natural mixtures of lipids. Table 2 gives examples of the carbon skeletons of the types of fatty chains found in human sebum, and Table 3 gives the range of carbon chain lengths and quantitative data for each type of chain. Most internal tissues contain the normal saturated C_{14}, C_{16}, and C_{18} acids as well as oleic and linoleic acids. These acids also occur in human surface lipids (see Table 4). The total sum of these "biologically valuable" acids is 37 percent, but the remaining acids, some 200 species, are not normally encountered in internal tissues. Many of these acids, such as those with odd numbers of C-atoms, or those with the iso or anteiso structures do occur in internal tissues in very small amounts. They do not, however, make up the bulk of the fatty chains as they do in human skin surface lipid.

The branched acids (items 5 and 6 of Table 3) constitute a large number of compounds even though they make up but a small portion of the total fatty acids. These acids also occur in vernix caseosa, which makes it improbable that they are contaminants of human skin (*14*). In the preen gland lipids of many birds, not only do mono- and dimethyl branched acids occur, but fatty acids with three, four, and five methyl branches are sometimes major components (*15*).

Normally, internal tissues of animals, especially nervous tissue, synthesize some fatty acids to a length of C_{24} as components of the sphingolipids that form membranes. In skin, however, fatty chain lengths are extended far beyond C_{24}. Fatty acids are found in adult human skin surface lipids in decreasing amounts up to C_{30}. In vernix caseosa, in mouse skin, in rat skin, and in the skins of many other animals significant amounts of fatty acids with extremely long chains occur (*16–18*). In rat skin, for example, lengths up to C_{38} are detectable (*18*). The upper limit of chain length reported in studies of this type often depends not so much on what the investigator has found in the sample, but how long he or she has been willing to wait for another peak to emerge from the gas-liquid chromatographic column.

It is certain that the lower ends of the ranges of chain length listed in Table 3 do not represent the shortest

Table 2. Examples of the carbon skeletons of the acids occurring in human surface lipids.

Carbon skeletons	Abbreviated formula
Straight chains with an even *number of C-atoms*	
	C_{14}
	C_{16}
Straight chains with an odd *number of C-atoms*	
	C_{15}
	C_{17}
Iso chains	
	iso-C_{16}
	iso-C_{17}
Anteiso chains	
	anteiso-C_{15}
	anteiso-C_{17}
*Other monomethyl branched chains**	
	4-Me-C_{14}
	6-Me-C_{13}
Dimethyl branched chains	
	4,14-diMe-C_{15}
	2,12-diMe-C_{14}
	6,8-diMe-C_{12}

* These methyl branches appear only on the even numbered C-atoms, the C-atom of the carboxyl group being counted as 1.

chain lengths of each skeletal type actually present. The methyl esters of acids in the C_{10} range and below are quite volatile and will be lost in the usual techniques of fatty acid isolation and methyl ester preparation unless special precautions are taken to prevent this. The values listed are those we have obtained in our laboratory during experiments in which no special precautions were taken to avoid such losses. Weitkamp *et al.*, in their analysis of human hair fat, a sample similar to skin surface lipid, reported the presence of acids with chain lengths as short as C_7 (*19*). It is also very probable that the components responsible for the offensive odor of bromhidritic feet are acids in the C_4 and C_5 range. The point of this discussion is not to establish exactly the range of the chain lengths but to note that it is extremely wide, extending from very low to very high, such a range being a unique feature of the human surface lipids.

Before I discuss the unsaturated acids, which also have a number of features that are unique to the skin lipids, some discussion of methods and definition of terms and symbols is in order. The position of a double bond in a monoene fatty chain can be conveniently determined by reductive ozonolysis. In this procedure the molecule is split at the double bond, and the number of C-atoms in each fragment indicates the position of the double bond. If the double bond of a fatty acid is between the ninth and the tenth C-atoms, the C-atom of the carboxyl group being counted as number 1, this position is indicated as Δ9. If the acid has 18 C-atoms, the abbreviated formula or structure is shown as $C_{18:1\Delta9}$ or, more simply, as $C_{18:\Delta9}$ (see Table 4). The formula $C_{18:2\Delta9,12}$ indicates a fatty acid with 18 C-atoms and two double bonds whose positions are between the ninth and tenth C-atoms and the 12th and 13th C-atoms. Many acids are biosynthesized from others by extending the chain at the carboxyl group in units of two C-atoms. Thus if $C_{16:\Delta9}$ were extended successively by several C_2 units one would get $C_{18:\Delta11}$, $C_{20:\Delta13}$, $C_{22:\Delta15}$, and so on. A group of such acids which differ from each other in structure only by an integral number of C_2 units constitutes what I am here calling a family. The family is named after the member considered to be the one from which the others are derived. The members of each family have the same number of C-atoms from the methyl end of the chain to the double bond. All acids of human surface lipid are considered to have the *cis* configuration unless otherwise indicated.

The presence of double bonds at Δ6 in the monoenoic fatty acids of human skin surface lipid is a striking feature (*19, 20*). In most naturally occurring monoenoic acids the double bond position is at Δ9. Monoenoic acids with the Δ6 double bond are extremely rare, occurring only in human skin, the sebaceous tissues of some other animals, and the seed fats of the parsley family and some other rare plants (*21*).

The commonly occurring Δ9 double bond is present in appreciable amounts in vernix caseosa lipid (*16, 22*) and to a small extent in adult human skin surface lipid. But the Δ9 double bond does not occur to any great extent on a C_{18} chain to form the major monoenoic acid of the biological world, namely, oleic acid. Instead, it occurs mainly on C_{16} chains to form palmitoleic acid ($C_{16:\Delta9}$) and its extension products. Although palmitoleic acid does occur in many internal tissues, it is usually a minor component. Its extension products are even more rare. In vernix caseosa, however, it appears that a very large proportion of palmitoleate chains are extended to $C_{18:1\Delta11}$, $C_{20:1\Delta13}$, $C_{22:1\Delta15}$, $C_{24:1\Delta17}$, $C_{26:1\Delta19}$, and $C_{28:1\Delta21}$ and beyond, since these acids occur in large amounts. Thus there is present a family of very long chain unsaturated acids all with double bonds between the seventh and eighth C-atoms from the methyl end of the chain, an unusual occurrence.

The $C_{16:1\Delta9}$ and the $C_{16:1\Delta6}$ families make up the largest portion of fatty acid monoenes of vernix caseosa and of human skin surface lipid. But many other families are also present in both lipid samples (Fig. 3). Not only do the straight even chains form families, but so do straight odd chains, iso chains, and anteiso chains. In fact

Table 3. Carbon skeletal types of the acids of human surface lipids. Structures are shown in Table 2. Data from (*11*).

Carbon skeletal type	Saturates		Monoenes		Dienes	
	Range	%	Range	%	Range	%
1. Straight chain*	C_{10} to C_{30}	35.8	C_{14} to C_{24}	36.4	C_{16} to C_{22}	2.8
2. Straight chain†	C_{11} to C_{27}	5.4	C_{15} to C_{25}	3.9	C_{17} to C_{19}	0.1
3. Iso	C_{12} to C_{28}	4.0	C_{14} to C_{26}	5.3		
4. Anteiso	C_{11} to C_{27}	1.4	C_{15} to C_{25}	1.3		
5. Monomethyl branched	C_{11} to C_{26}	2.6	C_{17} to C_{25}	0.2		
6. Dimethyl branched‡	C_{13} to C_{24}	0.8	?			
Totals		50.0		47.1		2.9

* Straight chains with even numbers of C-atoms. † Straight chains with odd numbers of C-atoms. ‡ Very small amounts of trimethyl branched acids are also present.

Table 4. Twenty-one of the fatty acids of human skin surface lipids. Each of the fatty acids listed constitutes at least 0.5 percent of the total.

Name	Abbreviated formula	Amount (%)
Palmitic*	n-C_{16}	25.33
cis-Hexadec-6-enoic	$C_{16:1\Delta6}$	21.70
cis-Octadec-8-enoic	$C_{18:1\Delta8}$	8.75
Myristic*	n-C_{14}	6.88
cis-15-Methylpentadec-6-enoic	iso-$C_{16:1\Delta6}$	3.96
Pentadecanoic	n-C_{15}	3.95
Stearic*	n-C_{18}	2.89
cis-Octadec-6-enoic (petroselenic)	$C_{18:1\Delta6}$	1.87
Oleic*	$C_{18:1\Delta9}$	1.87
cis-Heptadec-6-enoic	$C_{17:1\Delta6}$	1.31
12-Methyltetradecanoic	anteiso-C_{15}	1.13
Octadeca-5,8-dienoic (sebaleic)	$C_{18:2\Delta5,8}$	1.12
cis-Tetradec-6-enoic	$C_{14:1\Delta6}$	1.06
Heptadecanoic	n-C_{17}	1.06
cis-Heptadec-8-enoic	$C_{17:1\Delta8}$	0.82
cis-14-Methylhexadec-6-enoic	anteiso-$C_{17:1\Delta6}$	0.81
cis-16-Methylheptadec-8-enoic	iso-$C_{18:1\Delta8}$	0.78
4-Methyltetradecanoic	4-Me-C_{14}	0.70
Linoleic*	$C_{18:2\Delta9,12}$	0.53
cis-Eicos-10-enoic	$C_{20:1\Delta10}$	0.52
cis-Eicos-7,10-dienoic	$C_{20:2\Delta7,10}$	0.51
Total†		87.55

* "Biologically valuable" acids (total, 37 percent). † Some 200 additional acids make up the remaining 12.45 percent.

every chain length from each skeletal type of saturated fatty acid which undergoes either Δ6 or Δ9 desaturation can form a family. These families include not only elongation products but degradation products as well, giving rise to a very large number of fatty acid monoenes. From the fatty acids of wax esters of vernix caseosa, we have been able to identify no less than 24 families representing 54 different molecular species. The sterol ester fatty acids yielded 28 families and 124 molecular species. Human surface lipids showed a similar array of acids for these lipid classes (*23*). These are, indeed, unique features as far as the composition of fatty acids in natural mixtures is concerned. They are unique with regard to where the position of unsaturation occurs in the fatty chain; they are unique with regard to the number of skeletal types of fatty chains in which these types of unsaturation occur; they are unique with regard to the lengths of chains in which the unsaturation occurs—that is, the degree to which the chains are extended; and, finally, they are unique in that so many different fatty acids are made.

The unusual fatty acids discussed above are products of sebaceous glands; however, the epidermis contributes some unusual fatty acids to the skin surface by a different method. This became apparent when we examined the fatty acids of the living layer and stratum corneum obtained from sole skin. At least 80 percent of the acids of the living epidermis were of the "biologically valuable" type, whereas in stratum corneum these were reduced to about 60 percent. Of the acids remaining in the stratum corneum, many had odd numbers of C-atoms, were branched, and were longer than C_{20} (*24*). One can rationalize these results as follows: While epidermal cells are still viable and actively synthesizing keratin, the fatty acids produced or obtained from the diet are of the type used internally—that is, they are biologically valuable. In late stages of keratinization, when the epidermal cells are somewhat removed from the dermis, which is their source of nutrients, the subcellular membranes begin to disintegrate, and the biologically valuable fatty acids that make up these membranes are oxidized by the remaining mitochondria to yield the adenosine triphosphate required to complete the keratinization process. The leftover fatty acids then make their small contribution to the surface lipid.

Skin Lipid Biochemistry

In this section I point out in somewhat greater detail than previously the manner in which skin modifies normal biochemical processes to synthesize its unusual lipids. I shall begin with the types of fatty chains listed in Table 3. Normally, fatty chains are built up by a system of enzymes called the fatty acid synthetases. The process is initiated by a starter which is a derivative of coenzyme A (CoA). The hydrocarbon chain is built up by adding to the starter CoA derivative a number of C_2 units. These C_2 units are derived from malonyl-CoA. The carbonyl (CO) group to which the C_2 unit is added remains as such in the lengthened chain. This carbonyl group is then reduced to CH_2 by a cycle of four reactions before the next C_2 unit is added (Fig. 4A). Thus, palmitate is formed when seven C_2 units are added to acetyl-CoA, the starter. Had either six or eight C_2 units been added to acetyl-CoA instead of seven, then myristic (C_{14}) or stearic acid (C_{18}) would have resulted. Note that since the acetyl group of the starter has an even number of carbon atoms, a fatty chain with an even number of C-atoms is produced.

Chains with an even number of C-atoms are the major products of the fatty acid synthetase system of most internal tissues. In skin, however, a variety of other types of chains are also built up when different starters are used (Table 5). For example, if the starter is propionyl-CoA a chain with an odd number of C-atoms results. Chains with odd numbers of C-atoms can also be formed by decarboxylation of chains with even numbers of C-atoms after they have been α-hydroxylated (*25*). Since propionyl-CoA can form or be formed from methylmalonyl-CoA (*26*), which is needed in the synthesis of some of the

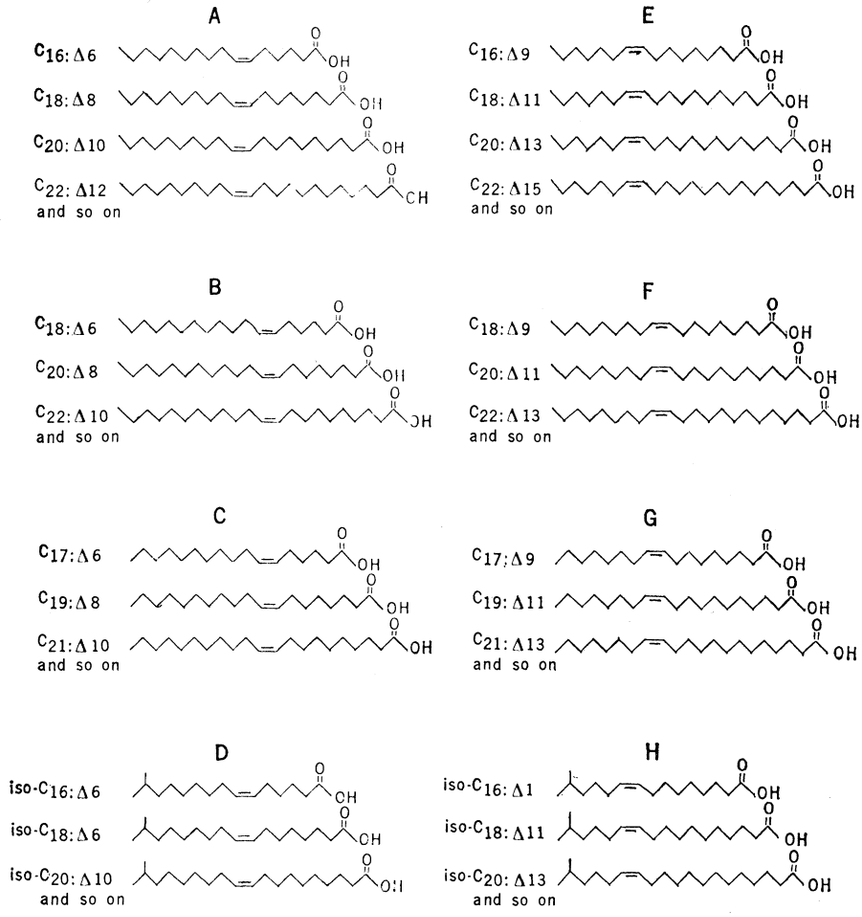


Fig. 3. Some families of fatty acids occurring in sebum. Acids within a family differ from each other in structure only by an integral number of C_2 units. The family is named after the member considered to be the one from which the others are derived. On the left are Δ6 families. (A) The $C_{16:\Delta6}$ family. (B) The $C_{18:\Delta6}$ family. (C) The $C_{17:\Delta6}$ family. (D) The iso-$C_{16:\Delta6}$ family. On the right are Δ9 families. (E) The $C_{16:\Delta9}$ family. (F) The $C_{18:\Delta9}$ family. (G) The $C_{17:\Delta9}$ family. (H) The iso-$C_{16:\Delta9}$ family. [Courtesy W. Montagna and W. C. Lobitz, Jr.; © Academic Press]

branched products found in skin, it would seem biologically more efficient to synthesize odd chains from propionyl-CoA. Iso and anteiso fatty chains are formed from starters that originate, respectively, from the amino acids valine and isoleucine. These amino acids first undergo a transamination reaction to form keto acids. The keto acids are then oxidatively decarboxylated to form, respectively, isobutyryl-CoA and α-methylbutyryl-CoA (*27*), which serve as starters of the chain building process (Fig. 4). A small amount of the iso acids with an *odd* total number of C-atoms, found in vernix caseosa, could arise from isovaleryl-CoA as the starter derivative which in turn could be formed analogously from leucine (*14*). Thus, by varying the starter the skin can synthesize most of the types of carbon skeletons found in its fatty chains.

Biochemically, a methyl branch can be placed throughout a fatty chain by substituting a molecule of methylmalonyl-CoA for malonyl-CoA. The branched acids of the preen glands of the goose are known to utilize this pathway (*28*). In the buildup of 4-methylhexadecanoic acid, for example, acetyl-CoA would react successively with five molecules of malonyl-CoA, utilizing five cycles of the fatty acid synthetase system (Fig. 5). But on the sixth cycle, a molecule of methylmalonyl-CoA would be used. On the seventh cycle a molecule of malonyl-CoA would again be used. If substitution of methylmalonyl-CoA for malonyl-CoA occurred in the fifth cycle instead of the sixth, then the product would be 6-methylhexadecanoic acid. This process always places a methyl branch on an even-numbered C-atom from the carboxyl group of the fatty acid. It is precisely on the even-numbered C-atoms that these extra methyl branches have been found even though we made intensive efforts to look for methyl groups on the odd-numbered C-atoms. If the starter acid were propionyl-CoA, the main chain would then have an odd number of C-atoms, but the methyl branch would still appear on the even-numbered C-atoms (*14*). Maximum amounts of methyl branching appear on the fourth C-atom for all chain lengths. The significance of this is not known.

Table 5. Examples of starters (derivatives of CoA) that initiate the formation of fatty chains in the skin.

Starter	Type of fatty acid formed
$CH_3-C-CoA$ (Acetyl-CoA)	An acid with an even number of C-atoms
$CH_3-CH_2-C-CoA$ (Propionyl-CoA)	An acid with an odd number of C-atoms
$CH_3-CH(CH_3)-C-CoA$ (Isobutyryl-CoA)	An iso acid with an even number of C-atoms
$CH_3-CH(CH_3)-CH_2-C-CoA$ (Isovaleryl-CoA)	An iso acid with an odd number of C-atoms
$CH_3-CH_2-CH(CH_3)-C-CoA$ (α-Methylbutyryl-CoA)	An anteiso acid with an odd number of C-atoms

Dimethyl branched acids can be made by inserting two molecules of methylmalonyl-CoA into the fatty chain. One of the two branches could also be provided by the starter moiety (Table 5). Very small amounts of trimethyl branching have been found, which could be formed similarly, and it appears that methyl chain branching other than iso or anteiso occurs on chain lengths up to C_{24} and beyond (*11*). Thus it is apparent that a large number of compounds can be made by utilizing an occasional methylmalonyl-CoA in the buildup of fatty chains.

The desaturation processes occurring in skin also exhibit some unusual biochemistry. It was pointed out above that few tissues can insert a double bond into a saturated fatty chain at the $\Delta 6$ position. If a double bond preexists at $\Delta 9$, however, a new double bond can be added in the $\Delta 6$ position. In the biosynthesis of arachidonic acid, for example, an early step is desaturation of linoleic acid at $\Delta 6$ to form *cis*-6,9,12-octadecatrienoic acid. Many internal tissues have the capability of inserting a double bond at $\Delta 6$, but only when there already exists *cis* unsaturation at $\Delta 9$. Indeed, Brenner (*29*) has proposed a model for what he calls a 6-olefinase where substrate binding to the enzyme at the *cis*-$\Delta 9$ double bond brings the chain to the $\Delta 6$ desaturating site of the enzyme. If the preexisting double bond is at $\Delta 8$, the new double bond forms at $\Delta 5$; if at $\Delta 7$, the new one forms at $\Delta 4$. The new double bond will always be placed in the chain on the carboxyl side of the existing double bond and will be separated from it by a CH_2 group. Thus, in these desaturations the fact that the new double bond landed at the $\Delta 6$ position is simply a consequence of the existing *cis*-double bond having been at $\Delta 9$, a highly favored position of the unsaturated fatty acids of most internal tissues.

Little is known about the skin enzyme (or enzymes) capable of forming monoenoic acids with *cis*-$\Delta 6$ unsaturation; however, we can already define some of its properties. The enzyme must be capable of recognizing the distance from the carboxyl group to the sixth C-atom for a variety of saturated chain types. Apparently it does not matter what the structure is on the terminal part of the chain just so long as there are six free C-atoms on the carboxyl side.

The unique diene, sebaleic acid (*cis*-5,8-octadecadienoic acid) occurs nowhere in nature, to the best of our knowledge, except in the surface lipid of human skin (*30*), its name reflecting

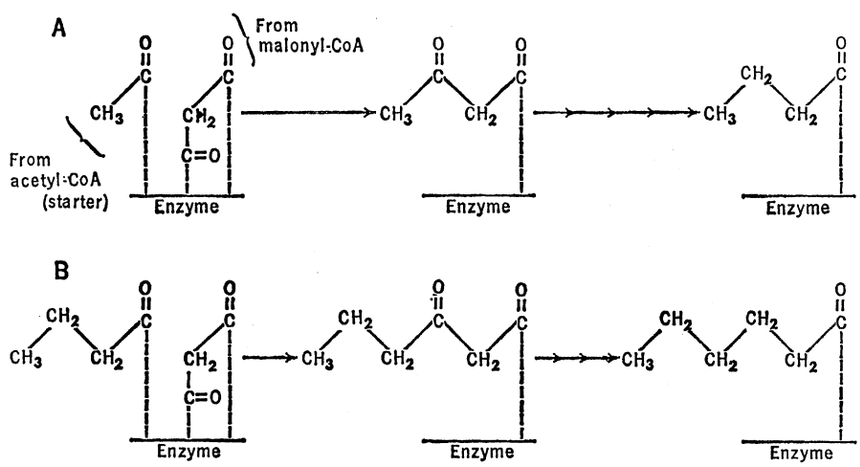


Fig. 4. Biosynthesis of the fatty chains of sebum. (A) The first cycle of the buildup of a fatty chain; (B) the second cycle. Further cycles add additional C_2 units to build the chain to its final length.

its origin from sebum. Its biosynthesis can easily be interpreted in terms of the above processes: The major monoene $C_{16:1\Delta6}$ is first formed by whatever processes skin uses to accomplish this *cis* desaturation, then it is extended by a C_2 unit to $C_{18:1\Delta8}$, and finally desaturated in the manner described above for acids with existing *cis* unsaturation to form $C_{18:2\Delta5,8}$.

Fatty acid chains are elongated biologically by the addition of C_2 units to the carbonyl group of the acid in a manner somewhat analogous to the original buildup of the fatty chain. For example, the $C_{16:\Delta6}$ family could be formed if palmitic acid were first desaturated at $\Delta6$ then elongated in C_2 units. Although the $C_{16:\Delta6}$ family could theoretically start from $C_{14:\Delta4}$ this seems highly unlikely since so little unsaturation occurs at $\Delta4$ compared to $\Delta6$. By shortening $C_{16:\Delta6}$ by a C_2 unit, $C_{14:\Delta4}$ could be formed. Chain degradation, a common biological process, could also account for the very short chains discussed earlier. Chain lengthening and shortening can apply to saturated acids as well as monoenes and to all the carbon skeletal types found in sebum.

Thus the skin has many biochemical techniques which, when used in combination, can vary enormously the types of fatty chains produced. It can use a variety of starter moieties, place one or two extra methyl groups into the chain with methylmalonyl-CoA, place a double bond at either $\Delta6$ or $\Delta9$, extend chains up to C_{30} and beyond and, possibly, degrade them down to C_4. This results in truly a vast number of possibilities.

The fatty alcohols are synthesied by reduction of fatty acids (*31*). The fatty alcohols of sebum show the same skeletal types as occur in the fatty acids, which suggests a common chain building process. The double bond positions are mainly ten C-atoms from the methyl end of the chains as they are in the $C_{16:1\Delta6}$ family of acids (*32*). But alcohol chains average four C-atoms longer than the acids. α-Hydroxy fatty acids are made by hydroxylation of fatty acids, and alkane diols by reduction of α-hydroxy acids.

All the above processes occur in sebaceous glands and are the results of primary biosynthetic activity of these glands. Some secondary processes also occur in sebaceous gland ducts and on the skin surface. It was pointed out earlier, for example, that the triacyl glycerols are hydrolyzed to a variable

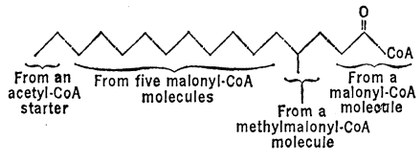


Fig. 5. An example of the origin of the carbon moieties of a monomethyl branched fatty acid.

extent in the ducts and on the skin surface by lipases. These lipases originate mainly, if not entirely, from skin surface microflora (*33*), and the amount of hydrolysis increases the longer the lipids remain on the skin. In the comedo, a lesion that can develop into acne vulgaris, this hydrolysis is almost complete (*34*). Indeed, many workers feel that the free fatty acids resulting from this hydrolysis play a role in the pathogenesis of acne, but the exact nature of this role, if it exists, remains obscure.

Another secondary process is the formation of sterol esters. Since sterol esters occur in the upper layers of sole skin, they are most probably formed late in the process of keratinization of epidermis. The fatty acids occurring in the sterol esters of comedos are both of the epidermal type and of the sebum type (*34*). Thus it appears that the large fraction of free cholesterol made by the epidermis undergoes a complex form of esterification in which fatty acids from both epidermis and sebum are utilized. These processes are summarized in Fig. 6.

Functions of the Unusual Lipids

An outstanding property of the unusual lipids of sebum is its extraordinary complexity. Why are so many compounds made? What functional significance could this have? One possible answer is that these substances serve as olfactory messages—a means for chemical communication in the biological world. Because of the huge

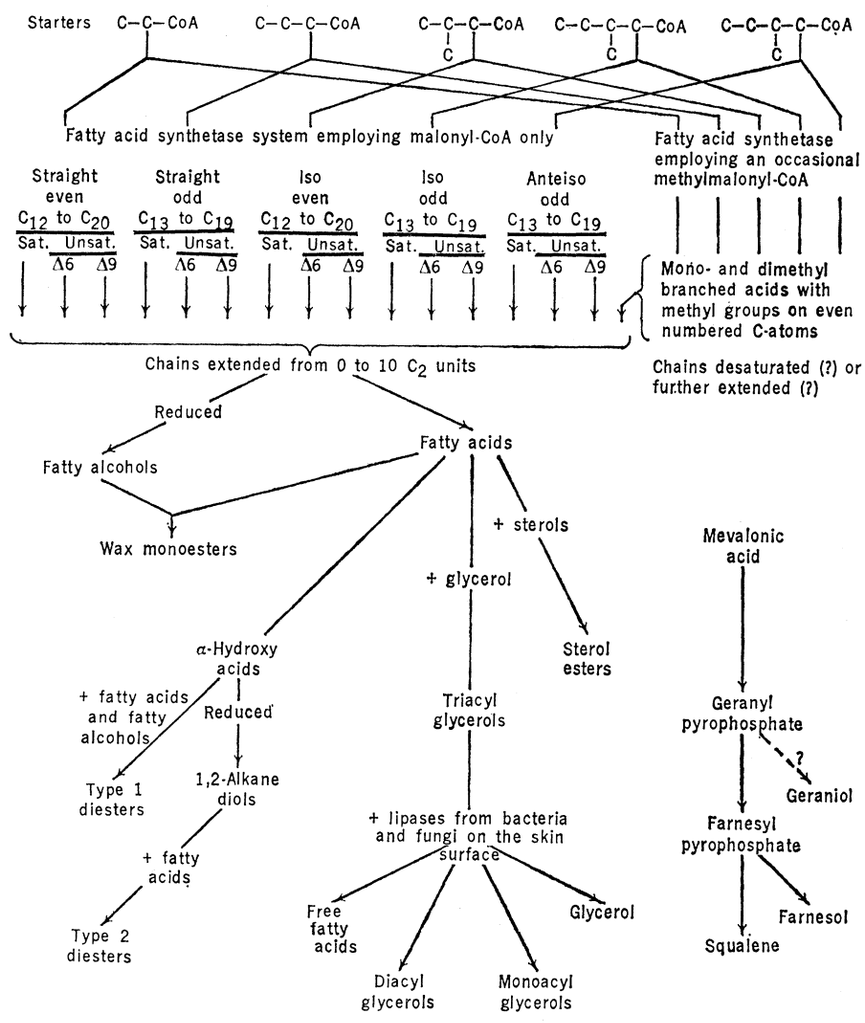


Fig. 6. Formation of lipids in human sebaceous glands and on the skin surface.

number of fatty acids that the skin can make, especially among the mono- and dimethyl branched acids and the unsaturates, it is extremely unlikely that any two individuals will make exactly the same substances in exactly the same proportions. Slight normal variations in enzyme concentrations, *p*H, body temperature, or cofactor concentrations, for example, could easily and markedly affect the final concentration of each fatty chain and thus provide an individual with a "chemical signature." This would be the distinctive odor by which a dog that had once sniffed a person would recognize and remember that person thereafter.

It is well known that in the animal world chemical communication plays a vital role, such as in recognition of members of the same and other species, in warning of the approach of a predator, and in sexual attraction. In man the sense of smell, although of variable sensitivity among different individuals, is generally poor compared to that of other animals. However, as was pointed out elsewhere (*35*), farnesol, which occurs on the human skin, is used as a perfume and presumably is of some value in human sexual attraction.

Besides complexity, skin lipids manifest a kind of perversity. Why, for example, does the sebaceous gland invest so much energy in setting up a unique enzymatic mechanism that enables it to place a double bond on a saturated fatty acid at the $\Delta 6$ position on a C_{16} chain instead of the usual $\Delta 9$ position on a C_{18} chain? Does this have any survival value? Do odd chain lengths, or iso and anteiso chains, or chains that are extremely long confront a would-be pathogenic microorganism with a metabolic problem? Do free fatty acids determine the type of microorganisms which can survive on the skin surface? Do the intermediates on the pathway to cholesterol synthesis prevent microorganisms from utilizing the cholesterol which they must get from the environment? I believe that the answers to all these questions are in the affirmative. Also, I do not believe that it is an accident that at all natural orifices of the body, at all points of possible entry, some form of sebaceous gland exists. There is already evidence that some of the substances present in human surface lipids prevent the growth of pathogens (*36*). This is not to say that these lipids are toxic to microorganisms in general, for obviously the skin is loaded with microorganisms, but the ones that survive are the ones that are compatible with normal healthy skin, and the unique lipids of the human skin surface help to maintain this ecological balance.

Skin lipids undoubtedly have other functions. Excretion of waxes assists animals and birds in preventing their fur or feathers from becoming wet. If this were not so, life would be difficult, if not impossible. Whether this function is significant for man is a subject that has been debated (*37*).

Finally, it is possible that free fatty acids and mono- and diacyl glycerols help retain moisture in the skin by forming monomolecular films over microdrops of sweat, thereby delaying evaporation. Furthermore, mono- and diacyl glycerols, as well as glycerol itself, released during hydrolysis of the triacyl glycerols will retain moisture because of their hygroscopicity.

Summary

Two key words characterize the uniqueness of skin lipids: complexity and perversity. Each suggests a function. Complexity manifests itself in the large number and variety of both saturated and unsaturated fatty chains synthesized by human skin. Functionally, this allows each individual to have a distinct odor or chemical fingerprint. Perversity manifests itself when one compares the lipids synthesized by skin with those synthesized by internal tissues. For example, skin makes odd instead of only even chains, branched instead of only straight chains, free instead of only esterified acids, places double bonds in unusual positions in the fatty chains, extends chains to extreme lengths, and accumulates intermediates in the synthesis of a biologically valuable compound such as cholesterol. Functionally, these products may pose metabolic problems to potential pathogens and thus contribute to the survival of only compatible microorganisms.

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