SYNTHESIS AND COMPOSITION OF SURFACE LIPIDS OF HUMAN SKIN*

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The composition and biosynthesis of lipids in human skin have long been of considerable interest, both because of the highly unusual structures these lipids contain and because of the possible involvement of the lipids in disease processes of the skin. Despite the exceptionally complex nature of surface lipid, its composition is now known in detail. In addition, since efficient procedures are now available for analyzing the class composition of skin lipid and the individual members of these classes, the changes in composition that may occur under specialized circumstances can now be studied in large groups of subjects.

Because of the unusual compounds in skin lipids, the pathways of lipogenesis that have been elucidated in other tissues cannot confidently be accepted for skin. Therefore, direct examination of lipogenesis in skin has been desirable, but the numerous studies that have been conducted have been only moderately successful in delineating pathways of lipid biosynthesis. Moreover, because of the extreme variation in surface lipid composition between species, studies of sebaceous lipogenesis in other animals have only limited application to man. However, useful inferences regarding the biosynthesis have been based on observations of the composition of human surface lipid collected under specific conditions. This review emphasizes the results of investigations of the composition of surface lipids from human skin from which deductions concerning their origin and biosynthesis can be made. Some of the more direct examinations of lipogenesis based on in vitro studies in skin are discussed by Wheatley (this issue).

SOURCES OF SKIN SURFACE LIPID

Surface lipid is derived from two principal sources: the sebaceous glands and the epidermis. In adults with normally active sebaceous glands, sebum is the predominant lipid on most areas of the skin surface; the amount of surface lipid on a particular area of the skin reflects the size and density of distribution of the sebaceous glands in that area (Rothman, 1954; Kligman and Shelley, 1958). Both the distributional density of the glands and the amount of surface lipid are found in descreasing order on the face, back, chest, abdomen, arms, and legs. The palms and soles, though devoid of sebaceous glands, normally carry some sebum transferred from other areas.

The surface film also contains lipid derived from the keratinizing epidermis. Since this lipid is, at least initially, an intracellular material, the amount obtained in a sample will probably depend on the method of collection. Furthermore, sebum on the skin surface might act as a solvent for the extraction of lipid from epidermal cells onto the surface.

Some of the lipids in the surface film may be produced by the dense population of bacteria resident on the skin and in the follicles, but evidence is lacking for the presence of such bacterial lipids in skin surface samples. Likewise, it has not been established how much lipid is derived from apocrine and eccrine sweat. Soaps and cosmetics are frequent sources of contamination of skin surface lipids. In addition, the environment contributes variable quantities of lipid to the skin surface film, notably petroleum hydrocarbons.

THE COMPOSITION OF LIPID CLASSES ON THE SKIN SURFACE

The compounds in human surface lipid can be separated and quantified by adsorption chromatography, either on a column (Nicolaides and Foster, 1956; Haahti, 1961) or on thin-layer plates (Downing, 1968). Lewis and Hayward (1971) have recalculated the analyses of several workers who examined the composition of surface lipid collected by various procedures from several sebum-rich areas. Their figures (Table I) indicate a remarkable agreement in these analyses. This concurrence is further enhanced by combining the values for free fatty acids and mono-, di-, and triglycerides into an aggregate figure (total glyceride acids) which eliminates the variable effect of triglyceride hydrolysis by microorganisms in the skin (Marples et al., 1970, 1971, 1972). Lewis and Hayward (1971) also eliminated from their calculations the figures for the minor constituents of surface lipid which derive principally from the epidermis. With this procedure, the three major components (squalene, wax esters, and total glyceride acids) are left to represent the composition of sebum (Table I). Although this calculation allows the composition of any surface lipid sample to be represented as a point on a triangular graph, it is surely an oversimplification and it is not clear how it can be used in any quantitative sense.

FACTORS INFLUENCING THE CLASS COMPOSITION OF SURFACE LIPIDS IN HUMAN SKIN

Sampling Procedures

Several authors have reviewed the sampling procedures for collecting skin surface lipid (Wheatley, 1963; Nicolaides, 1963; Nicolaides and Kel-

TABLE I

Analyses by various workers of surface lipid from sebum-rich areas

(adapted from Lewis and Hayward, 1971)

Lipid class	Percentage of total sebum recovered					
	Back[1]	Back[2]	Scalp[1]	Scalp[3]	Forehead[4]	Face[5]
Squalene	11.4	15.6	12.8	11.7	12.0	11.6
Wax esters	21.5	} 24.2	20.2	21.8	25.0	22.6
Sterol esters	2.9		3.3	2.6	2.1	2.5
Free fatty acids	16.0	16.2	29.6	33.1	16.4	27.2
Sterols	1.8	1.4	2.4	1.5	1.4	0.7
Glycerides	46.4	42.6	31.7	29.3	43.2	35.4
Saturated hydrocarbons	1.3	1.8	0.8	0.6	—	1.3
"Three-component sebum":						
squalene	12.0	16.3	13.6	12.2	12.3	11.9
wax esters	22.5	22.3	21.4	22.7	25.9	23.4
total glyceride acids*	65.5	61.4	56.0	65.1	61.8	64.7

[1] Lewis and Hayward (1971): acetone irrigation, column chromatography.

[2] Haahti (1961): acetone irrigation, column chromatography.

[3] Nicolaides and Foster (1956): ether dip, column chromatography.

[4] Downing et al. (1969): ether in cup, thin-layer chromatography.

[5] Felger (1969): dry wipe, column chromatography. Total glyceride acids = mono-, di-, and triglycerides plus free fatty acids.

lum, 1965; Lewis and Hayward, 1971). Theoretically, the composition of the collected sample could be affected either by minimizing the inclusion of epidermal cell lipid or by efficiently extracting it into the sample. Nicolaides (1963) has stated that nonpolar solvents such as hexane will not penetrate the moist layers of the epidermis and thus will extract only surface lipid, whereas highly polar solvents such as chloroform:methanol mixtures will penetrate and remove lipids from deep in the epidermis. On this basis, the solvent of choice for obtaining surface lipids should be hexane, and the most polar solvents should be used when surface lipid plus deep epidermal cell lipid are to be extracted together. Thus the use of "mildly penetrating diethyl ether" (Nicolaides, 1963) would result in the partial removal of cellular lipids along with the surface material. In practice, however, no differences in the composition of surface lipid appear to result from the use of different extracting solvents (Table I).

Skin lipids collected on absorbent materials such as cigarette paper (Strauss and Pochi, 1961) are claimed to be almost pure sebum (Nicolaides and Kellum, 1965) since the collection site is first defatted and the lipid is then absorbed directly from the pilosebaceous orifice; however, no accurate quantitative analysis has been made of lipid collected in this way.

Kellum (1967) isolated whole sebaceous glands from scalp biopsies and extracted the lipids for qualitative analysis. As with surface lipid, the major constituents were squalene, wax esters, and triglycerides; free fatty acids were absent. The yield of lipid per sebaceous gland (about 10 μg) would be sufficient for quantitative analysis by photodensitmetry of thin-layer chromatograms (Downing, 1968). However, the composition of this

lipid might differ from true sebum since it was extracted from sebaceous cells at all stages of development, from the germinative layer to their collapse in the lumen.

As epidermal cells progress from the basal layer and keratinize to form the stratum corneum, they undergo a marked change in their lipid composition, including an almost complete elimination of phospholipids (Kooyman, 1932; Reinertson and Wheatley, 1959), which suggests the existence of a mechanism for conserving phosphorus. This change means that any analysis of lipid extracted from full-thickness epidermis is of limited value in determining how much the epidermis contributes to lipids recovered from the skin surface. Nevertheless, the observation of Nieminen et al. (1967) that full-thickness epidermis from the forearm contains very little squalene or wax ester supports the conclusion that these lipids are not of epidermal origin, whereas the presence of substantial amounts of sterols, sterol esters, glycerides, and phospholipids suggests that these are true epidermal lipids, which may or may not reach the skin surface. The saturated hydrocarbons consistently found in epidermal and surface lipids (Nicolaides and Foster, 1956; Haahti, 1961; Nieminen et al., 1967) appear to be predominantly, if not entirely, petroleum hydrocarbons. Such hydrocarbons are absent from the skin lipids (vernix caseosa) that accumulate before birth (Downing, 1965) and from lipid extracted directly from isolated sebaceous glands (Kellum, 1967). In studies of the biosynthesis of skin lipids from ^{14}C-acetate, the saturated hydrocarbons were consistently found to be unlabeled (Lewis et al., 1965; Nicolaides and Kellum, 1966; Summerly and Woodbury, 1971); therefore, these hydrocarbons are probably exogenous contaminants.

Effect of Anatomic Origin on Surface Lipid Composition

In a summary of much of the earlier work, Rothman (1954) indicated that sebaceous glands vary widely in size and density of distribution over the surface of the human body, and that the amount of recoverable surface lipid is directly proportional to the abundance of sebaceous gland tissue below the area of collection. Surface lipid from areas with few sebaceous glands should, therefore, contain a higher proportion of lipid from the epidermis; this could affect the composition of the collected lipid. Boughton and co-workers (1957) measured the amounts of cholesterol and squalene in surface lipid samples extracted with acetone from the backs, chests, abdomens, arms, and legs of 8 volunteers. When they plotted the concentration of cholesterol against total lipid for each sample, they found a constant level of cholesterol (2.5%) at surface lipid levels between 50 and 150 μg/sq cm of skin, whereas at lower lipid levels the cholesterol content rose dramatically to over 30 percent. Conversely, the concentration of squalene
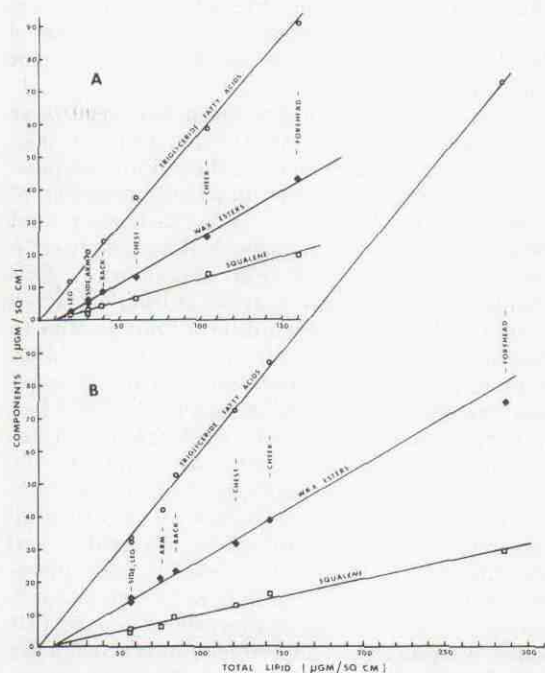

FIG. 1: Anatomic variation in the amount and composition of human skin surface lipid. For each anatomic area the average weight per sq cm of each major lipid component was plotted against the average amount of total surface lipid per sq cm in that area. Extrapolation to the abscissa indicates that no sebum constituents (wax esters and squalene) would be present at total surface lipid levels below 10 μg per sq cm and can, therefore, be taken to represent the level of lipid derived from the epidermal cells. Section A shows the results for three subjects washed with soap and water 3 hr before extraction of the surface lipids with hexane on two separate occasions. Section B shows the results similarly obtained from two additional subjects washed 12 hr before collection of surface lipid on two occasions.

in the samples, though much more variable, fell significantly at surface lipid levels below 50 μg/sq cm. These results concur with the theory that the cholesterol in surface lipid is principally a product of the epidermis and that squalene arises from the sebaceous glands.

With the recent development of quantitative thin-layer chromatography, the amount and lipid class composition of individual samples from different anatomic areas can be measured (Greene et al., 1970). Several methods of graphical interpretation (Figs. 1, 2) indicate that the epidermis contributed about 5–10 μg of lipid/sq cm to the collected samples and that, in agreement with Boughton et al. (1957), this produced significant effects on the composition of the mixture at surface lipid levels less than about 50 μg/sq cm. Thus, the proportions of squalene and wax esters at the level of 20 μg/sq cm of surface lipid suggest that at this level the collected lipid consists of roughly equal proportions of sebum and epidermal cell lipid. From these figures, the approximate composition of epidermal (horny layer) lipid can be calculated (Table II). At surface lipid levels higher than 100 μg/sq cm, the compositions of the mixtures from different anatomic sites were almost identical (Fig. 2), an indication that the sebum produced at these sites was similar in composition and was mixed with insignificant proportions of epidermal lipid. Therefore, the average composition of lipid from these sites (Table II) probably closely approximates that of pure sebum.

Attempts have been made to define the anatomic origin of surface lipid constituents by radioisotopic labeling experiments. In incubations of separated epidermis and corium from the forearm and palms with ^{14}C-acetate, Nicolaides and Rothman (1955) found that all specimens synthesized fatty acids, sterols, and squalene, but the specimens in which sebaceous glands were absent formed only minor amounts of squalene. Summerly and Woodbury (1971) incubated full-thickness skin biopsies from the back with [1-^{14}C]-acetate and then dissected out the sebaceous glands by the method of Kellum (1967). Lipids extracted from the isolated glands and from the epidermis both showed labeling in each of the classes of lipid found on the skin surface (except in the saturated hydrocarbons). Moreover, the ratios of labeling in triglycerides, wax esters, and squalene were the same in both epidermal and sebaceous lipid. From this, it must be concluded that there was extensive cross-contamination of lipid from the two anatomic sources so that the sites of specific lipogenesis could not be defined.

Effect of Variation in Synthesis on Surface Lipid Composition

We have seen that wherever sebaceous glands are sparse, epidermal contribution to the surface film can be sufficient to modify its composition. On the other hand, the sebum found where seba-
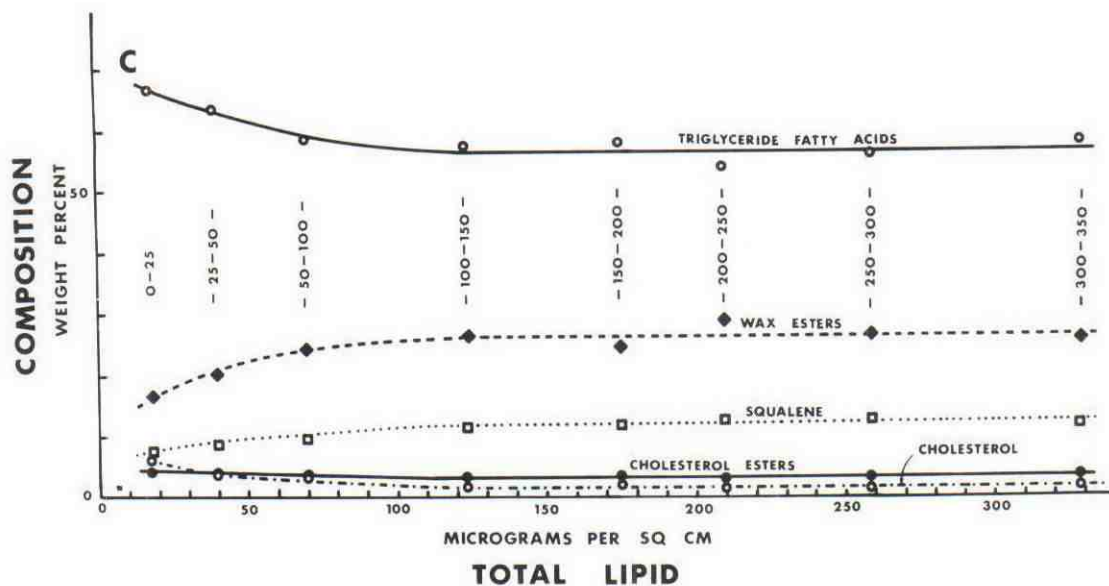
FIG. 2: Variation in the composition skin surface lipid in relation to the total amount of recoverable lipid. In all the surface lipid samples from various anatomic sites described in Figure 1 the average composition was calculated for groups of samples falling within the ranges of total lipid per sq cm as shown, and the average percentage of each constituent was plotted against the average weight of total surface lipid in each group.

TABLE II

Approximate composition of sebum and surface epidermal lipids

Constituents	Sebum (wt%)*	Surface epidermal lipid (wt%)
Glycerides plus free fatty acids	57.5	65
Wax esters	26.0	—
Squalene	12.0	—
Cholesterol esters	3.0	15
Cholesterol	1.5	20

* Greene et al. (1970).

ceous gland density is low does not appear to differ in composition from that in areas of high gland density. However, sebum production in normally sebum-rich areas is sometimes low enough to reveal the presence of epidermal lipid. Furthermore, in certain circumstances, actual changes in sebum composition have been observed.

Changes in sebum production during childhood. Several studies have correlated the low rate of sebum production in prepuberal children with an elevated level of cholesterol in skin surface lipid during childhood (Eckstein, 1972; Nicolaides and Rothman, 1952; Washburn and Liese, 1953; Boughton et al., 1957). Moreover, squalene concentrations in surface lipid tend to be low before puberty (Nicolaides and Rothman, 1952; Boughton et al., 1957). Ramasastry et al. (1970) measured the proportion of each lipid class in forehead surface lipid of individuals from birth to sexual maturity and found that surface lipid appeared to undergo

two distinct changes during development. At five days after birth, the composition resembled that of adult sebum, presumably because of a high rate of sebum production from sebaceous glands activated by maternal hormones. Between 2 and 8 years of age, the characteristic components of epidermal lipid (cholesterol and its esters) were more prominent and the sebaceous lipids (wax esters and squalene) had declined, so that the proportions of wax esters and squalene were about one-third and one-half, respectively, of their concentration in adult sebum. In this age range, then, sebum apparently constitutes less than half the surface lipid of the forehead, assuming that the sebum of children is not significantly different in composition from that of adults. In the age group of 8–10 years, wax esters and squalene rose to about two-thirds of the adult level, and at 10–15 years the surface lipid resembled that of adults.

Figure 3 shows how the concentrations of the four principal constituents in each of the surface lipid samples vary during development. The variability in the composition of the surface lipid of children is probably due to variations in the ratio of sebum and epidermal lipid. However this does not explain the greater variability in the squalene concentrations or the lower ratio of wax esters to squalene than in adult sebum. The combined values for surface lipid glycerides plus free fatty acids are also somewhat higher in children from 2–8 years than in adults, an indication that this aggregate figure is higher in epidermal lipid than in sebum. A similar conclusion was reached in a study of the anatomic variation of adult surface lipid (Greene et al., 1970).

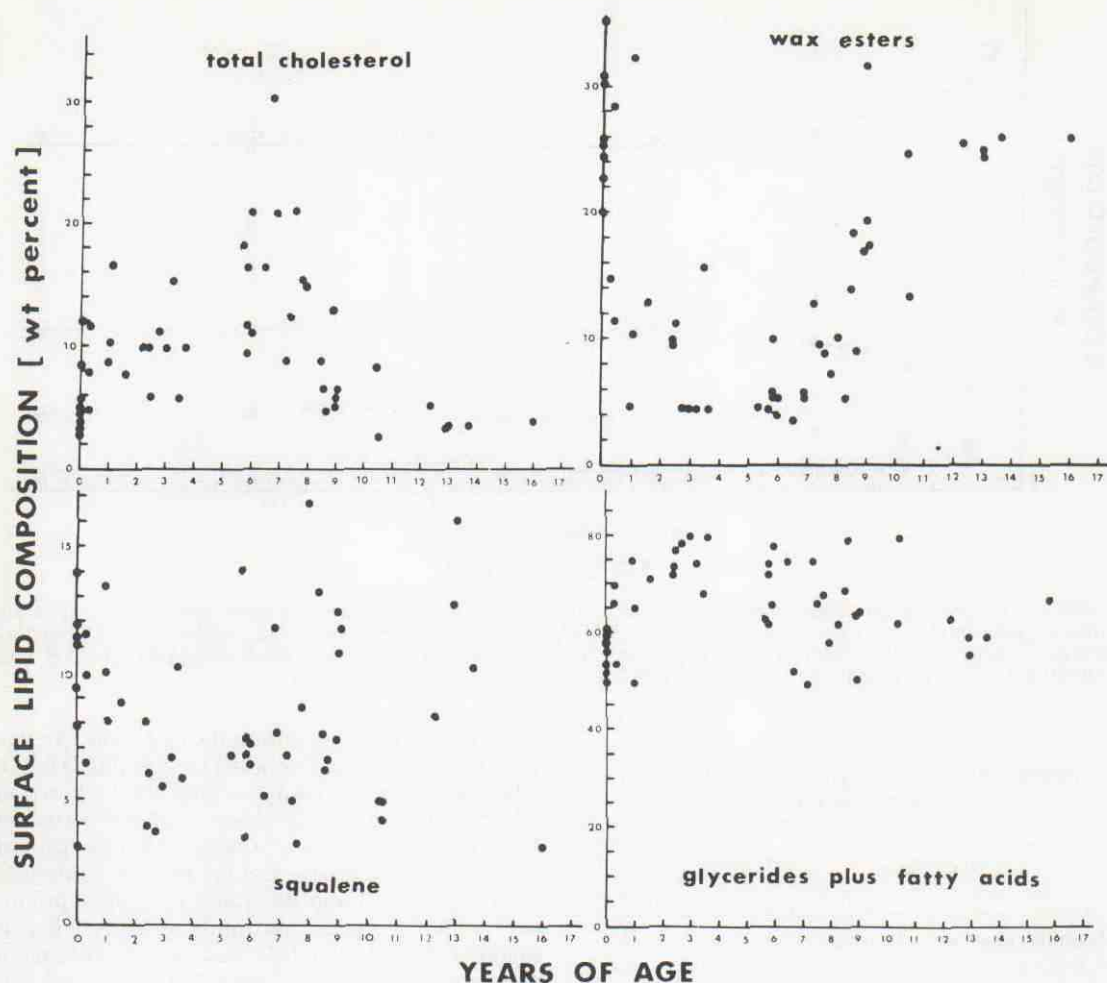Analyses of lipid class composition for the sur-

FIG. 3: Variation in the composition of human skin surface lipid during childhood. For each sample of surface lipid, the concentrations of each of the four major constituents were plotted against the age of the subject.

face lipid (vernix caseosa) present on neonates (Kärkkäinen et al., 1965) revealed 9 percent squalene, 12 percent wax esters, 33 percent sterol esters, 7 percent diesters, 26 percent triglycerides, 9 percent free sterols, and 4 percent of other lipids by weight. These data suggest the presence of sebum (providing squalene and wax esters) with a high proportion of epidermal lipid (providing sterols and sterol esters). However, the composition of vernix cannot be explained simply on this basis because the concentration of glycerides plus fatty acids is less than half of that in either sebum or epidermal lipid. Moreover, in vernix the ratio of squalene to wax esters is much higher than in sebum and the ratio of esterified sterols to free sterols is higher than in epidermal lipid.

Effect of variation in sebum synthesis on surface lipid composition in adults. Although the rate of sebum synthesis, which is under hormonal control (Strauss and Pochi, 1963), varies widely in normal adults, the composition of the skin surface lipid from sebum-rich areas varies little in normal subjects (Downing et al., 1970a; Lewis and Hay-

ward, 1971). In addition to confirming the negligible influence of epidermal lipid, these observations indicate that the composition of the sebum produced is not affected by the variation in the rate of sebaceous lipogenesis that results from differences in hormonal stimulation. Indeed, the maximum changes in sebum production produced by oral administration of estrogens or androgens have not had a noticeable effect on sebum composition (Strauss, Pochi, and Downing, unpublished). Furthermore, Strauss et al. (1967) have shown that sebum production can be reduced as much as 60 percent by the oral administration of eicosa-5,8,11,14-tetraynoic acid, a powerful inhibitor of prostaglandin biosynthesis (Ahern and Downing, 1970; Downing et al., 1970b), without changing the surface lipid composition (unpublished observations). Therefore, changing the rate of sebaceous lipogenesis does not necessarily change the composition of the lipid mixture being synthesized.

Pochi et al. (1970) found that when obese subjects underwent prolonged starvation, their rate of

sebum synthesis decreased by an average of 40 percent without any decrease in the amount of squalene produced. This selective inhibition of sebaceous lipogenesis resulted in a dramatic rise in the *proportion* of squalene in the surface lipid (Fig. 4). The same effect occurs in normal subjects (Downing et al., 1972) (Fig. 5). Although sebum composition began to change after about five days of fasting, an average of two weeks elapsed before the rate of sebum production (measured by the method of Strauss and Pochi, 1961) decreased. These results provoke several lines of speculation:

(a) The observed delay before a change in surface lipid composition occurs after the commencement or conclusion of fasting does not necessarily result from a similar delay in the alteration of lipid synthesis. The 5 or 6 days that elapse before the change in composition is seen probably represent the time lapse between synthesis of a particular lipid molecule in the sebaceous gland and its appearance on the skin surface. No one seems to have studied the time required for this translocation in man, but in the sheep and the gerbil the radioactivity of skin surface lipid rises to a sharp maximum 6 days after the intradermal injection of ^{14}C-acetate (Downing and Ramasastry, unpublished). Therefore, the true response of sebaceous lipid *synthesis* to these profound dietary changes is probably much more rapid than is apparent from the data of Pochi et al. (1970) and Downing et al. (1972). Significantly, Hsia and co-workers (1966) found that the in vitro incorporation of $[1\text{-}^{14}C]$-acetate into lipids of excised human skin specimens was impaired after only 18 hr of fasting. Perhaps even normal overnight inanition causes a significant change in sebum synthesis, which, because of the extended period during which mixing in the pilosebaceous follicle can occur, does not result in any circadian variation. Certainly, squalene concentration, the most variable aspect of sebum composition, might be affected by normal discontinuity in caloric intake.
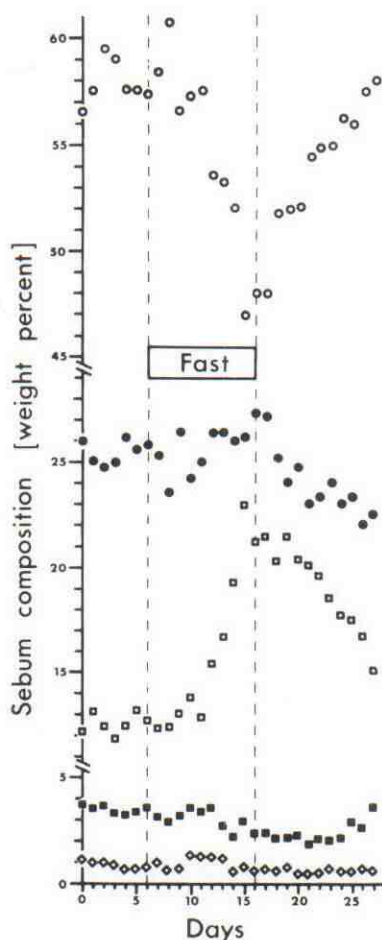

FIG. 5: Changes resulting from fasting in the composition of the skin surface lipid of human subjects of normal weight. The average composition of daily samples of surface lipid from 4 normal subjects was plotted in relation to the time of commencement of fasting or recommencement of feeding. O = triglycerides + diglycerides + free fatty acids; ● = wax esters; □ = squalene; ■ = cholesterol esters; ◇ = cholesterol.

(b) The delay of several weeks between the commencement of fasting and the onset of a reduction in sebum *secretion* is not as easily explained as the shorter time before the change in composition occurs. Elucidation of this question may require a better understanding of the mechanism by which differences between individuals are produced in the method used to assess sebum production rate. It seems unlikely that the lipid collected on cigarette papers pressed to the skin represents material forced to the surface by the pressure of lipogenesis. The amount of lipid collected might be proportional to the amount contained in the pilosebaceous ducts, the volume of which could, in turn, be proportional to the lipogenic activity of the sebaceous glands. A change in lipid synthesis (a metabolic event) would therefore occur much more rapidly than a change in the volume of the ducts (a morphologic process), which
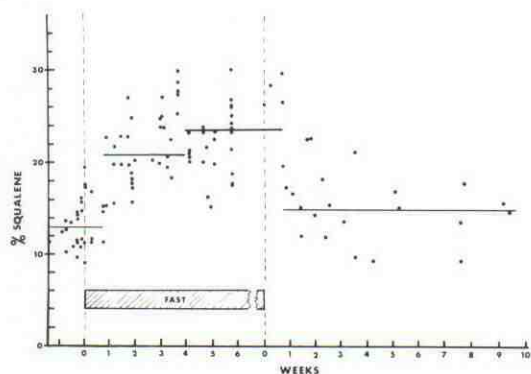

FIG. 4: Changes resulting from fasting in the concentration of squalene in the skin surface lipid of obese human subjects. For each individual sample of surface lipid, the concentration of squalene was plotted in relation to the time the subject commenced fasting or recommenced feeding.

may require weeks to adjust to a new level of sebaceous activity.

(c) In obese and normal subjects alike and at both the beginning and the end of fasting, changes in the amounts of wax esters appeared to lag behind the changes in squalene and triglycerides by about a week. This could indicate that wax esters are synthesized much earlier in the life cycle of the sebaceous cell than the other major constituents. If the waxes are produced in the sebaceous cells shortly after their separation from the basal layer and the squalene and triglycerides not until the commencement of fatty degeneration, when the cells are approching the lumen, about a week could elapse between these events. Under these circumstances, the simultaneous suppression of the synthesis of triglycerides and wax esters would not affect the amount of triglycerides reaching the skin surface for about 5 or 6 days, and a further week could pass before the amount of wax esters in the surface lipid was reduced.

COMPOSITION AND BIOSYNTHESIS OF THE ALIPHATIC CONSTITUENTS OF HUMAN SKIN SURFACE LIPID

Fatty Acids

1. *General composition.* The complexity of the mixture and the presence of unusual structures in the fatty acids of human surface lipid were first established by Weitkamp and his co-workers (1947). They studied only the free fatty acids of lipid extracted from barber-shop sweepings, reasoning that contaminating lipids from hair dressings and other extraneous sources would be unlikely to contain significant quantities of free acids. They showed that the monounsaturated acids isolated from their distillation fractions contained double bonds in the highly unusual 6,7 and 8,9 positions. However, in the C_{18} fraction, the fatty acid carrying the double bond in the more usual 9,10 position was the most abundant isomer and it was thought that this may have come from an extraneous source. The unsaturated acids, ranging in chain length from 11–20 carbon atoms, and the saturated acids, from 7–22 carbon atoms, were both identified as series of straight-chain compounds, including members, unique at that time, having an odd number of carbon atoms.

James and Wheatley (1956) were the first to use gas chromatography to examine skin lipids. Using surface lipid from the human forearm, they obtained quantitative analyses of the free fatty acids containing 7–18 carbon atoms, confirmed the presence of saturated and unsaturated acids with both odd and even numbers of carbon atoms, and detected the presence of two series of branched-chain saturated acids. The fatty acids occurring as esters in the surface lipid also appeared to consist of a similar mixture of compounds. Gas chromatograms obtained by Haahti (1961) showed that in surface lipids from the human back similar mixtures of fatty acids occur free as well as in the

triglycerides and in the mixture of wax and sterol esters. These series of saturated and unsaturated acids of odd and even chain length and the branched-chain saturated acids were also detected in gas chromatograms of methyl esters of the free fatty acids from human earwax (Haahti et al., 1960a) and of the esterified fatty acids from vernix caseosa (Haahti et al., 1960b).

In all of these earlier gas chromatographic studies, detection of unsaturated acids depended on comparisons of gas chromatograms of fatty acid methyl esters before and after the unsaturated compounds were obliterated by bromination. Haahti and Horning (1963) separated the methyl esters of the total fatty acids from surface lipid of the back into saturated and unsaturated compounds and analyzed both fractions by gas chromatography. These studies indicated the presence of saturated acids with straight chains, with two types of methyl branching, and with two types of double chain-branching. The unsaturated acids appeared to consist of a mixture of straight-chain compounds, singly methyl-branched isomers, and diunsaturated acids. However, no quantitative results were published.

Quantitative gas chromatographic analyses carried out on the fatty acids of vernix caseosa after the unsaturated acids had been separated as the mercuric acetate adducts (Downing, 1963) showed that the saturated acids contained a much higher proportion of branched-chain compounds (78%) than the saturated acids of adult surface lipid (12%). It was, therefore, unlikely that the branched compounds were of bacterial origin or derived from dietary branched acids. This work also revealed that a small porportion of branched constituents (15%) was present among the unsaturated acids and that both saturated and unsaturated acids extended to much longer chain lengths than previously detected. The fatty acid fraction of vernix caseosa was also found to contain a small proportion of 2-hydroxyacids which were apparently completely saturated and predominantly branched.

Structures of the branched-chain acids. From the time branched acids were first found in human skin lipids (James and Wheatley, 1956), they were assumed to include the iso and anteiso series identified by Weitkamp (1945) in wool lipid of sheep. In examining the fatty acids from wool lipid, Weitkamp isolated two series of methyl-branched fatty acids and deduced their position of branching from the melting-point behavior of binary mixtures of the compounds with straight-chain acids of various chain lengths. In one of these series with an even number of carbon atoms, the methyl branch was attached to the penultimate carbon atom of the chain (iso series). In the other series, consisting of compounds with an odd number of carbons, the methyl group was attached to the antipenultimate carbon (anteiso series). Iso and anteiso reference compounds could be distinguished by their slightly different gas chromato-

graphic retention times (Downing et al., 1960), which were identical with those of compounds in human surface lipid (Haahti et al., 1960a; Downing, 1963). Other types of branched acids were noted by James and Wheatley (1956), and subsequent gas chromatographic studies indicated that as many as five series of branched acids might be present (Nicolaides and Ray, 1965). Recently, Nicolaides (1971) reported a study of these minor constituents, in which combined gas chromatography–mass spectrometry procedures were used. His findings supported the accepted iso and anteiso structures for the bulk of the branched acids in wax esters of vernix caseosa. This study also indicated the presence of other series of branched acids in which a single methyl branch is located on any one of the even-carbon positions of the chain, except the 2 position. It should be noted, however, that even with pure branched-chain acids the location of methyl branching by mass spectrometry can be uncertain (Abrahamsson et al., 1963). Recently, in trying to analyze mixtures of methyl-branched acids similar to those in skin lipids, six major mass spectrometry laboratories have failed to identify the constituents (Ackman and Hooper, 1972). The proposed structures of the singly-branched fatty acids of human skin surface lipid are shown in Figure 6. In addition to these, compounds with two methyl branches were found, one of which was attached to carbon 4 of the chain, the other to one of the more distant even-numbered positions.

Origins of the branched-chain acids. Kärkkäinen et al. (1965) separated the various classes of lipid occurring in vernix caseosa and analyzed the constituent fatty acids of the wax esters, sterol esters, diesters, and triglycerides (Table III). Each fraction contained a considerable proportion of branched acids that extended to chain lengths of 26 carbon atoms; in the wax ester fraction, however, fatty acid chain lengths greater than 18 carbons were almost nonexistent. Fatty acids from the sterol esters combined the highest proportion of branched chains and the greatest amounts of material in the longer chain lengths. Similar differences in chain-length distribution were shown to exist between the wax ester and sterol ester fatty acids of vernix caseosa by Nicolaides et al. (1972). These studies support the theory that the wax and sterol ester fractions in vernix are from different anatomic origins, the sebaceous glands and the epidermis, respectively. Wax esters are undoubtedly a product of the sebaceous glands and probably do not occur in epidermal lipid. A moderate proportion of branched components is present in the wax ester fatty acids from both vernix and adult surface lipid (26.3% and 26.7%, respectively) (Nicolaides et al., 1972), and the sebaceous glands can therefore be accepted as a source of branched acids.

The sterol esters, however, probably arise both from the sebaceous glands and from the epidermis. The similarity in fatty acid composition between

iso (C_{12} to C_{18})

$$CH_3\underset{\underset{CH_3}{|}}{CH}-CH_2CH_2CH_2CH_2CH_2CH_2(CH_2)_nCO_2H \qquad n = 1 \text{ to } 6$$

4-methyl-C_{11} to C_{17}

$$CH_3CH_2CH_2CH_2CH_2CH_2(CH_2)_n\underset{\underset{CH_3}{|}}{CH}-CH_2CH_2CO_2H \qquad n = 1 \text{ to } 6$$

6-methyl-C_{11} to C_{16}

$$CH_3CH_2CH_2CH_2(CH_2)_n\underset{\underset{CH_3}{|}}{CH}-CH_2CH_2CH_2CH_2CO_2H \qquad n = 1 \text{ to } 5$$

8-methyl-C_{11} to C_{17}

$$CH_3CH_2(CH_2)_n\underset{\underset{CH_3}{|}}{CH}-CH_2CH_2CH_2CH_2CH_2CH_2CO_2H \qquad n = 1 \text{ to } 6$$

10-methyl-C_{12} to C_{17}

(anteiso when n = 1)

$$CH_3(CH_2)_n\underset{\underset{CH_3}{|}}{CH}-CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CO_2H \qquad n = 1 \text{ to } 6$$

12-methyl-C_{14} to C_{16}

(anteiso when n = 1)

$$CH_3(CH_2)_n\underset{\underset{CH_3}{|}}{CH}-CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CO_2H \qquad n = 1 \text{ to } 3$$

14-methyl-C_{16}

(anteiso)

$$CH_3CH_2\underset{\underset{CH_3}{|}}{CH}-CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CO_2H$$
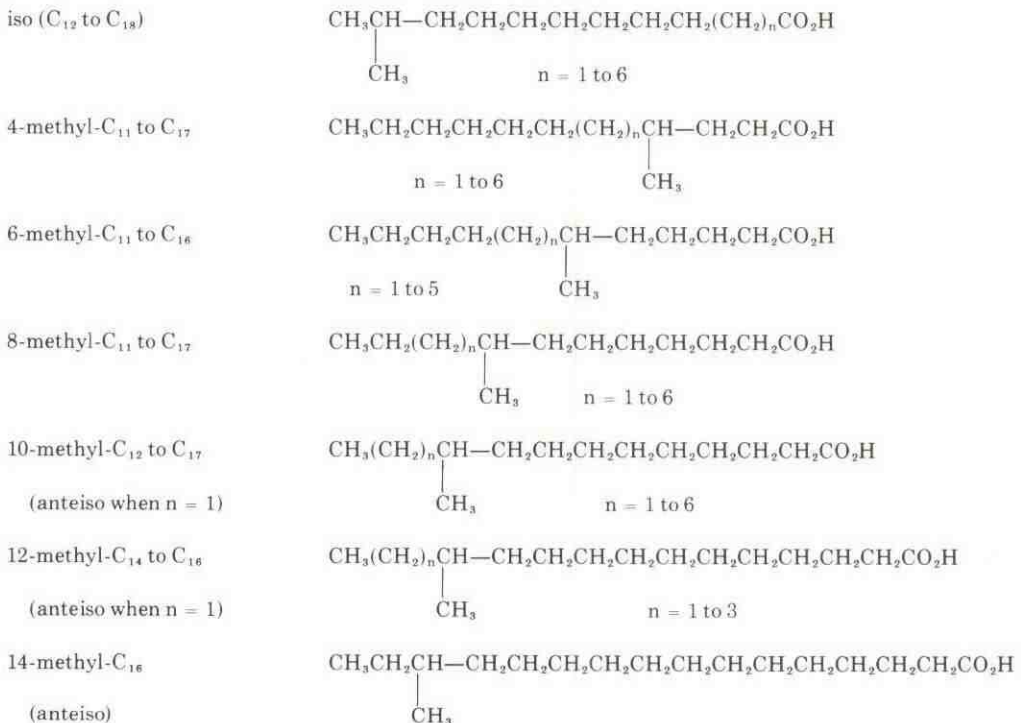
FIG. 6: Structures of the singly-branched fatty acids of human skin surface lipids identified by Nicolaides (1971). Numerous acids of longer chain length and presumably similar branching have been detected by many investigators.

TABLE III

Straight and branched chain fatty acids of the lipid classes from vernix caseosa*

(recalculated from the results of Käkkäinen et al., 1965)

	Wax esters		Sterol esters		Triglycerides		Diol esters	
	straight	branched	straight	branched	straight	branched	straight	branched
Saturated	18.9	25.6	11.6	55.7	49.6	21.4	32.4	32.3
Total	44.5		67.3		71.0		64.7	
Unsaturated	50.9	4.8	32.5	1.0	27.9	0.2	21.9	1.0
Total	55.7		33.5		28.1		22.9	

* Data are expressed as % for each class.

wax esters and sterol esters in adult surface lipid (Nicolaides et al., 1972) supports the proposal (Greene et al., 1970) that sebum is the principal source of sterol esters in the surface lipid from sebum-rich areas. On the other hand, the sterol ester fatty acids in vernix contain a much higher proportion of branched acids (62.2%) than the sterol esters in adult surface lipid (20.2%) or the wax esters of either vernix or adult lipid. This indicates that the vernix sterol esters are principally epidermal in origin and that the epidermis is a richer source of branched acids than the sebaceous glands.

Biosynthesis of the branched-chain acids. The biosynthetic pathways for the branched-chain acids in human surface lipid have yet to be established. The structures of the iso and anteiso acids suggest the possibility that they are formed from the short-chain branched acids produced by deamination of amino acids such as valine, leucine, and isoleucine. Wheatley et al. (1961) demonstrated in dog skin that labeled isoleucine was preferentially incorporated into a C_{15} acid, presumably the anteiso compound. Wheatley et al. (1967) extended this study to include other amino acids. Nicolaides (1971) has advanced the alternative suggestion that the branched acids bearing methyl groups in the 4 position and/or in even positions beyond this could be synthesized by substituting methyl malonyl CoA for malonyl CoA in one of the chain-elongation steps involved in the synthesis of fatty acids. In this system, the odd-carbon anteiso acids would be formed if methyl malonyl CoA was incorporated in the initial condensation with acetyl CoA. Methyl malonyl CoA could be formed from propionyl CoA, which could also be the source of odd-carbon straight chain acids if it substituted for acetyl CoA in the initial condensation with malonyl CoA. However, Nicolaides's proposal does not explain the origin of even-carbon anteiso acids or of either the odd- or even-carbon iso acids.

Actually, the biosynthesis of all of the branched-chain and odd-carbon acids known to be present in human surface lipid can be explained by combining the suggestions of Wheatley and Nicolaides and by using precursors that are known to be direct metabolites of the major amino acids. This scheme is shown in Figures 7–10. Thus, valine would be the precursor of the even-carbon iso acids via isobtyryl CoA, leucine would give odd-carbon iso acids via isovaleryl CoA, and isoleucine would produce odd-carbon anteiso acids via 2-methyl butyryl CoA. Methyl malonyl CoA, known to be a direct metabolite of valine, would produce the branched acids bearing a methyl branch on one or more of the even-carbon atoms of the chain, including odd-carbon anteiso acids. Propionyl CoA, a metabolite of isoleucine and methionine, would be available for the production of additional methyl malonyl CoA as well as the odd-carbon straight-chain acids. This scheme would provide for the derivation of all the chain types claimed to be present in human surface lipids except even-carbon anteiso acids. It may be significant that even-carbon anteiso acids were not detected in the study by Nicolaides (1971), but he did not comment on their absence.

Structures of the unsaturated fatty acids

(a) *Monounsaturated acids.* The free unsaturated fatty acids of human hair lipid isolated and identified by Weitkamp et al. (1947) included compounds of both odd and even chain lengths bearing double bonds in either the 6,7 ($\Delta6$) or 8,9 ($\Delta8$) positions. In addilion, the mixture of C_{18} isomers included the acid with a 9,10 ($\Delta9$) unsaturation. These structural assigments were based on identification of the monocarboxylic acid fragments obtained by oxidative splitting of the individual acids at the point of unsaturation. Nicolaides et al. (1964) confirmed these structural assignments in the free fatty acids of adult hair lipid by reductive ozonolysis of preparative gas chromatographic fractions of the unsaturated fatty acid methyl esters. Their observations extended to both straight- and branched-chain acids having 13–26 carbon atoms. In the lower-chain lengths $\Delta6$ unsaturation was predominant, but in longer-chain compounds the double bond was displaced from the carboxyl group by the addition of one or more 2-carbon units. Again, oleic acid ($18\Delta9$) was the only isomer found which did not fit this pattern of unsaturation. A similar series of structures was found in the C_{13}–C_{19} unsaturated fatty acids of vernix caseosa.
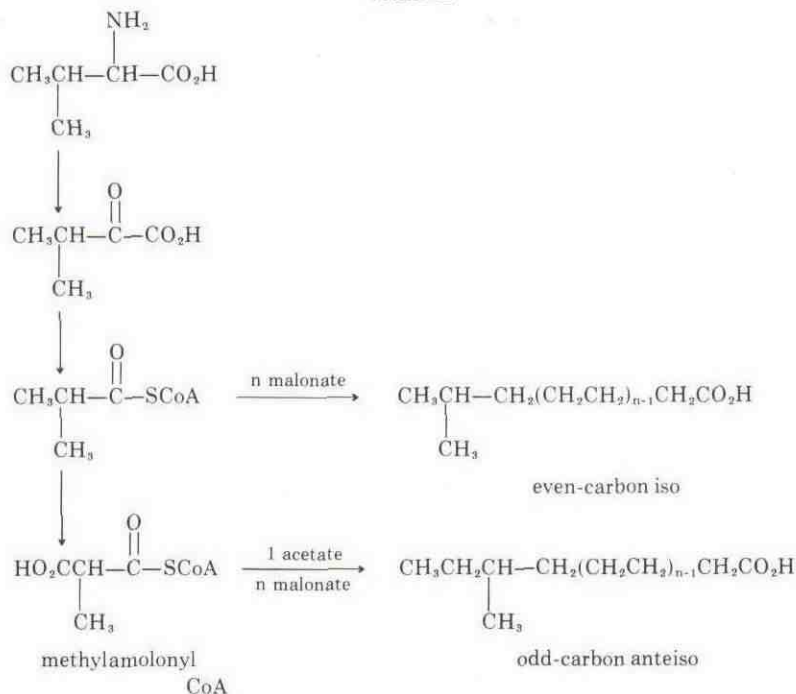
VALINE

$$CH_3CH-\overset{\overset{\displaystyle NH_2}{|}}{CH}-CO_2H$$
$$\underset{\overset{|}{CH_3}}{}$$

$\downarrow$

$$CH_3CH-\overset{\overset{\displaystyle O}{\|}}{C}-CO_2H$$
$$\underset{\overset{|}{CH_3}}{}$$

$\downarrow$

$$CH_3CH-\overset{\overset{\displaystyle O}{\|}}{C}-SCoA \xrightarrow{\text{n malonate}} CH_3CH-CH_2(CH_2CH_2)_{n-1}CH_2CO_2H$$
$$\underset{\overset{|}{CH_3}}{} \qquad\qquad\qquad\qquad \underset{\overset{|}{CH_3}}{}$$

even-carbon iso

$\downarrow$

$$HO_2CCH-\overset{\overset{\displaystyle O}{\|}}{C}-SCoA \xrightarrow[\text{n malonate}]{\text{1 acetate}} CH_3CH_2CH-CH_2(CH_2CH_2)_{n-1}CH_2CO_2H$$
$$\underset{\overset{|}{CH_3}}{} \qquad\qquad\qquad\qquad\qquad \underset{\overset{|}{CH_3}}{}$$

methylamolonyl   odd-carbon anteiso
CoA

FIG. 7: Proposed biosynthesis of branched-chain fatty acids from valine.

LEUCINE

$$CH_3CH-CH_2\overset{\overset{\displaystyle NH_2}{|}}{CH}-CO_2H$$
$$\underset{\overset{|}{CH_3}}{}$$

$\downarrow$

$$CH_3CH-CH_2\overset{\overset{\displaystyle O}{\|}}{C}-CO_2H$$
$$\underset{\overset{|}{CH_3}}{}$$

$\downarrow$

$$CH_3CH-CH_2\overset{\overset{\displaystyle O}{\|}}{C}-SCoA \xrightarrow{\text{n malonate}} CH_3CH-CH_2CH_2(CH_2CH_2)_{n-1}CH_2CO_2H$$
$$\underset{\overset{|}{CH_3}}{} \qquad\qquad\qquad\qquad\qquad \underset{\overset{|}{CH_3}}{}$$
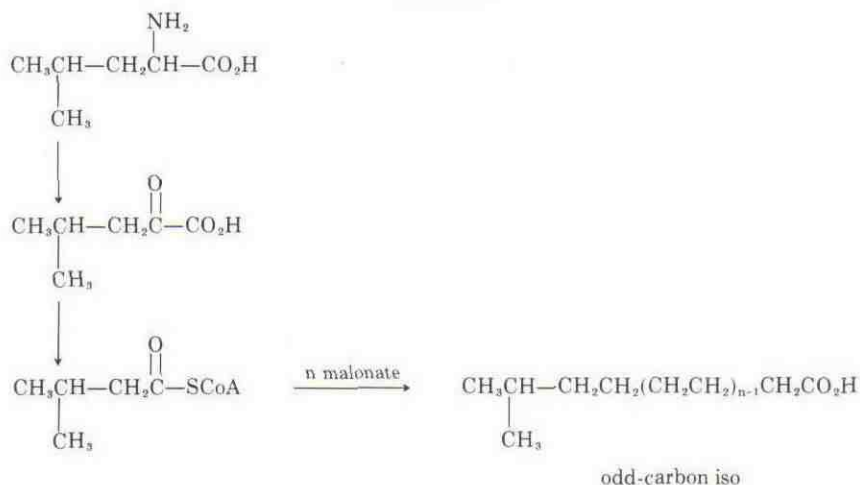
odd-carbon iso

FIG. 8: Proposed biosynthesis of branched-chain fatty acids from leucine.

Using preparative gas chromatography, Downing and Greene (1968a) isolated pure-chain-length fractions of up to 30 carbon atoms from the unsaturated fatty acid methyl esters of vernix caseosa. The double-bond positions in these mixtures of isomers were determined by splitting at the point of unsaturation with periodate-permanganate and analyzing the acidic fragments by pyrolysis of their tetramethylammonium salts to methyl esters in the gas chromatograph (Downing and Greene, 1968b). This procedure avoided the loss of volatile fragments and allowed the structures and relative amounts of the double-bond isomers to be determined. This showed that both $\Delta 6$ and $\Delta 9$ unsaturation were present in the shorter-chain lengths; in the longer-chain compounds, there were isomers derivable by addition of 2-carbon units to both of these bond positions, the $\Delta 9$ extension products being predominant.

Polyunsaturated acids. In the 18-carbon fatty

acids from human hair lipid, Weitkamp et al. (1947) detected the presence of compounds containing two and three double bonds and concluded that the dienoic acid was not the 9,12-linoleic acid usually found in animal tissues. Nicolaides and Ansari (1969) isolated the dienoic acids from the total acids of human hair lipid and showed that each chain length consisted of a mixture of isomers, the most abundant of which were $C_{18}\Delta5,8$, $C_{18}\Delta9,12$, and $C_{20}\Delta7,10$ (Table IV).

Biosynthesis of the unsaturated fatty acids. The biogenesis of the unusual double-bond positions in the unsaturated acids of human surface lipid has yet to be elucidated by direct methods. Predominance of the $\Delta6$ pattern of unsaturation found in fatty acids from human hair (Weitkamp et al.,

1947) made it unlikely that these acids were of bacterial origin. Nicolaides and Ray (1965) confirmed this by demonstrating that the $\Delta6$ pattern was predominant in some of the shorter-chain unsaturated acids of vernix caseosa. However, detailed quantitative analysis of the double-bond positions of the fatty acids of vernix showed that the $\Delta9$ unsaturation pattern was predominant in chain lengths greater than C_{17} (Downing and Greene, 1968a). In spite of the complexity of this mixture of unsaturated acids, the biogenesis of each compound detected can be explained on the basis of three enzymic reactions, outlined in Figure 11. This scheme requires only that preformed saturated acids be desaturated at either the 6,7 or 9,10 positions and then be extended by the famil-
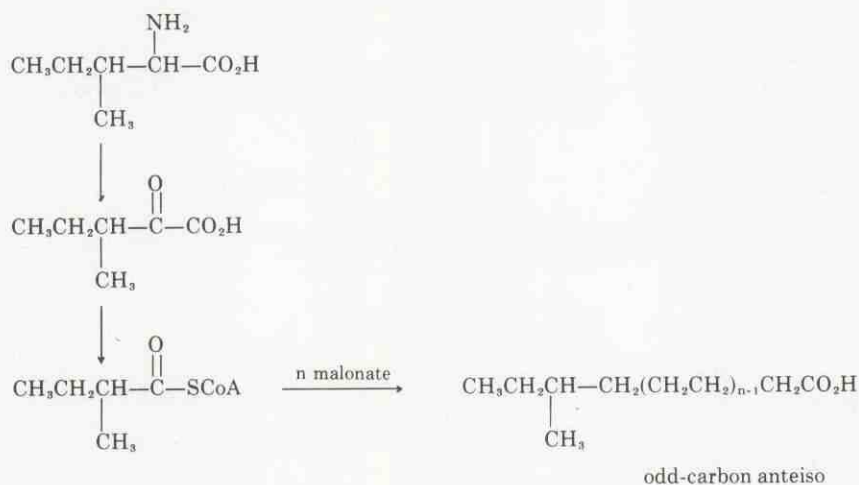
ISOLEUCINE

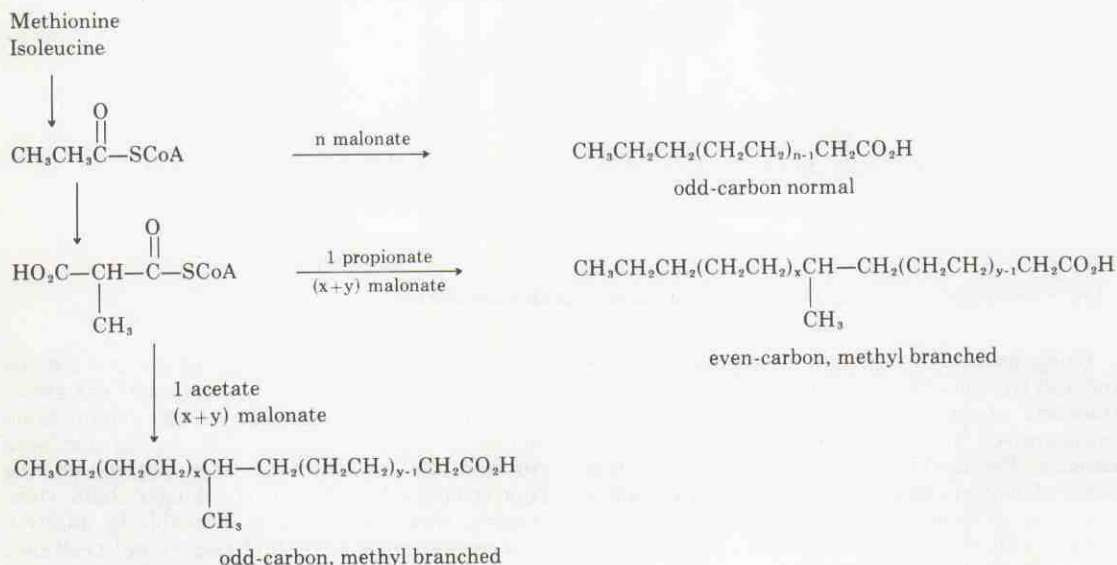FIG. 9: Proposed biosynthesis of branched-chain fatty acids from isoleucine.

FIG. 10: Proposed biosynthesis of branched-chain fatty acids from methionine and isoleucine via propionate.
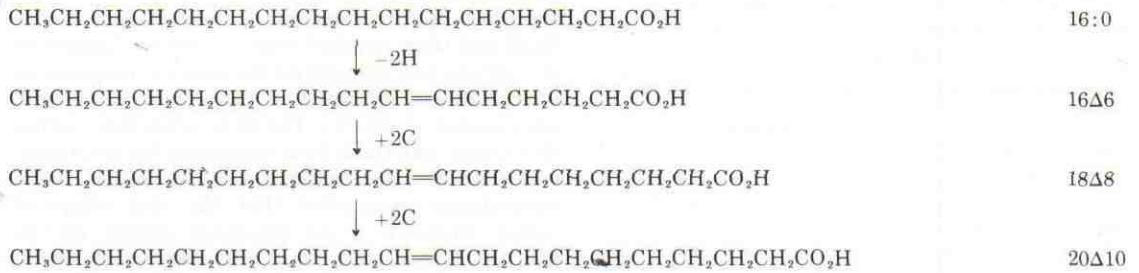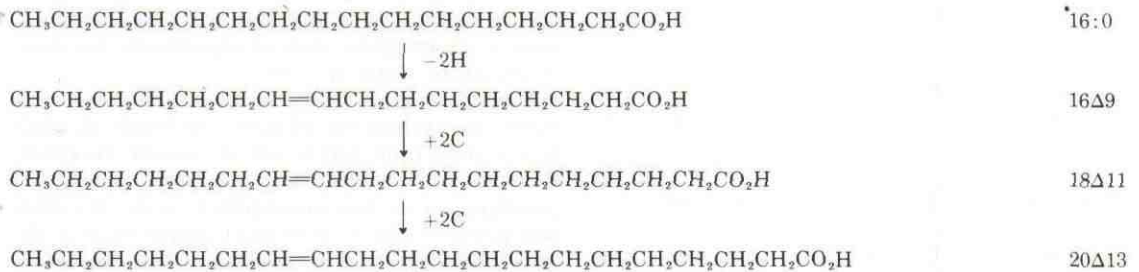
(a) *Biogenesis of fatty acids of the $\Delta 6$ series*

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CO_2H$ 16:0

$$\downarrow \;\; -2H$$

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCH_2CH_2CH_2CH_2CO_2H$ 16Δ6

$$\downarrow \;\; +2C$$

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCH_2CH_2CH_2CH_2CH_2CH_2CO_2H$ 18Δ8

$$\downarrow \;\; +2C$$

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CO_2H$ 20Δ10

(b) *Biogenesis of fatty acids of the $\Delta 9$ series*

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CO_2H$ 16:0

$$\downarrow \;\; -2H$$

$CH_3CH_2CH_2CH_2CH_2CH_2CH=CHCH_2CH_2CH_2CH_2CH_2CH_2CH_2CO_2H$ 16Δ9

$$\downarrow \;\; +2C$$

$CH_3CH_2CH_2CH_2CH_2CH_2CH=CHCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CO_2H$ 18Δ11

$$\downarrow \;\; +2C$$

$CH_3CH_2CH_2CH_2CH_2CH_2CH=CHCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CO_2H$ 20Δ13

FIG. 11: Proposed pathways for the biosynthesis of the monounsaturated fatty acids of human skin surface lipid. (a) Biogenesis of fatty acids of the Δ^6 series. (b) Biogenesis of fatty acids of the Δ^9 series.

iar 2-carbon addition involving acetate or malonate. Minor amounts of acids occur that could have arisen by chain shortening after desaturation. Downing and Greene (1968a) noted that both the $\Delta 6$ and $\Delta 9$ desaturation systems show a preference for a 16-carbon substrate (palmitic acid), whereas *in other animal tissues the 18-carbon acid* (stearic) is more readily desaturated.
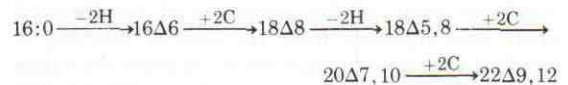
A similar biosynthetic scheme requiring one additional enzymic reaction can be invoked to explain the biogenesis of each of the dienoic acids of human surface lipid detected by Nicolaides and Ansari (1969) (Table IV). Thus the dienoic acids having double bonds in the 5,6 and 8,9 positions ($\Delta 5,8$), could be formed by $\Delta 5$ desaturation of the $\Delta 8$ monounsaturated acids previously produced by chain extension of $\Delta 6$-desaturated acids, e.g.:

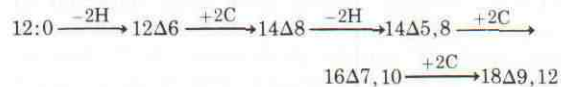$$16\Delta 6 \xrightarrow{+C_2} 18\Delta 8 \xrightarrow{-2H} 18\Delta 5,8$$

This proposal has the advantage that the additional unsaturation is introduced closer to the carboxyl group and separated by one methylene group from the original double bond. The production of polyunsaturated acids according to these criteria occurs widely in animal tissue (Mead, 1968). An enzyme system with the same characteristics could produce the $\Delta 6,9$ dienoic acids, which occur in human surface lipid in the C_{16} to C_{20} chain lengths, and also the two instances of $\Delta 4,7$ unsaturation, eg.:

$$18\Delta 9 \xrightarrow{-2H} 18\Delta 6,9$$

$$17\Delta 7 \xrightarrow{-2H} 17\Delta 4,7$$
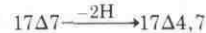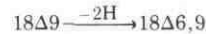
Finally, all of the remaining dienes, which have one of the double bonds more distant from the carboxyl group than the 9,10 position, can be accounted for by chain extension of either a $\Delta 5,8$ or $\Delta 6,9$ precursor by one or more 2-carbon units. The overall system would thereby be as follows:

$$16:0 \xrightarrow{-2H} 16\Delta 6 \xrightarrow{+2C} 18\Delta 8 \xrightarrow{-2H} 18\Delta 5,8 \xrightarrow{+2C}$$
$$20\Delta 7,10 \xrightarrow{+2C} 22\Delta 9,12$$

It is particularly intriguing that this system would include the possibility:

$$12:0 \xrightarrow{-2H} 12\Delta 6 \xrightarrow{+2C} 14\Delta 8 \xrightarrow{-2H} 14\Delta 5,8 \xrightarrow{+2C}$$
$$16\Delta 7,10 \xrightarrow{+2C} 18\Delta 9,12$$

The compound thus produced would be linoleic acid, an essential fatty acid required in the diet of animals because of the inability of animal tissues to directly introduce unsaturation further from the carboxyl group than the 9,10 position. Therefore, a pathway exists in skin whereby the metabolic blockage of linoleic acid synthesis could be bypassed. Although the presence in skin lipids of the necessary $12\Delta 6$ precursor has not been established, its synthesis can be inferred from the occurrence of $26\Delta 20$ and $28\Delta 22$ (Downing and Greene, 1968a).

TABLE IV

Proportions (mole %) of double-bond isomers in individual chain lengths of the dienoic acids of human scalp surface lipid

(adapted from Nicolaides and Ansari, 1969)

Double bond positions	Chain Length					
	Even carbon				Odd carbon	
	16	18	20	22	17	19
5,8	88	55	2		59	86
7,10	5	2	78	16	8	5
9,12		26	4	70	3	1
11,14			2			
Subtotal	93	83	86	86	70	92
4,7			1		10	
6,9	7	5	1		12	5
8,11		4	5	5	8	3
10,13		8	7	9		
Subtotal	7	17	14	14	30	8
Total	100	100	100	100	100	100
% of total dienes	1.8	70.5	22.6	1.2	1.6	1.8

Wilkinson (1970) incubated isolated human epidermal cells with 1-^{14}C-acetate and then separated the monoenoic and dienoic acids to determine the degree and position of labeling. Linoleic acid (18Δ9,12) was the most heavily labeled isomer, but the data clearly indicate that labeling occurred almost entirely by acetate exchange, since the monocarboxylic fragment from oxidative fission was not significantly labeled. The absence of net synthesis even in the nonessential unsaturated acids was apparent from the lack of labeling in 18Δ5,8, which is not labeled by acetate exchange because of the proximity of the double bond to the carboxyl group. In this study even the monounsaturated acids were labeled mainly by acetate exchange. Similar results have been obtained in incubations of human preputial skin and mouse skin with ^{14}C-acetate (Wilkinson, 1972); that is, labeling of polyunsaturated fatty acids occurred only by acetate exchange. There is, therefore, no direct evidence as yet that net synthesis of essential fatty acids can occur in skin.

Origins of the unsaturated fatty acids. The double-bond positions in fatty acids from specific lipid classes have been examined to determine which lipid classes are of sebaceous or epidermal origin. Wilkinson (1969) separated the cholesterol esters from the surface lipids of adult human forearm and found that 96 percent of the component unsaturated acids showed the Δ6 desaturation pattern. He therefore concluded that the sterol esters in the surface lipid were synthesized in the sebaceous glands. Nicolaides et al. (1972) examined the unsaturated acids from the wax and sterol esters of both vernix caseosa and adult surface lipid and from the diol esters of vernix (Ansari et al., 1970a) and calculated the relative proportions of the Δ6 and Δ9 series of monoenoic acids from each source (Table V). The fatty acids from vernix diol esters, like those from epidermal lipids (Ansari et al., 1970b) were mainly Δ9 unsaturated. They nevertheless concluded that the diol esters of vernix originate in the sebaceous glands, on the basis that diol esters are major components of the sebaceous lipids of other animals. This conclusion is not in accordance with the observation that diol esters are not detectable in normal adult sebum but form up to 10 percent of vernix caseosa lipids and are sometimes detectable in surface lipid samples from adults with an abnormally low level of sebaceous gland activity.

Nicolaides et al. (1972) also concluded that wax esters are exclusively sebaceous in origin in adult skin surface lipid and in vernix caseosa. However, this does not explain why the proportion of Δ9 desaturation in the unsaturated acids of vernix wax esters (12.23 %) is so much higher than in the unsaturated acids of adult surface lipid wax esters (1.42 %).

Nicolaides et al. (1972) do not accept the theory that sterol esters can be synthesized in the sebaceous glands. They account for the very high proportion of Δ6 desaturation in the sterol esters of adult surface lipid with the hypothesis that free sterols in the keratinizing epidermis become esterified with fatty acids derived from sebum.

Most investigators of human surface lipids have sought to describe the anatomic origins of specific lipid classes, and of their constituent fatty acids, on the basis of their derivation, under all conditions, from either the epidermis or the sebaceous glands. A less rigorous approach appears to provide a more rational scheme for the derivation of surface lipid constituents. Thus if we accept the premise that the unsaturated acids of sebum are almost exclusively of the Δ6 pattern and that those of the epidermis are almost entirely of the Δ9 pattern, we can calculate that surface lipid sterol ester in which the unsaturated fatty acids contain 11 percent Δ9 unsaturation and 89 percent Δ6 pattern

TABLE V

Relative proportions (mole %) of Δ6 and Δ9 unsaturation patterns in the monoenoic fatty acids of the wax and sterol esters of adult scalp surface lipids and in the wax esters, sterol esters and diol esters of vernix caseosa

(adapted from Nicolaides et al., 1972 and Ansari et al., 1970)

Type of unsaturation	Wax esters		Sterol esters		Diol esters
	Vernix	Adult	Vernix	Adult	Vernix
Δ6	87.07	98.28	30.35	88.92	12.76
Δ9	12.23	1.42	69.65	10.99	87.24

(Table V) could be obtained from a mixture of 98 percent sebum (containing 3% sterol esters) and 2 percent epidermal lipid (containing 15% of sterol esters). In the sterol esters of vernix caseosa, the unsaturated fatty acids contain 30.35 percent of the $\Delta 6$ and 69.35 percent of the $\Delta 9$ pattern. Therefore, we can assume that approximately two-thirds of these sterol esters are derived from epidermal lipid.

The wax esters of adult surface lipid contain unsaturated fatty acids, 98 percent of which are derived from $\Delta 6$ desaturation, as might be expected in a sebaceous lipid. However, the wax ester unsaturated acids in vernix contain 12 percent of the $\Delta 9$ pattern compounds. This might be explained by considering that under the special conditions in utero the epidermis might synthesize lipids which in the adult arise only from the sebaceous glands. The suggestion that mixing of fatty acids between esters occurs after secretion in utero is less attractive because of the very distinctive chain-length distribution of the wax ester fatty acids in vernix (Kärkkäinen et al., 1965; Nicolaides et al., 1972).

Hydroxyacids. Downing (1963) reported that 10 percent of the total acids in vernix caseosa were 2-hydroxyacids which appeared to be completely saturated and predominantly branched, and ranged in chain length from $C_{10}-C_{26}$. Other investigators have not reported the isolation of more than trace amounts of hydroxyacids from vernix, but Ansari et al. (1970a) found that the fatty acids from stratum corneum and the living layer of the epidermis contained about 5 percent of 2-hydroxyacids with 14–26 carbon atoms. However, they did not report on the presence of branched chains or unsaturation in this fraction. Nevertheless, it would appear that 2-hydroxyacids are characteristic of epidermal lipid rather than sebum, where their occurrence has not been described.

Fatty Alcohols

Composition. The structures of the fatty alcohols in human surface lipid bear a close relationship to those of the fatty acids. The chain structures include odd and even members of both the straight- and branched-chain series, in which there are unsaturated members where the unsaturation is predominantly of the $\Delta 6$ pattern. Brown et al. (1954) identified some of these structures in human hair lipid by mass spectrometry. Hougen (1955) isolated twelve pure alcohols from the same source by amplified distillation. He identified six saturated straight-chain compounds ($C_{14}-C_{24}$) and three saturated branched-chain structures of 20, 22, and 24 carbon atoms, as well as three straight chain unsaturated alcohols having 20, 22, and 24 carbon atoms in which the double bond positions were identified as $\Delta 10$, $\Delta 12$, and $\Delta 14$, respectively. The gas chromatographic studies of Boughton and Wheatley (1959), Haahti (1961), and Haahti and Horning (1963) indicated that the chain lengths of the alcohols ranged from $C_{12}-C_{24}$, with C_{20}, C_{22}, and C_{24} as the most abundant unsaturated homologues. Downing (1965) found that the saturated fatty alcohols in vernix caseosa consisted of approximately equal amounts of straight and branched compounds of 14–27 carbon atoms and this has been confirmed by Nicolaides (1967) for adult surface lipid fatty alcohols. Other studies have established that in the unsaturated fatty alcohols of adult surface lipid the positions of unsaturation are similar to those in the surface lipid fatty acids in all chain lengths (Haahti and Horning, 1963; Nicolaides, 1967).

Origins and biosynthesis of the fatty alcohols. In human surface lipid, monohydric fatty alcohols have been found only as esters with fatty acids, forming the wax ester fraction. They are thus identifiable as products of the sebaceous glands. However, they also occur as diesters with the 2-hydroxyacids found in vernix caseosa (Downing, 1963) and in epidermal lipids (Ansari et al., 1970a), in which case they might also be synthesized by epidermal cells. Free fatty alcohols have not been found in human surface lipids, apparently because of the inability of epidermal or bacterial lipases in the skin to hydrolyze wax esters (Downing, 1970).

The fatty alcohols are presumably produced by enzymic reduction of fatty acids, although the mechanism of this process is not understood. Nicolaides (1963) suggested that fatty acids would have to be extended by two 2-carbon units before reduction to account for the relationship between the structures of the acids and alcohols. However, it is now known that equivalent structures of acids and alcohols, both saturated and unsaturated, already exist in all chain lengths and require only the reduction step for formation of the alcohols. Nevertheless, some selectivity on the part of the reducing enzyme must be postulated to account for the difference in chain-length distributions between the fatty acids and fatty alcohols. These relationships must be considered separately for the saturated and unsaturated compounds:

In the saturated fatty acids, the most abundant constituents are the C_{14} and C_{16} straight-chain homologues, whereas in the alcohols the even-carbon straight-chain members from $C_{14}-C_{24}$ are all prominent constituents. There is, therefore, little apparent chain-length specificity on the part of the reducing enzyme presumed to be responsible for converting the saturated acids to saturated alcohols, although a profound depletion of the longer-chain saturated fatty acids does occur.

In the unsaturated fatty acid series, the C_{16} monoene is the principal homologue, with much smaller proportions of C_{14} and C_{18}, whereas in the unsaturated alcohols the C_{20} monoene is most abundant, with smaller but substantial amounts of C_{27} and C_{24}. However, in any one chain length the positions of double bonds and the relative amounts of the double-bond isomers are similar in the fatty acid and fatty alcohol series. Thus the differences in the chain-length distributions of the unsatu-

rated acids and alcohols could arise if the reducing enzyme showed a preference for selecting longer-chain acids for reduction to alcohols.

Kärkkäinen et al. (1965) and Nicolaides et al. (1972) noted the paucity of fatty acids above C_{18} in the wax esters compared with the acids of other esters. This could result from depletion of the longer-chain acids by conversion to alcohols, but only if wax ester synthesis were in some way isolated from the formation of other esters. There is some indication that such isolation does exist, in that wax esters might be formed at a much earlier stage in the life of the sebaceous gland cells than other major lipids.

Nicolaides (1963) has discussed the apparent selectivity which leads to the combination of acid and alcohol pairs in such a way that wax esters containing a saturated straight chain in both the acid and alcohol moieties are rare (Haahti, 1961). The presence of branching or unsaturation in one or both of the wax ester chains produces esters that are more likely to be liquid at skin temperature.

Fatty diols. The unsaponifiable fraction of vernix caseosa has been reported to contain 8 percent of 1,2-diols, which were isolated as their acetone adducts and analyzed by gas chromatography in this form (Downing, 1965). In this work the positions of the two hydroxyl groups were established by oxidizing the diols with periodate-permanganate and following this procedure with gas chromatographic analysis of the methyl esters of the fatty acid products. The results indicated that each diol molecule had lost one carbon atom in the oxidation. This work also established the absence of any significant amount of unsaturated diols, and allowed a quantitative analysis of the chain-length distribution, which extended from 18–26 carbon atoms and consisted mainly of branched-chain compounds (92.7%). Kärkkäinen et al. (1965) also analyzed the diols from vernix and concluded that they consisted of saturated straight-chain compounds. However, Fu and Nicolaides (1969) have confirmed Downing's conclusion that the diols of vernix are predominantly saturated, branched-chain alkane-1,2-diols.

The diols occur in vernix caseosa as diesters, in which they are esterified with two fatty acid moieties, and form up to 10 percent of the total lipid. They are not detectable in adult surface lipid except when the proportion of sebum is exceptionally low. Therefore, it seems reasonable to suppose that they are of epidermal origin, but their presence in lipids from this source has not been reported. The double-bond positions in the fatty acids of the diesters have been reported to be mainly of the $\Delta 9$ pattern and would thus support the proposal of an epidermal origin.

SQUALENE AND STEROLS IN HUMAN SKIN SURFACE LIPID

In the human sebaceous gland the biosynthetic pathway to the sterols appears to be at least partially inhibited in such a way that the last acyclic intermediate, squalene, accumulates. In most animal tissues, lanosterol, the product of cyclization of squalene, is converted to cholesterol by removal of three methyl groups, shifting of the ring-unsaturation from $\Delta 8$ to $\Delta 5$, and finally, reduction of the side-chain unsaturation. This is known as the Bloch pathway. Wilson (1963) has discussed the evidence that in rat skin and preputial gland, a different pathway may operate, in which all of the cyclic intermediates are esterified, and reduction of the side-chain unsaturation of the lanosterol esters is the first step. This system, the Kandutsch–Russell pathway, appears to operate in the skin of sheep, where esters of lanosterol and dihydrolanosterol are major constituents. In human skin, the presence of a small proportion of 7-dehydrocholesterol has been suggested (Miller and Baumann, 1954; Reinertson and Wheatley, 1959). This vitamin D_3 precursor would fit the Kandutsch–Russell scheme in that the side chain has been reduced before completion of the ring transformations, but it apparently occurs only among the free sterols. In addition, there is much evidence that transformations of steroidal hormones occur in human skin, and this has been the subject of several reviews (Grant, 1969; Milne, 1969; Strauss and Pochi, 1969; Reisner and Sansone-Bazzano, this issue).

CONCLUSIONS

Investigators concerned with the composition of human surface lipid have often taken the lead in applying novel techniques to elucidate the chemistry of this complex mixture. At least equally inspired investigations will be required to reveal the biosynthetic pathways leading to the unusual lipids produced in skin. Evidence regarding the precursors for lipid synthesis and the control of lipogenesis in human skin is accumulating, but the pathways proposed for the generation of the unusual lipid structures that have been found are almost entirely speculative. The biosynthesis of the branched-chain acids and alcohols, their unique patterns of unsaturation, the indications of unusual pathways of sterol biosynthesis, and the accumulation of sterol intermediates are all matters of considerable interest. Though often debated, the question remains: Do these constituents of human surface lipid have any biologic function?

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