Skin Lipids. IV. Biochemistry and Function

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Abstract

Metabolic processes and other types of alterations of the lipids of human skin are summarized and discussed.

Known functions of skin lipids are discussed, and in the light of new data presented in the papers of this series, new functions are speculated upon.

Biochemistry of Skin Lipids

SEBACEOUS GLAND LIPIDS. Human sebaceous glands excrete triglycerides, wax esters (i.e. fatty alcohols esterified to fatty acids), squalene, farnesol, a hydrocarbon that gives the Liebermann-Burchard test, possibly sterol esters and unidentified substances (1). Isotope incorporation studies of skin slices (2–6) show that acetate is incorporated into the fatty acids, squalene and sterols of human epidermis, dermis and total skin. Perfusion experiments with dog skin (7) show that uniformly labelled isoleucine is incorporated into the fatty acids of dog skin much more actively than is acetate. Furthermore, the incorporation of isotope is greatest in what appears to be a saturated "monobranched" C_{15} acid, an acid that is present in greatest amount in dog hair lipid (8) and most likely a sebaceous gland product. Straight chain acids as well as acids with chains having the *iso* and *anteiso* skeleton can be synthesized by addition of malonyl CoA to a number of substrates by enzyme preparations obtained from a variety of tissues (9). These syntheses require TPNH (reduced triphosphopyridine nucleotide). Enzymes responsible for the production of TPNH (i.e. glucose-6-phosphate dehydrogenase, 6-phosphogluconic acid dehydrogenase, of the hexose monophosphate shunt for the aerobic oxidation of glucose, and isocitric acid dehydrogenase of the Krebs cycle) are highest in the sebaceous gland compared to epidermis, dermis (superficial or deep), hair follicle, or sweat gland as determined by quantitative histochemical techniques (10). These facts indicate that active synthesis of fatty material is occurring in sebaceous glands.

The free fatty acids constitute the fraction of human surface lipid the components of which have been most completely chemically characterized. These acids result from a lipolytic breakdown of sebaceous gland triglycerides. This occurs in the duct of the gland and on the skin surface (11). Together with the acids of the triglycerides, the non esterified fatty acids comprise approximately 70% of the total fatty acid content of human surface lipid (the remainder occur in mono- and diglycerides, wax esters and sterol esters). Some data concerning structures and amounts are given in (12) and in Tables I and II. [The data in Table I are hitherto unpublished and were obtained by previously described methods (12,13)].

It is thus very probable that human sebaceous glands synthesize acids with the following types of carbon skeletons:

a) saturated straight chains with an even or an odd number of carbon atoms,

b) saturated chains with the *iso* structure and an even number of carbon atoms,

c) saturated chains with the *anteiso* structure and an odd number of carbon atoms,

d) saturated chains of an unknown type of branching of both an even and an odd number of carbon atoms,

e) *cis* unsaturated chains with carbon skeletons as in a), b) and c) at C_6 or derivable from this position by addition or subtraction of an integral number of C_2 units from the carboxyl group.

Based on well-established biosynthetic pathways, the following scheme can be postulated for the biosynthesis of these fatty acid carbon skeletons:

Acetyl CoA + malonyl CoA ——→ straight chain fatty acids with an even number of carbon atoms

Propionyl CoA + n malonyl CoA —→ straight chain fatty acids with an odd number of carbon atoms

Isobutyryl CoA + n malonyl CoA —→ *iso* acids with an even number of carbon atoms

α-Methyl-butyryl CoA + n malonyl CoA ——→ *anteiso* acids with an odd number of carbon atoms

where n is a number such as to produce fatty acids mainly of chain lengths from C_{14} to C_{18}. Chain lengths from C_8 to C_{30} have been reported (all references in 12). It is not known whether the short chain acids are synthesized as such or are breakdown products of longer acids. The amino acids valine and isoleucine could be the source of isobutyryl CoA, and α-methyl-butyryl CoA respectively (9). The double bond pattern of the unsaturated acids suggests that

TABLE I

Composition of the Free Fatty Acids of Human Surface Lipid[a]

	%
Saturated fatty acids	
Straight chain even	40
Straight chain odd	7
Branched chain	11
Total saturated	58
Monoenoic fatty acids	
Straight chain even	32
Straight chain odd	3
Branched chain even	2
Branched chain odd	2
Total monoenes	39
Polyenoic fatty acids	
Dienes	3
Tri- and tetraenes	traces
Total branched (saturated and unsaturated)	15
Total straight (saturated and unsaturated)	85

[a] Principal chain lengths C_{14} to C_{18}.

TABLE II

Neutralization Equivalents (N.E.) and Iodine Values (I.V.) for Various Acids and Alcohols from Adult Human Skin Surface Lipid[a]

	Lipid obtained by					
	Scalp soaks				Pooled cut hair extracts	
	(Male)		(Female)		(Male)	
	N.E.	I.V.	N.E.	I.V.	N.E	I.V.
1. Acids						
Free	261	45.3	266	44.6	261	48.3
From triglycerides	262	43.4	262	44.9	269	53.5
From di- and mono-glycerides (early)[b]	279	52.9	—	—		
From di- and mono-glycerides (late)[b]	282	53.6	320	—	333	—
From wax esters	255	82.8	255	80.1	258	73.4
From branched chain esters[c]	268	78.8	267	75.5	263	56.4
2. Alcohols (wax)	300[d]	37.2	300[d]	36.8	—	34.3

[a] Reproduced by permission of JAOCS (14).
[b] "Early" and "late" refer to a rechromatographed fraction in which "early" was richer in diglycerides and "late" was richer in monoglycerides (Ref. 14).
[c] Includes 50% sterol ester.
[d] Reference 15.

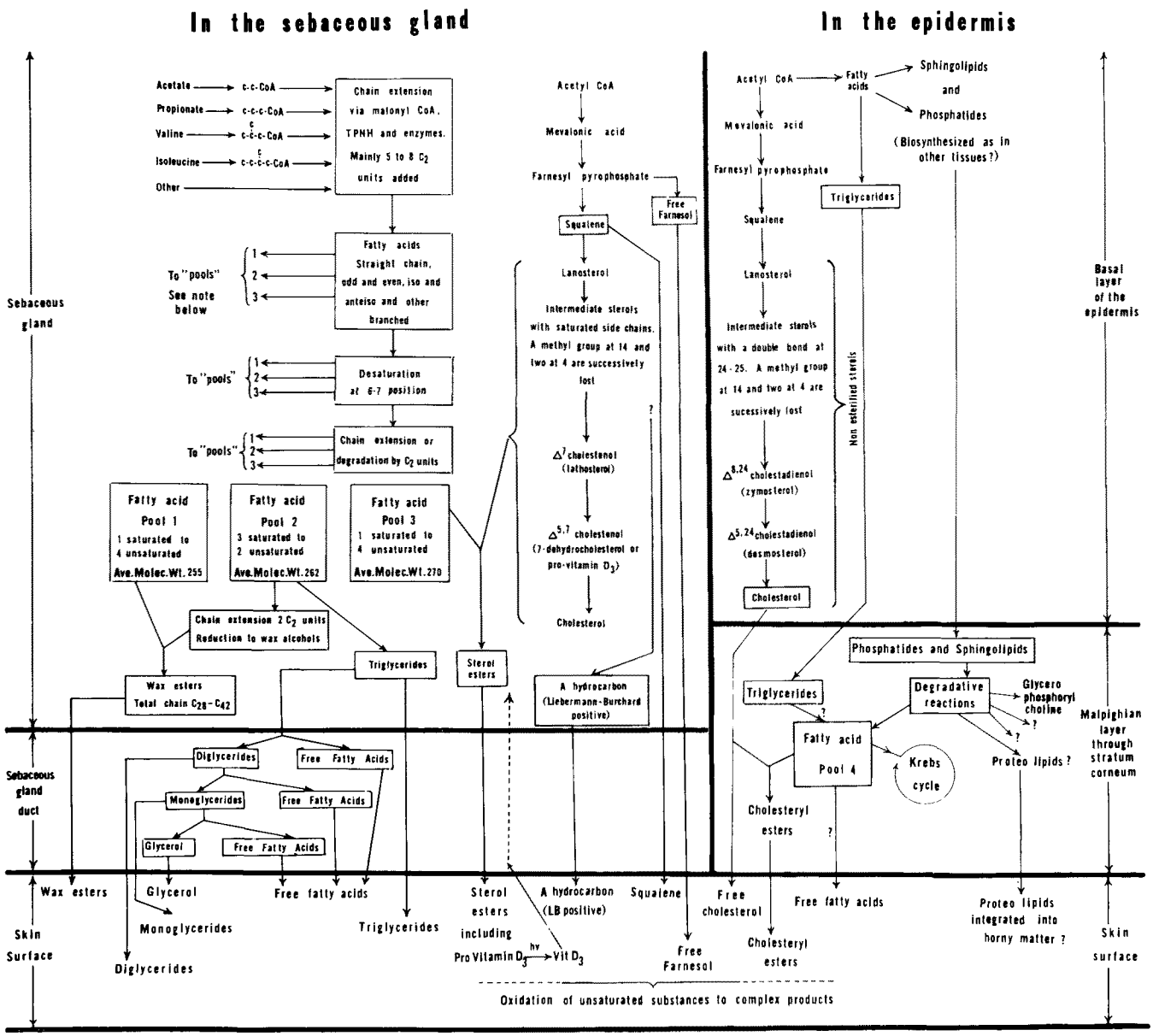

FIG. 1. Biochemistry of lipids of human skin. Note: Fatty acid "pools" are not necessarily specific geographic localization. They are *most* likely the result of enzymatic selection.

these are desaturated at the 6–7 position and either elongated or degraded by an integral number of C_2 units, elongation being preferred (13).

Different groups of these saturated and unsaturated fatty acids constitute what are here being called Pools 1, 2, 3 and 4 of Fig. 1. The evidence for the delineation of 3 groups of fatty acids into "Pools" in the sebaceous gland is given in Table II. Differences in iodine values and neutralization equivalents for the fatty acids listed show that certain acids are esterified to glycerol, certain others to the wax alcohols and still others to the sterols. The word "Pool" is not intended to imply a specific location of entire groups of fatty acids in the cell, although this possibility should not be ruled out. It is used here in the sense of a group that is probably delineated by virtue of enzyme specificity.

Some of the longer chain acids (possibly from Pool 2) could be extended two C_2 units and reduced to form fatty alcohols (wax alcohols). This is postulated because the structures of the fatty alcohols parallel those of the fatty acids of this Pool except that the alcohols average two C_2 units greater in length than the acids (11). The detailed mechanism for the formation of the alcohols is not known.

The fatty alcohols are esterified with the fatty acids of Pool 1 to form wax esters. The total chain length of acid plus alcohol moieties of the wax esters extends from C_{28} to C_{42} (16,17). Combinations of fatty acid and fatty alcohol are such as to produce esters that melt at skin temperature (11). Acids of Pool 1 have a rather high degree of unsaturation. No free wax alcohols are found in skin lipids which suggests that the esterification is very likely accomplished immediately after the alcohol is formed, perhaps through some terminal reactive derivative.

Other acids or their CoA derivatives of Pool 2 form triglycerides. Sebaceous glands also appear to syn-

thesize sterol esters, but not free sterols (see biosynthesis of epidermal lipids). The fatty acids esterified to these sterols are of the more unsaturated ones of the sebaceous gland type (18,14).

Farnesol would presumably be formed by hydrolysis of its pyrophosphate used in the synthesis of squalene. The hydrocarbon that gives the Liebermann-Burchard test could be formed in the process of formation or degradation of sterols.

Epidermal Lipids

a) Sterols and sterol esters. The extensive study of the metabolism of sterols of the skins of animals and of man has been reviewed recently by Kandutsch (19). He discussed the various intermediates and pathways that can lead to the formation of cholesterol from lanosterol, one of the first cyclization products formed by squalene. Bloch and his colleagues have shown that the predominant pathway for the synthesis of cholesterol for most tissues is via intermediates with unsaturation in the side chain (i.e. a double bond at 24–25). In this pathway all transformations of the nucleus of the sterol occur prior to the reduction of desmosterol ($\Delta^{5,24}$ cholestadienol) to form cholesterol. These transformations involve loss of three methyl groups (one at carbon 14 and two at carbon 4) and migration of double bond from either Δ^7 or Δ^8 to Δ^5. Kandutsch and Russell (20) showed, however, that in a tumor of the mouse preputial gland, cholesterol can be synthesized by an alternate sequence of reactions. The intermediates in this case, save for lanosterol, all involve saturated side chains. Two of these intermediates are Δ^7 cholestenol (lathosterol) and $\Delta^{5,7}$ cholestadienol (7-dehydrocholesterol or provitamin D_3). Wilson (21) pointed out that the intermediates of the Kandutsch-Russell pathway are primarily in the esterified form and ". . . that a fundamental difference between these pathways may be that the reactions of the Kandutsch-Russell pathway utilize only esterified intermediates whereas the Bloch pathway can utilize free intermediates." He presented evidence for the existence of both pathways in rat skin and the Kandutsch-Russel pathway in rat preputial gland.

Wilson also noted that ". . . since free sterols are found primarily in the epidermis and esterified sterols in the dermis, it seems logical that the free pathway is present in the epithelium whereas the sebaceous gland of the dermis may utilize the esterified pathway for cholesterol synthesis." (Since sebaceous glands normally lie fairly deep in the dermis, they would remain in dermal tissues in most techniques to separate it from epidermis.) In support of this view histochemical data show that sterol esters but not free sterols (as defined by the Liebermann-Burchard color reaction) are present in the sebaceous gland of the rat (22), of the human ear canal (23), and the ordinary sebaceous glands of man (24). Haahti et al. also state (18) that the fatty acids of the sterol esters resemble those of the sebaceous glands. These are presumably acids of the type listed above for sebaceous glands and not of the type as found by Reinertson and Wheatley (25) for epidermis.

Sterol esters also occur in the keratinizing epidermis. Kooyman (26) observed that sterols of the basal layer of the epidermis from soles, (sebaceous glands absent) were largely non esterified (~90%) but those from the cornified layer of soles had become more esterified (~80% were free). Our data (1) and those of Reinertson and Wheatley (25) also show that the keratinizing epidermis of soles produce sterol esters. Reinertson and Wheatley reported that 7-dehydrocholesterol, an intermediate of the Kandutsch-Russell pathway, occurred in lipids of the sole epidermis. Also, Δ^7 cholestenol (another such intermediate) occurred in mouse skin in both free and esterified forms (27), although it occurs free to a much smaller extent (10%). Thus, the hypotheses that a) the Kandutsch-Russell pathway operates *only* via esterified intermediates and that b) these intermediates are *only* of sebaceous gland origin must be accepted with some reservation, although they may apply to the bulk of the sterols found in skin tissue. Sterol esterases may complicate the issue.

The conflicting results of Reinertson and Wheatley (25) and those of Gaylor and Sault (28) should be mentioned. Reinertson and Wheatley conclude that the site of 7-dehydrocholesterol synthesis, which is pro vitamin D_3 and an intermediate of the Kandutsch-Russell pathway, was largely in the Malpighian layer. Gaylor and Sault, however, conclude that the site of 7-dehydrocholesterol synthesis is the sebaceous gland in rat skin and in human skin. There is also the possibility that the pro vitamin is generated in both sources.

That sterol esters may be intimately involved with the process of keratinization of human epidermis is suggested by recent work of Gara et al. (29). They found that the ability of the human skin surface to esterify cholesterol in patients with psoriasis, a disease of excessive and abnormal keratinization, was one tenth that of normal human controls.

b) Other epidermal lipids. Besides free sterols and sterol esters other lipids of human epidermis are triglycerides, free acids, sphingolipids and phosphatides (1). It is assumed that the phosphatides, sphingolipids and triglycerides are synthesized by processes known to occur in other tissues. The fatty acids for these syntheses (25) appear to be those found in other internal tissues, presumably synthesized by similar routes.

Human Skin Surface Lipids. The lipids of the end product of the keratinization process plus those of the altered sebum excretion constitute the skin surface lipids (Fig. 1). Free fatty acids and sterol esters can originate from either keratinizing epidermis or from sebum. The contribution from epidermis should be small (1).

Some lipids undergo reaction on the skin surface. Squalene forms small amounts of a variety of complex oxidation products. Unsaturated fatty acids can also be oxidized. However, surface lipid is surprisingly stable. Perhaps the ferric chloride reducing substance found by MacKenna et al. (30,1) serves as an antioxidant. Since the acid number of surface fat increases as it remains on the skin surface, glyceride lipolysis can go further toward completion. Resident microflora may assist in this process. They may also alter other constitutents and add some of their own products to skin surface lipids as well.

Lipids of Other Sebaceous Type Glands in Man and Animals. Thus far the formation of lipids by ordinary human sebaceous glands has been considered. The lipids produced by specialized human sebaceous structures can differ from these markedly (1). Even the lipids of supposedly ordinary sebaceous glands show some variation. For example, lipids expressed from the large sebaceous glands of the nose and cheek have much less triglyceride and much more free acid than surface lipids of the scalp (Fig. 2). This might be a consequence of exposure of sebum to larger ducts and more lipase. Meibomian gland lipids differ even

more dramatically. A mixture of wax ester and sterol ester constitutes about three quarters of this sebaceous excretion and little squalene or triglyceride is present (1). This mixture is a semisolid in contrast to sebum which is an oil at body temperature.

Additional variations in the fatty chains than those mentioned above appear in other types of sebaceous excretions in man and animals. At least three types of hydroxylated fatty chains are found, for example. α-Hydroxy fatty acids have been found in *vernix caseosa* (31), in wool wax (32,33), and in total rat skin (34). The α, β diols as well as α-hydroxy fatty acids have been found in wool wax (32). The fatty chains of the α, β-diols can be straight (with an odd or an even number of carbon atoms), *iso* or *anteiso*. Such chains with two functional groups are probably components of diesters (1).

Other types of chain branching than those mentioned above also exist in sebaceous excretions. *Iso* fatty acids with an *odd* number of carbon atoms have been found in wool wax (33). These structures could be biosynthesized from isovaleryl CoA (derived from leucine) and malonyl CoA (9). The fatty acids of the wax esters of the preen gland of the goose are poly branched, i.e. 2,4,6,8-tetramethyldecanoate and 2,4,6,8-tetramethylundecanoate. Murray (35) suggested that the branched C_{14} acid (the main component) is biosynthesized by adding to one acetate moiety 4 propionate units whereas the C_{15} acid is built up from 5 propionate units (35). Noble et al. (36) showed that the C_{14} acid is formed by incorporation of propionate in the predicted manner.

Functions of Skin Lipids

Epidermal Lipids. Lipids serve two major functions: as an energy source and as building units for cellular membranes. In the epidermis the germinative layer (basal layer) is the site of mitotic activity and lipids must be synthesized for all new membranes. The energy for these syntheses undoubtedly comes from circulating glucose which seeps up from the capillaries of the dermis. Cell organelles begin to degrade as the cell moves outward to keratinization (37). The lipids of the membranes of the cell organelles are also degraded and appear to be utilized as a significant energy source as the cells get farther away from external nutrients. Evidence for this comes from respiration studies where glucose and protein cannot account for all the oxygen utilized. This implies a significant utilization of lipid (38–40). In a study of the incorporation of P^{32} into the phosphate esters of guinea pig ear skin (40), Yardley and Godfrey found significant activity of P^{32} in phosphoryl choline if glucose was absent, but not if glucose was present. They suggested that this activity came from lecithin breakdown whereupon the released fatty acids served as substrate for utilization of oxygen.

Sebaceous Gland Lipid. The lipids that comprise the sebum of an animal can make it a free flowing oil or a semisolid wax at body temperature. Squalene and possibly triglycerides will lower the melting point, for squalene melts well below 0C, and triglycerides with one *cis* unsaturated fatty acid in the β position, as is the usual case, will ordinarily be an oil at body temperature. Chain branching will also lower the melting point and saturation of *cis* double bonds will raise it. Diesters of fatty chains with two functional groups, e.g. the hydroxy fatty acids, may raise the melting points above those of simple esters if greater intermolecular contact can be established. Thus, sebaceous

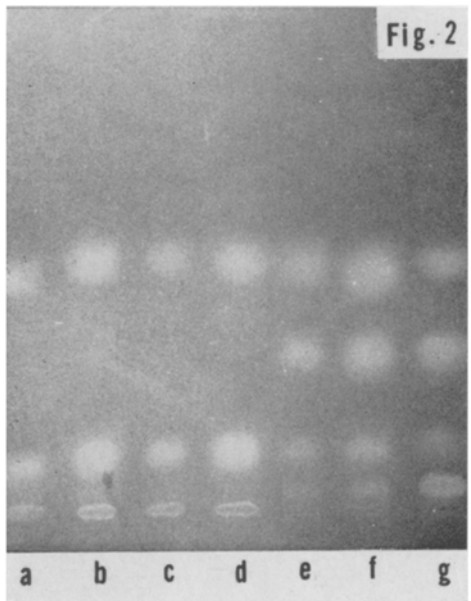

FIG. 2. The plate was developed in hexane/ether 4/1 (v/v) containing 1% acetic acid as previously described (1). The chromatograms are a) and b) 50 and 100 μg lipid expressed from a large sebaceous gland from the nose of a young man, c) and d) 50 and 100 μg, respectively, of lipid expressed from the cheek of the same young man, e) and f) are 50 and 100 μg of human scalp surface lipid and g) is 20 μg cholesteryl palmitate, 30 μg triglyceride, 20 μg palmitic acid and 20 μg free cholesterol. The material expressed from human sebaceous glands shows very little triglyceride and much free acid. This is in contrast to human surface fat from the scalp where there is a considerable amount of material in the triglyceride region as well as less free acid. This indicates that there is a significantly greater amount of lipase in these large sebaceous glands of the face and nose than there is in other normal glands such as those of the scalp.

type glands have a variety of means to prepare "oilier" or "waxier" surface by utilizing such devices as saturation vs. unsaturation, branching vs. straight chain and from one to three ester groups in the molecule as in a simple wax ester or sterol ester, a diester wax or a triglyceride.

Skin surface can be viewed as aqueous, oily, or waxy. An aqueous skin surface is a high friction, sticky surface. Palms and soles have a thick stratum corneum, a rich supply of sweat glands, and no sebaceous glands or hair, a combination that makes an ideal surface for grasping. An oily surface would best suit areas where two skin surfaces are in contact, e.g. the axilla or the anogenital region. Thus rat preputial gland lipid contains squalene and triglyceride but none of the higher melting more polar wax esters found on the skin surface (1). Also horse smegma contains an appreciable amount of squalene (41). A waxy surface would better serve a waterproofing function than an oily one, for a solid would not float away at an aqueous interface as readily as an oil would. Such waxes could be the long chain more saturated wax esters or the diesters of the hydroxy fatty acids. Hydroxy fatty acids comprise ~10% of *vernix caseosa* fatty acids but none of human surface lipid. Since the fetus bathes in anmionic fluid, a more waterproofing skin surface might better serve it. The semisolid wax produced by Meibomian glands can help to seal in the moisture of the eyes during sleep. Here too, as in the case of *vernix caseosa,* waxes would be superior to oils in this aqueous environment. An aqueous environment might account for some similarity in the composition of waxes of the Meibomian gland and *vernix caseosa* (1). Animals with a heavy pelage such as

the sheep or the rat would be better protected against wetting by a waxy rather than an oily excretion on their hair. Wax diesters could help maintain higher melting points. In the case of man where hair is only vestigial and eccrine sweat glands numerous, a free flowing oil which human sebum is (its squalene and triglyceride content are both high) would better spread over a moist skin surface. Birds must have a waterproof waxy coat. The manufacture of a rather high melting wax plus the instinct to preen meets this need successfully. Thus, the concept that a prime function of sebaceous glands is to prepare an oily or a waxy surface (or a sticky surface by absence of glands) explains not only variations in composition between species but variations within a given species at different anatomical sites.

The surface lipid film might serve additional functions. The lipid film may assist in the absorption of vitamin D. The free fatty acids of the surface lipid of man may help maintain a compatible microflora, although this issue has been debated (42,43). The rat and the goose apparently do not require much free fatty acid for this function for they do not have an appreciable amount. Most other animals, however, do not have an eccrine sweating mechanism that provides a moist and favorable environment for microfloral growth. Some of the more volatile components of skin surface lipid may serve a "social" function, such as sexual attraction or recognition. As was beautifully demonstrated in this Symposium, insects secrete such substances (44). Man himself uses farnesol—which occurs in his own surface lipid—as a perfume.

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REFERENCES

1. Nicolaides, N., Skin Lipids. II, JAOCS *42*, 691–702 (1965).
2. Nicolaides, N., O. K. Reiss and R. G. Langdon, J. Am. Chem. Soc. *77*, 1535 (1955).
3. Nicolaides, N., and S. Rothman, J. Invest. Derm. *24*, 125–9 (1955).
4. Patterson, J. F., and R. D. Griesemer, J. Invest. Derm. *33*, 281–6 (1959).
5. Griesemer, R. D., and R. W. Thomas, J. Invest. Derm. *41*, 95–8 (1963).
6. Griesemer, R. D., and R. W. Thomas, *Ibid. 41*, 235–8 (1963).
7. Wheatley, V. R., D. C. Chow and F. D. Keenan, Jr., J. Invest. Derm. *36*, 237–9 (1961).
8. Wheatley, V. R., and D. Sher, *Ibid. 36*. 169–70 (1961).
9. Horning, M. G., D. B. Martin, A. Karmen and P. R. Vagelos, Bioch. & Bioph. Res. Comm. *3*, 101–6 (1960).
10. Hershey, F. B., Chap. IX *in* "The Epidermis", Ed. W. Montagna and W. C. Lobitz, Jr., Academic Press, New York, 1964, pp. 145–60.
11. Nicolaides, N., Chap. XI *in* "The Sebaceous Gland," Vol. 4, Advances in the Biology of Skin. Ed. W. Montagna, R. A. Ellis and A. F. Silver, MacMillan, New York, 1963, pp. 167–87.
12. Nicolaides, N., and T. Ray, Skin Lipids: 3 (this symposium).
13. Nicolaides, N., R. E. Kellum and P. V. Woolley III, Arch. Biochem. Biophys. *105*, 634–9 (1964).
14. Nicolaides, N., and R. C. Foster, Jr., JAOCS *33*, 404–9 (1956).
15. Brown ,R. A., W. S. Young and N. Nicolaides, Anal. Chem. *26*, 1653 (1954).
16. Haahti, E., Scand. J. Clin. Lab. Invest. *13*, Suppl. 59 (1961).
17. Nicolaides, N., J., Invest. Derm. *37*, 507–10 (1961).
18. Haahti, E., T. Nikkari and K. Juva, Acta Chem. Scand. *17*, 538–40 (1963).
19. Kandutsch, A. A., Chap. XXV in "The Epidermis," Ed. W. Montagna and W. C. Lobitz, Jr., Academic Press, New York, 1964, pp. 493–506.
20. Kandutsch, A. A., and A. E. Russell, J. Biol. Chem. *235*, 2256–61 (1960).
21. Wilson, J. D., Chap. X *in* "The Sebaceous Gland," Vol. 4, Advances in the Biology of Skin, Ed. W. Montagna, R. A. Ellis and A. F. Silver, MacMillan, New York, 1963, pp. 148–65.
22. Montagna, W., and C. R. Noback, Am. J. Anatomy *81*, 39–60 (1947).
23. Montagna, W., C. R. Noback and F. G. Zak, Am. J. Anat. *83*, 409–35 (1948).
24. Suskind, R. R., J. Invest. Derm. *17*, 37–54 (1951).
25. Reinertson, R. P., and V. R. Wheatley, J. Invest. Derm. *32*, 49–59 (1959).
26. Kooyman, D. J., Arch. Derm. Syph. *25*, 444–50 (1932).
27. Brooks, S. C., and C. A. Baumann, Cancer Res. *16*, 357–63 (1956).
28. Gaylor, J. L., and F. M. Sault, J. Lipid Res. *5*, 422–31 (1964).
29. Gara, A., E. Estrada, S. Rothman and A. L. Lorincz, J. Invest. Derm. *43*, 559–64 (1964).
30. MacKenna, R. M. B., V. R. Wheatley and A. Wormall, Biochem. J. *52*, 161–8 (1952).
31. Downing, D. T., Aust. J. Chem. *16*, 679–82 (1963).
32. Downing, D. T., Z. H. Kranz and K. E. Murray, Aust. J. Chem. *13*, 80–94 (1960).
33. Pelick, N., and J. W. Shigley, paper presented at AOCS meeting, New Orleans. La., April 22, 1964.
34. Kishimoto, Y., and N. S. Radin, J. Lipid Res. *4*, 139–43 (1963).
35. Murray, K. E. Austr. J. Chem. *15*, 510 (1962).
36. Noble, R. E., R. L. Stjernholm, D. Mercier and E. Lederer, Nature *199*, 600–1 (1963).
37. Nicolaides, N., Chap. XXVI in "The Epidermis," Ed. W. Montagna and W. C. Lobitz, Jr., Academic Press, New York, 1964, pp. 511–35.
38. Freinkel, R., Chap. XXIV in "The Epidermis," pp. 485–92.
39. Cruickshank, C. N. D., M. D. Trotter and J. R. Cooper, J. Invest. Derm. *39*, 175–9 (1962).
40. Yardley, H. J., and G. Godfrey, J. Invest. Derm. *42*, 51–7 (1964).
41. Sobel, H., J. Invest. Derm. *13*, 333–8 (1949).
42. Rothman, S., and A. Lorincz, Ann. Rev. Med. *14*, 215–42 (1963).
43. Kligman, A. M., Chap. VII *in* "The Sebaceous Gland," Vol. 4, Advances in the Biology of Skin, Ed. W. Montagna, R. A. Ellis and A. F. Silver, MacMillan, New York, 1963, pp. 110–24.
44. Jacobson, M., Isolation and characterization of insect attractant lipids. This symposium.