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THE SKIN SURFACE LIPIDS OF MAN COMPARED WITH THOSE OF EIGHTEEN SPECIES OF ANIMALS*

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Investigators could study the biochemistry of human skin surface lipids more conveniently if they could discover experimental animals that produced the same lipids. Human skin surface lipids differ markedly from those of the animals previously studied such as the sheep (1), various rodents (rat, mouse, guinea pig, and rabbit) (2), and birds (3). Possible significance of these differences has been discussed (4–8). To define the details of similarities and differences, we compared, by thin layer chromatography (TLC), the skin surface lipids of adult humans, vernix caseosa, and 18 species of animals. We found additional differences, but more significantly, that only man produces a "triglyceride type" of sebum. In this report we describe and discuss these findings.

MATERIALS AND METHODS

Sampling. The animals studied, the number of samples and the manner they were taken are given in Table I.† In Method 1, the animal was physically restrained, anaesthetized, or freshly killed, the hair clipped with scissors (electric clippers were avoided because they contaminate the skin surface with hydrocarbon lubricant), an open bell jar 7 cm dia. pressed firmly to the exposed skin, and redistilled hexane quickly poured into the jar onto the skin surface. The solvent was irrigated over the skin with the aid of a large pipette equipped with a rubber bulb, taking care that no solution got into the bulb, then transferred to another container with the same pipette.

In Method 2, the animal was first anaesthetized with ether, its tail, feet and anus wiped with cotton pledgets soaked in hexane, then dipped up to its neck in a beaker of redistilled hexane or rolled on its back in a large shallow dish containing hexane.

The hexane solution from each of the above methods was filtered through a medium sintered glass funnel, the solvent removed on a rotary evaporator and the residual lipid weighed to constant weight.

Thin layer chromatography. The basic TLC procedures have been described elsewhere (6, 10) except for multiple development of plates. This consisted of developing the plate, with a sequence of solvents of decreasing polarity, the later less polar solvent being allowed to develop farther up the plate than the earlier more polar one. After the development with each solvent was complete, the plate was removed from the tank, air dried for 5 to 10 minutes, then put into another tank containing the next solvent of the sequence. Table II shows the two sequences of solvents we used and what each development accomplished.

The monoester region, where cholesteryl oleate migrated could be a mixture of sterol esters, a mixture of wax esters, or both. Both ester mixtures were usually complex in that a number of molecular species of sterols or wax alcohols were each esterified to a variety of fatty acids. Sterol esters could not be separated from wax esters on silicic acid/magnesium silicate 9/1 by weight, the main absorbent of this study, because differences in chain length, unsaturation, and other structural features of the various fatty acid, fatty alcohol and sterol moities caused the spots from each ester class to spread and overlap with each other. Better, although still incomplete, separation was achieved by TLC on alumina (11). The analysis of the monoesters were thus based on these two types of TLC and on the Liebermann Burchard test for sterol esters performed as follows. After alumina TLC, the dried plates were sprayed with conc. H_2SO_4, heated to 100°C for 5 minutes, the plate photographed, then resprayed with standard charring mixture (55% conc. H_2SO_4, 44.4% H_2O and 0.6% $K_2Cr_2O_7$ by weight, ref. 12).

Identification of the diesters, which migrated in the region between the monoesters and triglycerides (triolein), were based on TLC and infrared spectra of the saponification products of material obtained by preparative TLC (to be published at a later date).

RESULTS AND DISCUSSION

Figs. 1 through 4 are photographs of representative thin layer chromatograms of the surface lipids of four different groups of animals, each group of which is compared with

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TABLE I

Surface lipid sampling data for various species

	Sample site	No. of samples analyzed	Method lipid obtained*
1) Horse	Back and sides	2	Method 1
2) Cow	Back and sides	2	Method 1
3) Sheep	Back and sides	3	Method 1
4) Goat	Back and sides	1	Method 1
5) Pig	Back and sides	2	Method 1
6) Dog (mixed breed)	Back and sides	3	Method 1
7) Cat (domestic)	Back and sides	3	Method 1
8) Rat (Sprague-Dawley)	All body but head	4	Method 2 (dipped)
9) Mouse	All body but head	5	Method 2 (dipped)
10) Hamster	Back and sides	3	Method 2 (rolled)
11) Rabbit	Back and sides	2	Method 2 (rolled)
12) Guinea pig	Back and sides	2	Method 2 (rolled)
13) Chicken			
hen	Preen gland extrudate	7	Extrudate extracted with
rooster		4	$CHCl_3$/MeOH/ 2/1 (Ref. 6)
14) Duck	Preen gland extrudate	5	Extrudate extracted with $CHCl_3$/MeOH/ 2/1 (Ref. 6)
15) Goose	Preen gland extrudate	2	Extrudate extracted with $CHCl_3$/MeOH/ 2/1 (Ref. 6)
16) Turkey	Preen gland extrudate	2	Extrudate extracted with $CHCl_3$/MeOH/ 2/1 (Ref. 6)
17) Chimpanzee	Back	1	Method 1
18) Baboon	Back	1	Method 1
19) Man (adult)	Scalp	5	Diethyl ether soak (Ref. 9)
20) Man (fetus)	Lipid extract of vernix caseosa	3	Extracted as with preen gland extrudate (Ref. 7)

* See text for a description of Methods 1 and 2.

TABLE II

Multiple linear TLC development of surface lipid samples

	Developing solvent in parts by volume			Distance on plate to which solvent was developed	What the development accomplished
	Hexane	Diehyl Ether	Acetic Acid		
Sequence A*					
1st solvent	80	20	1	Half way up	Caused free acids to migrate above free cholesterol.
2nd solvent	95	5	0	To top	Spread out diesters between triglyceride and sterol esters.
3rd solvent	100	0	0	To top	Separated saturated hydrocarbons from squalene.
Sequence B†					
1st solvent	80	20	0	Half way up	Caused cholesterol to migrate from origin to an $R_F = \sim 0.1$
2nd solvent	95	5	0	To top	Same as 2nd solvent in sequence A
3rd solvent	100	0	0	To top	Same as 3rd solvent in sequence A

* The sequence used to produce the "A" versions of Figs. 1 to 4.

† The sequence used to produce the "B" versions of Figs. 1 to 4.

lipids of adult human scalp skin surface, vernix caseosa and to standards. As is seen from Table II and the figures, the chief difference between the "A" figures and the "B" figures is that the free fatty acids migrate to a position just above free cholesterol on the "A" figures but barely leave the origin in the "B" figures.

The thin layer chromatograms show the wide variety of lipid patterns that exist among these animals. No two are alike and all of them differ from human surface lipid. Details of lipid composition are now discussed.

1. In the squalene region, only human surface lipid and vernix caseosa show appreciable amounts. From other data, squalene is known to make up 7 to 17% of human surface lipid and 2–8% of vernix caseosa (6). Its presence could not be shown by TLC for any of the animal lipids studied. If present at all, it constitutes less than one percent. For rat skin surface lipid, which has been studied by several workers, (14, 6 and 2) a squalene content of 0.7%, 0.5% and 0.35% respectively has been reported. Since horse smegma was early reported to contain squalene (15) it was thought that squalene was also a constituent of equinal sebum (4). The assumption that one type of sebaceous excretion in an animal is the same as that of another excretion must now be considered unwarrented, since variations in chemical composition were found for different types of sebaceous gland excretions in the same animal (6). In the rat, too, there is a variation in the squalene content of its preputial gland and its surface lipids: the former contains at least 3 times the amount of squalene as the latter (6).

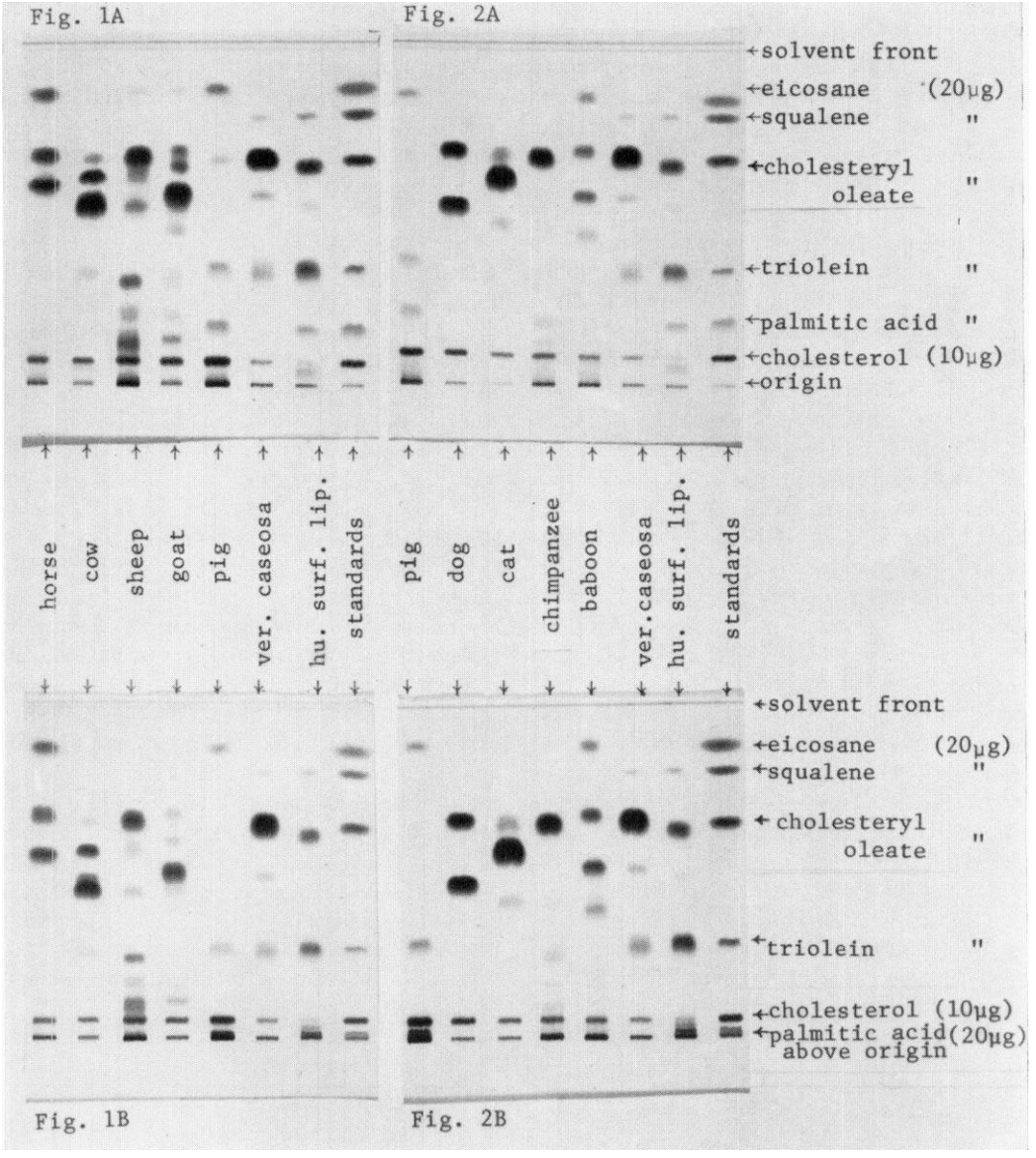
2. In the monoester region, all animals of this study and man show appreciable amounts of material except the domestic chicken and the turkey. All show more sterol esters than wax esters except the birds, the chimpanzee and human adults. It is noteworthy that even vernix caseosa shows 3 to 4 times as much sterol esters as wax esters, whereas adult scalp surface lipids contain about 10 times as much wax esters as sterol esters.

3. The diester region shows the greatest diversity among the specimens studied. All but the pig and the goose show material in this region. For man, both adult surface lipid and vernix caseosa show a faint spot of slightly different R_F values. Substances in this region are major components for many species.

4. In the triglyceride region, only adult human surface lipid, vernix caseosa and the pig surface lipid show substantial amounts. Small amounts of material are also seen for the surface lipids of the cow, sheep, goat, chimpanzee, rat, hamster and rabbit. When the material in this region is resolved further by using only solvent 2 of Table II, which gives higher resolution, the spots from these animals do not line up exactly with triglycerides of known composition or with triglycerides of human surface lipid. Thus it is questionable that these minor components are triglycerides. Wheatley has also concluded that rat, mouse, rabbit, and guinea pig have minimal amounts of triglyceride since he could find only traces of glycerol in the aqueous phase of the hydrolysate of these surface lipid samples (4). The method he used, however, was subject to considerable error since it involved evaporation of this aqueous phase to dryness *in vacuo* (16), and it is known that glycerol can be quantitatively removed by such a procedure (ref. 17 and other references quoted there). Thus his data do not allow him to conclude that glycerides were present in minimal amounts. In a detailed study of unhydrolyzed rat surface lipid, Nikkari showed that the material migrating in the triglyceride region was complex (containing a considerable amount of non-saponifiable matter) and concluded that triglycerides, if present, do not exceed 3% of the total sample (14).

It is noteworthy that Haahti *et al.* found significant amounts of material in the triglyceride region for the domestic duck and chicken but none for the goose (3). We find no material in this region for any of these fowls or for the turkey. The explanation of this discrepancy is uncertain. One possibility is, as we have found in some of our earlier preparations, that unless special precautions are taken to excise the preen gland widely and avoid any contact of the mouth of the gland whatsoever, as the contents of glands are extruded, they can easily be contaminated with subcutaneous fat.

5. In the free fatty acid region of the "A" Figures, adult human, pig, and chimpanzee surface lipid show appreciable amounts of ma-

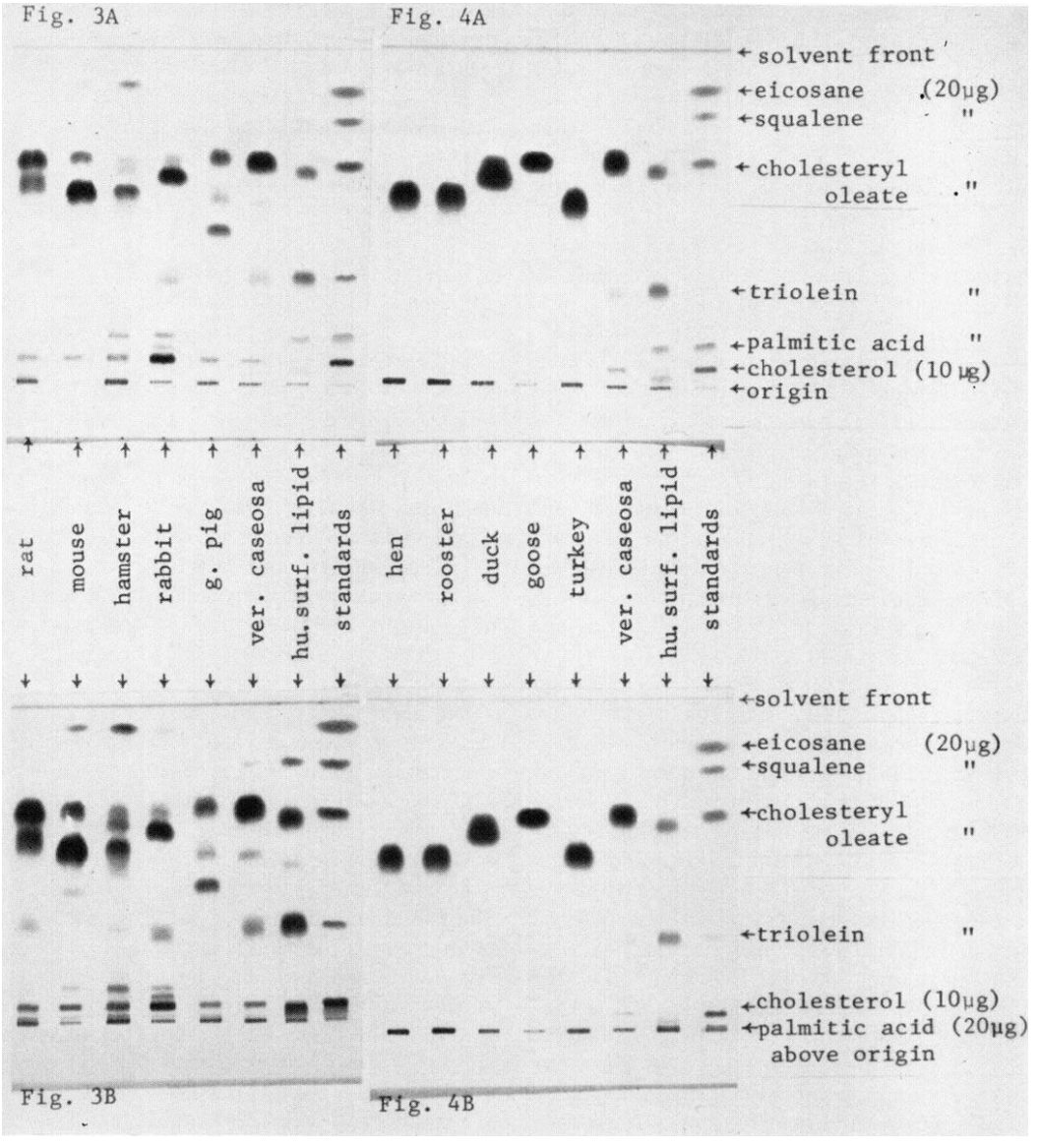


FIGS. 1–4 are photographs of thin layer chromatograms of the surface lipids and preen gland lipids of the species of animals and birds indicated. Standards are in the farthest lane to the right on each plate. Conditions for preparation and development of the plates are described in the text. In the "A" figures, free acids migrate to an R_F of about 0.25, whereas they remain at the origin in the "B" figures, all the other constituents migrating about to the same position in both the A and the B systems. The components migrating between cholesteryl oleate and triolein are the diesters. Other spots have not been identified.

terial. Most of the other samples show only minor amounts if any at all. It is noteworthy that vernix caseosa contains only very small amounts.

6. In the region where free cholesterol migrates, considerable amounts of material are found for all specimens but the fowls and adult human surface lipids.

Control of what touched the animal skin surface was not achieved in this study, thus the matter of prevention of contamination, though possibly minimized by taking the sam-



ple close to the skin surface after clipping off most of the surface hair, still could not be avoided. Besides the ingredients produced normally by the skins of the animals, contaminants could be present. For reasons previously discussed (18), saturated hydrocarbons (the uppermost spots of these chromatograms) are most probably of external origin but triglycerides are also likely contaminants since they, too, have a widespread occurrence. Interestingly, triglycerides are not a significant component of most of these lipid samples. A further fact which would tend to rule out excessive contamination for most of these samples was that replicate samples from animals housed at different facilities gave similar results.

COMMENT

Comparison of the surface lipids of man with those of animals reveals that only human surface lipid is predominantly of a glyceride type. We define the latter as a lipid containing primarily triglycerides and their breakdown products i.e. mono and diglycerides and free fatty acids. (Breakdown of a triglyceride also forms glycerol, a non lipid.) The glycerides plus the

free fatty acids make up about 60% of human scalp skin surface lipid, wax ester and squalene making up most of the remainder. Initially, triglycerides are synthesized by the sebaceous gland, then are degraded to form free acids and di and monoglycerides as they pass through the duct and on to the skin surface (first shown in ref. 19 and later substantiated in ref. 18). None of the other animals have significant amounts of triglycerides or free fatty acids in their skin surface lipid except the pig.

It is our belief, however, that this lipid sample of the pig represents primarily its hexane extractable epidermal lipids rather than those of its sebaceous glands whereas the other samples represent primarily sebaceous excretions. This conclusion is based upon the following reasoning. If one extracts human surface lipid from different parts of the body, the yield per unit area depends upon the total sebaceous gland volume underlying that portion of the skin. A high yield of lipid would indicate a high sebaceous glandular volume. A low yield of lipid is obtained from sites where there are no sebaceous glands, such as the palms and soles. This low yield is due to the fact that human epidermal cells do not contain much lipid, and since most of what is present is highly polar, it would not be extracted by momentary exposure to hexane as would the sebaceous gland lipids. In the case of the pig, the contribution of the sebaceous glands to the surface lipid must be minimal for it has a sparse pilosebaceous population on its back and sides, the sites where our samples were taken (20). Furthermore, the amount of lipid we got from the pig was only of the order of one tenth the amount per unit area obtained from other more furry animals. Additionally, pig surface lipid showed the same lipid classes as those obtained from the nonpolar epidermal lipid samples of different human sources, namely, sterol esters, triglycerides, free fatty acids, and free sterols for sole epidermis (6) for the living layer of leg epidermis (6) and for the wall and sac contents of epidermal cysts (10). The latter comparisons suggest that although sebaceous lipids may vary from species to species and from site to site, epidermal lipids are more similar to each other from animal to animal.

To summarize, it appears that only man produces a surface lipid primarily of sebaceous gland origin, in which triglycerides and its breakdown products are the major constituents, whereas the main products of the surface lipids of furry animals and the preen glands of birds are a wide variety of mono and diester waxes.

Why should only man and none of the other animals, including two primates, produce a glyceride type of surface lipid of sebaceous origin? Man has much less hair over most of his body than do furry animals; he is also the only animal with a generalized eccrine sweating system. Considering at least these two differences between human and animal skin, a better question might be, how can triglycerides and their breakdown products (along with some squalene and wax esters) serve a relatively moist and hairless human skin surface more adequately than say, solely some combination of wax and sterol mono and diesters as is found in the furry animals and birds?

Some possible answers follow. The triglycerides might be thought of as a reservoir for free fatty acids and for di- and monoglycerides, these breakdown products being mainly what is required. Monoglycerides are emulsifying agents and could conceivably function as such if combinations of sweat and sebum do, in fact, form emulsions. Free fatty acids will undoubtedly have a strong influence as to what microflora can survive on human skin. These acids could counteract what might otherwise be favorable conditions for growth, i.e. a moist, warm environment. Free acids along with diglycerides may form monomolecular films over a moist skin surface thereby preventing too rapid evaporation of the sweat. Such a film of lipid would enable the sweat to spread more thus providing a larger area for more even cooling.

The diversity of surface lipid waxes among the animals and birds merits comment. It is related to the fatty acid, fatty alcohol and sterol moieties as we shall report later. The variations may reflect a variety of functions these waxes might serve such as the waterproofing of fur, feather and skin, control of skin micro flora, a medium for the excretion of vitamin D precursor and intake of the vitamin, and possibly others. Whatever functions this variety of waxes may serve their various owners, they should also have certain physical properties. When they are synthesized and excreted in the sebaceous glands, they should

be liquid or capable of flowing. When spread out on feather or fur it would be better if the waxes were now solid so as not to cause clumping. Thus there should be a phase change at or about skin temperature. This would vary with different animals since their skin temperatures vary. Biochemical selection of certain structural features of the fatty moieties of the wax such as chain length, *cis* unsaturation, chain branching, or more than one ester group in the molecule could provide a suitable melting range along with whatever other properties the functions of these waxes demand.

SUMMARY

1. The composition of the surface lipids of the horse, cow, sheep, goat, pig, dog, cat, baboon, chimpanzee, rat, mouse, hamster, rabbit, guinea pig, and the preen gland lipids of the hen, rooster, goose, turkey and duck were compared to the lipids of adult human scalp surface and of vernix caseosa by thin layer chromatography.

2. The surface lipids of man are unique among the animals in several ways:

a) Only man produces a surface lipid (primarily of sebaceous origin) which consists predominantly of triglycerides and their breakdown products, i.e. di- and monoglycerides and free fatty acids.

b) Squalene is a major component in the surface lipids of man whereas it occurs in traces if at all in those of the other animals.

c) Adult scalp skin surface lipids show very small amounts of sterol esters but substantial amounts of wax monoesters, whereas the reverse is true for most animals and for vernix caseosa.

d) Most of the animals show substantial amounts of diester waxes in their surface lipids; these are minor components in the human samples.

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