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COMPARATIVE CHEMISTRY OF SEBUM*

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Sebum is the excretory product of the sebaceous glands. These glands are found in the skin of all mammals except whales and porpoises (Montagna, 1963). The bulk of sebum is a mixture of relatively nonpolar lipids, most of which are synthesized de novo by the gland. The lipid is excreted by a holocrine mechanism, whereby the lipid-laden cells disintegrate and pour their contents through the sebaceous duct onto the skin surface. One apparent function of sebum is to provide the skin surface and hair with a hydrophobic coating. The preen gland of birds has a similar function, but it is not a sebaceous gland. The preputial glands of rodents are specialized sebaceous glands, used primarily for the release of pheromones.

The chemistry of sebum is interesting, because many compounds not found elsewhere in the body have been identified in sebum. As first was clearly shown by Wheatley (1956), the composition of sebum is remarkably species specific. The application of modern chromatographic methods to studies of sebum has greatly expanded our knowledge of its chemistry during the last two decades. There have been some comparative studies (Wheatley and James, 1957; Nicolaides et al., 1968, 1970; Nikkari, 1969), but in most studies each investigator has worked on one species only. The present review summarizes the results of different authors in a form that allows easier comparison among various species.

METHODS

Collection of Sebum

It is practically impossible to obtain pure uncontaminated sebum. Even in studies of the lipid extract of isolated sebaceous glands (Kellum, 1966, 1967), there exists the possibility of dermal contamination; a large proportion of immature cells is also included in the specimen and it remains to be established whether there are any differences in lipid composition between these cells and mature sebum. It is easier to isolate the uncontaminated excretory product of preputial glands but, as will be discussed later, its composition differs strikingly from that of the true sebaceous glands.

In all studies reviewed in this paper, the sebum samples were obtained by extracting the skin surface or hair with organic solvents. The methods used for collecting the skin surface lipids and the problems associated with them have been discussed in detail (Nicolaides, 1963; Wheatley, 1963; Nicolaides and Kellum, 1965; Nikkari, 1965). It is generally agreed that at normal levels of sebum excretion the major constituents of the skin surface lipids are of sebaceous origin. The contribution of the desquamating epidermal cells to the skin surface lipids is quantitatively small but becomes important in studies of the minor constituents, e.g., the human skin surface sterols. These problems will be emphasized when appropriate in this review.

The Analysis of Sebum

The methods for analyzing sebum have been reviewed by Haahti (1961), Nicolaides (1963), Wheatley (1963), and Nikkari (1965). Slightly different methods have been applied by each author cited in the present review. Basically, two different approaches have been used:

Analysis of hydrolysis products. This involves saponification of the sebum sample by heating it in an aqueous alcoholic solution of potassium or sodium hydroxide so that the ester bonds in waxes, sterol esters, and glycerides are broken. The ether bonds in alkyl and alk-1-enyl glycerides are not affected by this treatment, and any squalene or other hydrocarbons present in the original sample will remain unchanged. The neutral saponification products ("nonsaponifiables") are subsequently extracted with an organic solvent of low polarity (usually *n*-hexane). The fatty acids ("saponifiables") are extracted similarly after acidification of the alcoholic phase. In some studies the saponifiable and nonsaponifiable fractions have been extracted together from the acidified alcoholic phase and have been separated subsequently by chromatographic procedures (Nikkari, 1965, 1969; Nicolaides and Ansari, 1968). Glycerol remains behind and is usually determined colorimetrically.

The nonsaponifiable material has been fractionated either by aluminum oxide column chromatography followed by colorimetric quantification of squalene and sterols (Wheatley, 1954; Wheatley and James, 1957; Nicolaides and Wells, 1957; Downing et al., 1960) and determination of 7-dehydrocholesterol by ultraviolet absorption (Wheatley and James, 1957; Boughton and Wheatley, 1959), or by thin-layer chromatography (TLC) on silica gel G followed by analysis of all fractions with gas–liquid chromatography (GLC) (Nikkari and Haahti, 1964; Nikkari, 1965, 1969); the sterols have been further fractionated by TLC on silica gel impregnated with silver nitrate and finally identified by means of GLC-mass spectrometry (Miettinen and Luukkainen, 1968).

The saponifiable material has been fractionated into unsubstituted and hydroxy fatty acids by thin-layer (Nikkari and Haahti, 1964; Nikkari, 1965) or column (Downing et al., 1960; Downing, 1963) chromatography. The fatty acids have usually been analyzed as their methyl esters by GLC. The unsaturated fatty acids have been identified by performing GLC before and after hydrogenation (Nikkari, 1965, 1969; Kärkkäinen et al., 1965; Wilkinson and Karasek, 1966); in the latter studies the peaks that did not correspond to straight-chain components were considered to be branched-chain acids. More accurate figures for unsaturated and branched-chain fatty acids have been obtained by fractionating the fatty acids before GLC on the basis of unsaturation on silicic acid impregnated with silver nitrate (see, e.g., Nikkari, 1965; Nicolaides and Ansari, 1968; Ansari et al., 1970; Nicolaides et al., 1972). Individual unsaturated fatty acids have been further isolated by preparative GLC, and their double-bond positions have been determined by GLC of the aldehyde fragments obtained by re-

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ductive ozonolysis (Nicolaides et al., 1964, 1972; Nicolaides and Ray, 1965; Nicolaides and Ansari, 1968; Ansari et al., 1970; Wilkinson, 1970).

Analysis of lipid class composition. This involves the study of unhydrolyzed sebum. After removal of the free fatty acids from an *n*-hexane solution of the lipid by extraction with dilute alkaline ethanol–water, the lipid classes have been separated from each other by silicic acid column chromatography (Nicolaides and Foster, 1956; Haahti, 1961; Nikkari, 1965; Kärkkäinen et al., 1965; Nicolaides, 1965; Nicolaides et al., 1970, 1972) or preparative thin-layer chromatography on silica gel G (Nikkari, 1965; Wilkinson and Karasek, 1966) followed by purification and quantification of the fractions. Monoester waxes and sterol esters have further been separated by urea fractionation (Nicolaides and Foster, 1956), preparative thin-layer chromatography on aluminum oxide (Nikkari, 1965; Kärkkäinen et al., 1965; Wilkinson and Karasek, 1966), or magnesium oxide column chromatography (Nicolaides et al., 1972). Downing et al. (1969) and Snyder and Blank (1969) separated the lipid classes by TLC, charred the spots with sulphuric acid, and quantified the fractions by photodensitometry.

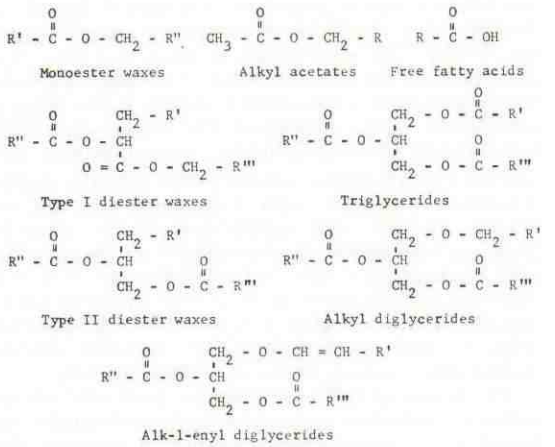

FIG. 1: The structures of the major aliphatic constituents of sebaceous materials.

THE SKIN SURFACE AND HAIR LIPIDS OF MAMMALS

The structures of the major aliphatic components found in sebaceous materials are shown in Figure 1. Table I, largely based on the classical data by Wheatley (1956), gives the available information of the composition of the hydrolysis products and Table II is a summary of the results obtained by different authors on the lipid class composition of skin surface lipids. Since there has been no conclusive evidence that the paraffinic hydrocarbons found in skin surface lipids are of endogenous origin, they will not be discussed.

Free Fatty Acids and Glycerides

Adult human sebum contains twice as much saponifiable as nonsaponifiable material, whereas in other species these fractions are about equal (Table I). This is in accordance with the finding of Wheatley (1956) that whereas more than half of human sebum is composed of triglycerides and free fatty acids, the seba of rodents, rabbit, and sheep contain less than 10 percent free fatty acids and practically no triglycerides. These findings have been confirmed by other authors (Table II), and no demonstrable amounts of free fatty acids or triglycerides were found in the hair lipids of chimpanzee, baboon, hamster, guinea pig, dog, cat, goat, or cow by qualitative TLC (Nicolaides et al., 1968).

TABLE I

Average percentage composition of the hydrolysis products of mammalian seba

	Human			Rat		Mouse	Guinea pig	Rabbit	Sheep		Ox
	Adult	Newborn*									
Reference:	*a*	*b*	*c*	*d*	*e*	*e*	*e*	*e*	*f*	*g*	*f*
Total nonsaponifiable	30	38	49	50	41	55	45	46	46	52	43
Squalene	12	2.6		0.4	0	0	0	0	0		
Sterols	2.6	20		25	10	13	21	3.9	26	39	14
Monohydric alcohols	12	5		17	18	5.9	5.0	31	9.0	4.9	
Alkane-1,2-diols		pr[h]		6	2.9	28	11	2.2	2.5	3.4	
Total fatty acids	68	44	40	50	59	44	55	53	55	48	53
α-hydroxy acids			4	6		0[h]	0[h]	pr[h]		14	
ω-hydroxy acids										2	

pr = present.

* Vernix caseosa.

a. Nicolaides and Wells, 1957: ethyl ether washings of the scalp

b. Wheatley, 1954

c. Downing, 1963

d. Nikkari and Haahti, 1964; Nikkari, 1965: pooled acetone extracts of fur

e. Wheatley and James, 1957: as in *d*.

f. Wheatley, 1956

g. Downing et al., 1960: hexane extract of wool

h. Nikkari, 1969: acetone extract of cut hair

TABLE II

Average percentage composition of unhydrolyzed lipids of sebaceous origin

	Skin surface									Preputial gland	
	Human					Rat		Mouse	Sheep	Rat	Mouse
	Adult			Newborn*							
Reference:	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*g*	*j*
Squalene	9.3	11	12	9		0.5	0.5		tr	1.5	
Free sterols	1.4	4.2	1.4	9		6	5	13	12†	2	3
Sterol monoesters	2.5 }	24	2.1	33	25	27 }	40	10	} 25†	} 14	5
Monoester waxes	20 }		25	12	16	17 }		5			48
Diester waxes, type I						10 }	25	} 65	9	0	
Diester waxes, type II				7	3	11 }			7†	0	
Glyceryl ether diesters									10†		14
Triglycerides	23	31	41	26		} 8†	7	6	6†	60	17
Free fatty acids	27	14	16	0.5		1	2			2	tr
Other	9	12	2				5			9	13
Unidentified	11	4				19	17		31	11	

* Vernix caseosa

† Identification has been made on the basis of chromatographic mobility only

tr = traces

a. Nicolaides and Foster, 1956: ethyl ether soaks of scalps (2 pools)

b. Haahti, 1961: acetone washings of backs of 4 subjects

c. Downing et al., 1969; ethyl ether washings of foreheads of 17 subjects

d. Kärkkäinen et al., 1965: three subjects

e. Nicolaides et al., 1970; 1972: one subject

f. Nikkari, 1965: three pooled acetone extracts of the fur

g. Nicolaides, 1965: pooled acetone extract of the fur

h. Wilkinson and Karasek, 1966: pooled acetone extract of the fur

i. Nikkari, unpublished data obtained by preparative TLC from an acetone extract of wool

j. Snyder and Blank, 1969: a duplicate analysis of a pooled sample

The free fatty acids are not present in sebaceous glands but are formed from the triglycerides through the action of lipases when the sebum reaches the skin surface (Nicolaides and Wells, 1957; Nicolaides, 1963; Kellum, 1967). Skin bacteria contain lipolytic enzymes (see, e.g., Freinkel and Shen, 1969; Marples et al., 1971). No other esters, either in human or animal sebum, are known to be hydrolyzed to the same extent as triglycerides (Nicolaides, 1963; Downing, 1970). One-fourth of vernix caseosa lipid is triglyceride, but free fatty acids are virtually absent, as in the preputial gland lipid of rat and mouse. The absence of triglyceride lipase from the sterile environments where these lipids originate favors a bacterial origin of this enzyme.

Monohydric Alcohols and Monoester Waxes

Aliphatic monohydric alcohols are present in all seba studied. They are constituents of both monoester waxes and type I diester waxes, and their concentration is roughly proportional to the content of these two waxes (Tables I and II).

The early work carried out on the elucidation of the composition and structure of monohydric alcohols in sheep and human sebum has been reviewed by Nicolaides and Ray (1965) and Nicolaides (1967). Four main types of chains have been found in both saturated and monounsaturated alcohols of human sebum (Nicolaides, 1967): (a) straight chains with an even number of carbon atoms, (b) straight chains with an odd number of carbon atoms, and monomethyl branched (c) iso and (d) anteiso chains. In addition, there are small amounts of other branched-chain alcohols. On the basis of gas chromatographic retention data, the same homologous series of monohydric alcohols occur in seba of all species studied so far. The chain lengths of these alcohols are generally relatively long, and increase in the following order: adult human sebum (C_{10}–C_{24}, maximum at C_{20}; Haahti and Horning, 1963; Nicolaides, 1967); vernix caseosa (C_{14}–C_{26}, maxima at C_{20} and C_{24}; Kärkkäinen et al., 1965); rat sebum (C_{14}–C_{32}, maximum at C_{24}; Nikkari and Haahti, 1964); and sheep sebum (C_{16}–C_{33}, maximum at C_{26-27}; Downing et al., 1960).

Monoester waxes occur in all seba, and when squalene is absent, they are the most nonpolar components. Their content is quite low in the seba of the mouse, the rabbit, and the cat, where diester waxes are especially prominent (Table III), and in goat and cow sebum (Nicolaides et al., 1968) where unidentified components, possibly diester waxes, form the bulk of the lipid. The monoester waxes of rat sebum were calculated to

possess an average molecular weight of 592 (Nikkari, 1965), which corresponds to an average chain length of 40 carbon atoms. The waxes of human sebum are somewhat shorter: they have an average molecular weight of 562 (Nicolaides and Foster, 1956) and a chain-length distribution from C_{26} to C_{42} with a maximum content at C_{34-36}, as determined from direct GLC of the waxes (Haahti, 1961).

TABLE III

Concentration and properties of diester waxes and triglycerides in mammalian seba[a]

	Content in sebum %	Average molecular weight*	Percentage molar distribution of chains*		
			Saturated		Unsaturated
			Straight	Branched	
Type I waxes					
Rat	10[b]	869	41	30	29
Rabbit	66[c]	835	90	7	3
Cat	66[c]	837	73	19	8
Sheep	9[d]				
Cow	35[c]		100[e]		
Type II waxes					
Vernix	8	852	21	52	27
Baboon	21[c]				
Rat	11[b]	854	46	39	15
Mouse	61[c]	869	54	13	33
Guinea pig	18[c]	892	47	19	34
Dog	46[c]	856	33	64	3
Sheep	7[d]				
Triglycerides					
Adult man	23[e]	846[e]			
Vernix[f]	26	828	47	25	28

* Calculated from average chain weights determined by GLC

a. Nikkari, 1969; *b.* Nikkari, 1965; *c.* Nicolaides et al., 1970; *d.* Nikkari, unpublished results; *e.* Nicolaides and Foster, 1956; *f.* Calculated on the basis of the data of Kärkkäinen et al., 1965.

Hydroxy Fatty Acids and Type I Diester Waxes

The sheep wool lanolin has long been known to contain appreciable amounts of hydroxy fatty acids. Downing et al. (1960) found that 30 percent of all fatty acids in lanolin were α-hydroxy fatty acids (Table II). The structures of the esters from which hydroxyacids are liberated upon hydrolysis were unknown for a long time, although as early as 1952 Tiedt and Truter were able to crystallize a lanolin fraction that, upon saponification, gave rise to hydroxy acids, unsubstituted fatty acids, and monohydric alcohols. On the basis of the finding that the molecular weight of wool wax esters was too high (about 800) to be accounted for by simple combination of the fatty acids and alcohols, Tiedt and Truter speculated that the fraction they had isolated could be a diester of the hydroxy fatty acid. Detection of α-hydroxy fatty acids in rat sebum (Nikkari and Haahti, 1964) and the observation that they were constituents of a polar wax fraction (Nicolaides, 1965; Nikkari, 1965) led to the final identification (Nikkari and Haahti, 1968) of what were called "type I diester waxes" (Nikkari, 1965), i.e., diesters of an α-hydroxy fatty acid with one molecule of unsubstituted fatty acid and one molecule of aliphatic monohydric alcohol (Fig. 1). Since small amounts of sterols were found in the chromatographically purified fraction, it was assumed (Nikkari, 1965) that part of the type I diester waxes contains a sterol in place of the aliphatic alcohol; however, the existence of these "type III diester waxes" has never been substantiated.

The presence of type I diester waxes in sheep sebum has been confirmed (Nikkari, unpublished results) and it has also been demonstrated that they form about two-thirds of rabbit and cat sebum and about one-third of the sebum of the cow (Nikkari, 1969; Nicolaides et al., 1970). There is a small amount of material with the chromatographic mobility of type I diester waxes in dog and goat sebum as well (Nicolaides et al., 1968). Although α-hydroxy fatty acids have been reported to occur in vernix caseosa (Downing, 1963; Kärkkäinen et al., 1965), type I diester waxes have been found neither in vernix caseosa nor in adult human sebum.

The average molecular weights of all type I diesters are quite similar with each other and with the molecular weights of type II diester waxes and triglycerides of sebaceous origin (Table III). Although the rat wax has a slightly higher average molecular weight than that of the rabbit and the cat, they all may possess similar physical properties because the rat wax has a large proportion of branched and unsaturated chains, whereas the hydrocarbon chains in rabbit and cat wax are mostly saturated straight chains. The chains of the type I diester wax of cow sebum are almost totally straight and saturated (Nicolaides et al., 1970).

In hypophysectomized rats, the sebaceous gland activity is at a minimum and therefore is not lowered by the administration of estrogen (Nikkari and Valavaara, 1969). The skin surface and hair of these rats still contain acetone-extractable lipids, the main components of which have TLC-mobilities of type I diester waxes and sterol esters (Nikkari and Valavaara, 1970). Thus, at least a part of the type I diester waxes may be of epidermal origin.

Alkane-1,2-diols and Type II Diester Waxes

Alkane-1,2-diols have been known to occur in wool wax (see Downing et al., 1960), and Wheatley demonstrated their presence in the seba of the rodents and the rabbit as well (Table I). They contain the same types of saturated and monounsaturated chains as the monohydric alcohols but there are large species differences in the distribu-

tion of these chains (Nikkari, 1969). In the sebum of the rat the 1,2-diols were found to be constituents of what were called "type II diester waxes" (Nikkari, 1965; Nikkari and Haahti, 1968), i.e., diesters of alkane-1,2-diols with unsubstituted fatty acids (Fig. 1). Type II diester waxes have since been discovered in all seba shown to contain diols and also in the sebum of the baboon and the dog (Nikkari, 1969; Nicolaides et al., 1970). Their concentration (Table III) shows a rough correlation with that given for the diols in Table I. Wheatley (1956) reported the occurrence of 2 percent alkane-1,2-diols on human skin surface, but they have not been found by other authors. No diester waxes have been detected in adult human sebum, although a small amount of type II diester waxes can be isolated from vernix caseosa (Tables II and III).

The "diol lipids" (for a review, see Bergelson, 1969) that occur in small concentrations in several animal tissues are basically similar structures to the type II diester waxes, but their component diols have much shorter chains (C_2–C_4) than those of the diester waxes. The maximum concentration of the latter is at C_{16} in rat sebum (average chain weight 262) and between C_{18} and C_{24} in the mouse, the guinea pig, the dog, the sheep, and vernix caseosa (average chain weights of 309, 324, 320, 328, and 340, respectively) (Nikkari, 1969; Downing et al., 1960). Although the chain lengths of the diols from rat and vernix caseosa differ by as much as 78 daltons, the average molecular weights of the parent diester waxes (Table III) are essentially equivalent (852 and 854, respectively) because of reciprocal differences in the length of the fatty acid chains.

Similarities and Differences Between Triglycerides and Diester Waxes

The average molecular weights of the type II diester waxes are similar to those of type I diester waxes and also to the average molecular weights of triglycerides of human sebum (Table III). By direct GLC of waxes it was found that the maximum concentration of type II diester waxes from rats (Nikkari and Haahti, 1968) and mice (Nicolaides et al., 1970) were at $C_{56\text{-}58}$ and $C_{58\text{-}60}$, respectively, and the maximum concentrations of the type I diester waxes from rats (Nikkari and Haahti, 1968), rabbits, cats, and cows (Nicolaides et al., 1970) were all at $C_{54\text{-}56}$. The average molecular weights of the triglycerides of adult human sebum and vernix caseosa correspond to an average carbon content of $C_{54\text{-}55}$ and $C_{52\text{-}53}$, respectively.

Because of their quite similar molecular sizes and chemical structures, the diester waxes and triglycerides could be expected to have similar physical properties. Why do most of the furred animals then have a sebum that contains diester waxes, and why does human sebum contain high concentrations of triglycerides? Teleologically it can be concluded that triglycerides must be beneficial for the bare skin surface and diester waxes for the hair. Free fatty acids liberated from the triglycerides (but not from the waxes) have been ascribed antifungal and antibacterial properties, and the monoglycerides may function as emulsifying agents on the skin (Nicolaides et al., 1970). Since diester waxes are slightly less polar than triglycerides, they may afford better protection against wetting of the fur, especially since they are not broken down in the same way as the triglycerides. However, the chimpanzee does perfectly well with a sebum that apparently contains neither triglycerides nor diester waxes and has monoester waxes or sterol monoesters as its major component (Nicolaides et al., 1968). More work on the chemical composition and physical properties of seba from different species is needed before any definite conclusions can be drawn about the significance and "function" of various sebum constituents.

The absence of free fatty acids and alcohols from the hair lipids of furred animals may be due to (a) the absence from the animal skin surface and hair of the same kind of lipases that are present on the human skin surface, (b) conditions on the animal skin surface that are unfavorable to the action of the lipases, or (c) resistance of the diester waxes to the action of these enzymes. Freinkel and Shen (1969) determined the pH optima of lipases produced by bacteria present on human skin. Otherwise, the skin surface lipases have not been characterized enzymologically. Pancreatic lipase hydrolyzes diesters of hexane-1,6-diol with two molecules of oleic acid (Mattson and Volpenheim, 1972), which bear some resemblance to the type II diester waxes. On the other hand, pancreatic lipase also splits esters of long-chain alcohols with fatty acids (Mattson and Volpenheim, 1969), i.e., monoester waxes, which the skin surface lipase does not do (Nicolaides, 1963; Downing, 1970).

The Fatty Acid Chains of Sebum

The same four homologous series of both saturated and monounsaturated chains that are present in the aliphatic alcohols of human sebum are also found in the fatty acids of seba of all animals studied so far; there are usually also small amounts of dienoic fatty acids. Nicolaides et al. (1972) have presented mass spectrometric evidence that in addition to the iso and anteiso branched chains, a small proportion of the fatty acids in both adult human sebum and in vernix caseosa has other monomethyl branched and dimethyl branched chains as well (see Table IV); the methyl group in the other monomethyl branched acids was predominantly at position 4. According to Wheatley and James (1957), the seba of rat, guinea pig, and rabbit contain "highly branched" fatty acids; however, their data were based on gas chromatographic mobilities only and have not been confirmed.

In Table IV, the fatty acid composition of the monoester waxes and sterol esters of adult human

sebum, vernix caseosa, and rat and mouse sebum are compared. There are two distinctly different patterns of hydrocarbon chain distribution within these fatty acids: (1) The fatty acids of monoester waxes from all the species studied and of sterol esters from adult human and mouse sebum resemble each other in showing a high content of monounsaturated chains and relatively low concentrations of branched chains. (2) The fatty acid spectra of the sterol esters from vernix caseosa and rat sebum are strikingly similar and differ from the others by having relatively high contents of branched saturated chains with lower percentage monounsaturated chains than the others; they also differ from the monoester wax fatty acids from the same sources by having longer chains.

Nicolaides' group has done pioneer work in elucidating the double-bond positions in the monoene fatty acids and alcohols of sebum (for references, see Table V). They have found that the double-bond position in human sebum monoenes follows preferentially the "Δ6 pattern," i.e., the double bond is located either at Δ6 or at a position that results when a $C_{14\text{-}20}$:Δ6 chain is either extended or degraded at the carboxyl group by an integral number of C_2 units; the main apparent precursor is C_{16}:Δ6. (In other tissues, Δ9 pattern is found with C_{18}:Δ9 as the main precursor.) In the sebaceous glands of the rat and the mouse, the Δ9 pattern is predominant; the chief apparent precursor is C_{16}:Δ9 in the rat and C_{18}:Δ9 in the mouse. In the vernix caseosa, the total monoenoic acids and the monoenoic acids of the monoester waxes show mostly the "human" Δ6 pattern, whereas more than two-thirds of the sterol ester monoenes and most of the diester wax monoenoic acids have the "rodent" Δ9 pattern. So far, the information is too sparse to determine whether the double-bond pattern is entirely species specific or whether the Δ6 pattern is typical of certain lipid classes (triglycerides and hence free fatty acids, monoester waxes, and certain sterol esters) and the Δ9 pat-

TABLE IV

Fatty acid composition of waxes and sterol esters of sebum

	Monoester waxes				Sterol monoesters			
	Human		Rat	Mouse	Human		Rat	Mouse
	Adult	Vernix			Adult	Vernix		
Reference:	*a*	*a*	*b*	*c*	*a*	*a*	*b*	*c*
Saturated								
Straight chain	19.2	10.3	6.0	27.0	26.9	7.7	12.1	25.1
Branched chain								
Iso and anteiso	5.3	12.7	29.4	21.1	8.5	57.7	52.5	12.3
Other	2.4	2.0			2.3	tr		
Monounsaturated	64.4	66.5	63.9	46.6	54.0	29.4	29.5	55.3
Straight chain	45.4	54.9			44.6	24.9		
Iso and anteiso	19.0	11.6			9.4	4.5		
Diunsaturated	4.0	6.0	0.2		8.3	3.7	5.9	2.0
C_{22} and longer chains	1.3	0.7	2.1	13.5	5.3	32.9	18.4	49.5

a. Nicolaides et al., 1972; *b*. Nikkari, 1965; *c*. Wilkinson and Karasek, 1966.

TABLE V

Double-bond positions in the monounsaturated fatty acids and alcohols of seba from different species

	Human		Rat	Mouse
	Adult	Vernix caseosa		
Fatty acids (FA)				
Total FA		Mostly Δ6[a]	Mostly Δ9[b]	Mostly Δ9[c]
Monoester wax FA[d]	98 mole% Δ6	87 mole% Δ6		
	1 mole% Δ9	12 mole% Δ9		
Sterol ester FA[d]	89 mole% Δ6	30 mole% Δ6		
	11 mole% Δ9	70 mole% Δ9		
Type II diester FA[e]		Mostly Δ9		
Free FA[f]	Mostly Δ6			
Monohydric alcohols[g]	Mostly Δ6			

a. Nicolaides and Ray, 1965; *b*. Nicolaides and Ansari, 1968; *c*. Wilkinson, 1970; *d*. Nicolaides et al., 1972; *e*. Ansari et al., 1970; *f*. Nicolaides et al., 1964; *g*. Nicolaides, 1967.

TABLE VI

Percentage composition of fatty acids of type II diester waxes

	Human vernix caseosa			Rat	Mouse			Guinea pig	Dog	
Reference:	*a*	*b*	*c*	*b*	*b*	*d*	*e*	*b*	*b*	*e*
Saturated										
Straight	32.4	28.4	37.9	34.8	38.3	50.9	45.5	57.9	35.6	32.0
Branched	33.7	37.0	28.1	46.1	13.7	7.2	13.5	17.8	60.2	68.0
Unsaturated	31.9	34.6	34.0	19.1	48.0	38.2	40.9	24.3	4.2	—
C_{14} and shorter	11.6	7.9	6.3	3.0	1.1	0.7	1.0	0.5	3.0	5.4
C_{22} and longer	8.4	14.1	3.7	43.8	24.6	14.4	28.9	30.7	10.1	2.6

a. Kärkkäinen et al., 1965; *b*. Nikkari, 1969; *c*. Ansari et al., 1970; *d*. Wilkinson and Karasek, 1966; *e*. Nicolaides, 1970.

tern to others (diester waxes and certain sterol esters). If the latter is true, then, e.g., the mouse would be exhibiting the Δ9 pattern mainly because of the high content of diester waxes in its sebum (Table III).

To account for the different fatty acid spectra of waxes and sterol esters in vernix caseosa (Tables IV and V), Nicolaides et al. (1972) speculated that the fatty acids of the waxes originate from the sebaceous glands, whereas a part of the sterol ester fatty acids are derived from the epidermis. According to Tables IV and V, it is also possible that there are two types of sterol esters: one is formed by the sebaceous glands, has the fatty acid spectrum shown in Table IV for the rat and vernix sterol esters, and possesses the Δ9 double-bond pattern; the other either is excreted by the sebaceous glands or, as suggested by Nicolaides et al. (1972), is formed on the skin surface by (trans) esterification of the free cholesterol, originating from the epidermis, with fatty acids of sebaceous origin present on the skin surface. In adult human sebum, which contains less sterol esters than vernix caseosa and rat sebum, the sterol esters formed by the latter mechanism would predominate, whereas the sterol esters of vernix caseosa and rat sebum would represent the former mechanism.

The fatty acid composition of Type II diester waxes shows a far greater species specificity than that of monoester waxes and sterol esters (Table VI); this specificity is not obscured by interlaboratory variations. One would expect to find some similarities in composition within related species, e.g., the rodents. However, the fatty acids from the rodents do not show any peculiarities that would distinguish them as a group from other mammals, but they can be easily differentiated from each other.

Sterols and Sterol Esters

An excellent review of cholesterol metabolism in the skin was written by Kandutsch (1964). Two pathways of cholesterol biosynthesis from lanosterol skin in rat and preputial gland were demonstrated by Wilson (1963). In whole rat skin, 87 percent of all the isolated intermediates belong to

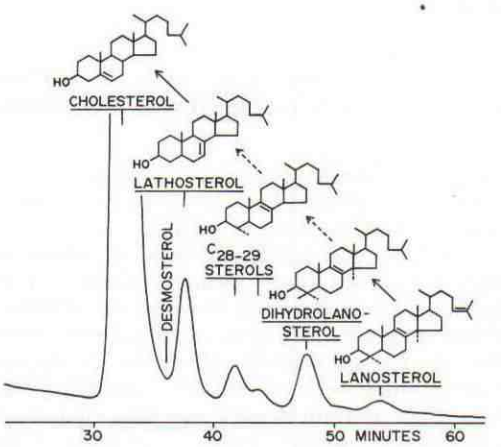

FIG. 2: The "Kandutsch–Russel" or "saturated side chain" pathway of cholesterol biosynthesis and a gas–liquid chromatogram of human skin surface sterols showing the presence of some members of this pathway. The position of reference desmosterol is also shown. The sample: trimethyl silyl ethers of digitonin-precipitable sterols of sebum obtained from foreheads of 6 subjects by acetone swabs. Column: 1% DC 560; 6 ft.; 230° C.

the "Kandutsch–Russel" or "saturated side chain" pathway (Clayton et al., 1963) with lathosterol alone contributing 37 percent, whereas desmosterol, the last intermediate in the "unsaturated side chain" or "Bloch" pathway, amounts to only 7 percent. The major intermediates found in human sebum (Fig. 2), human vernix caseosa, and the sebum of the rodents (Table VII) also belong to the Kandutsch–Russel pathway.

Human sebum has a relatively high concentration of squalene, which is virtually absent from seba of other mammals studied (Tables I and II). On the other hand, adult human sebum has very little cholesterol, either free or esterified, compared with the hair lipids of rodents and sheep (Tables I and II). The main site of squalene synthesis in human skin is the sebaceous gland whereas sterol synthesis occurs mainly in the keratinizing epidermis (Nicolaides and Rothman, 1955). Therefore, the high concentration of squalene in human sebum apparently results from an inability of the human sebaceous gland to convert it to sterols.

TABLE VII

Average percentage composition of total sterols in mammalian seba

	Human			Rat		Mouse	Guinea pig	Rabbit	Sheep	
	Adult		Newborn*							
Reference:	*a*	*b*	*c*	*d*	*e*	*e*	*e*	*e*	*b*	*f*
5α-cholestan-3β-ol(cholestanol)		2	1.7						10	
5-cholesten-3β-ol(cholesterol)	93	91	79	27	56	36	88	91	38	41
5,24-cholestadien-3β-ol(desmosterol)			0.08							
7-cholesten-3β-ol(lathosterol)	3.0		17	39	43	64	9	3		
C_{28-29} sterols	1.6		2.1	23						
C_{30} sterols	2.1	0.4		10					48	59
5,7-cholestadien-3β-ol (7-dehydrocholesterol)		0.08	0.1		<0.01	0.06	—	—		

* Vernix caseosa

a. Nikkari, unpublished work (see Fig. 2); *b*. Wheatley, 1956; Boughton and Wheatley, 1959; *c*. Miettinen and Luukkainen, 1968; *d*. Nikkari and Haahti, 1964; Nikkari, 1965; *e*. Wheatley and James, 1957; *f*. Downing et al., 1960.

Whether any sterols at all are synthesized by the human sebaceous gland is still unclear. According to Kellum (1967), there is only 0.7–1.0 percent cholesterol in the lipids of isolated sebaceous glands, but since these glands contain a high proportion of immature cells in which the synthetic pathway may not have gone to completion, these figures do not necessarily reflect the cholesterol content of final sebum. On the other hand, the figures given by Kellum may represent cholesterol coming from the dermis during the isolation of the sebaceous glands.

Human sebum from skin areas poor in sebaceous glands has a low squalene and a high free-cholesterol content, and sebum from skin areas rich in sebaceous glands has a high content of squalene and a very low content of total cholesterol, most of which is esterified (e.g., Greene et al., 1970). Accordingly, it has been assumed that the free cholesterol originates mainly from the epidermis and the esterified cholesterol, together with squalene, from the sebaceous glands (Nicolaides, 1963). This could be used to differentiate sterols of epidermal and sebaceous origin. However, the epidermis of several species is able to esterify cholesterol (Freinkel and Aso, 1969); moreover, a clear sterol ester fraction was observed in the skin surface lipids obtained from a rat in which sebaceous gland activity had been suppressed by hypophysectomy and estrogen treatment (Nikkari and Valavaara, 1970). It is therefore difficult to know what proportion of the skin surface and hair sterols arises from the sebaceous glands and from the desquamating epidermal cells. It was suggested by Wilson (1963) that the sebaceous glands preferentially use the Kandutsch–Russel pathway, the intermediates of which were known to be mainly esterified, and that the epidermis uses predominantly the Bloch pathway, the intermediates of which are generally free. However, on human skin surface, both the free and esterified sterols have the same percentage of lathosterol, the Kandutsch–Russel intermediate (Nikkari, unpublished observations). This means either that both free and esterified sterols are synthesized by the sebaceous glands or that the epidermis also uses the Kandutsch–Russel pathway; it does not exclude the existence in the epidermis of the Bloch pathway which could run easily to completion without any rate-limiting step between desmosterol and cholesterol. In any case, it appears that neither the degree of esterification nor identification with either one of the biosynthetic pathways can be used to assess the origin of the skin surface sterols.

The human vernix caseosa as well as rodent and sheep seba have appreciably higher contents of sterols than human skin surface lipids (Table I). In vernix and in rat sebum most of the sterols are esterified (Table II) and their fatty acids contain a high proportion of branched chains (Table IV), which are considered to be chiefly of sebaceous origin. The vernix and adult sebum resemble each other by their high squalene content; apparently the virtually complete block between squalene and sterols in adult human sebaceous glands is only partial in the fetal glands, or the vernix caseosa sterols have a nonsebaceous origin. In fetal skin there seems to be an additional rate-limiting step (Table VII), i.e., impaired conversion of lathosterol to cholesterol, which is also present in the skin of the rat and the mouse. The high content of lanosterol and dihydrolanosterol in sheep sebum indicates yet another site of impaired cholesterol biosynthesis. Only the skin of rabbit and guinea pig seems to be able to carry out complete cholesterol biosynthesis without accumulating large amounts of some precursors. However, the total sterol content in rabbit sebum is very low, and the high concentration of type I diester waxes (Table III), at least some of which obviously originate from the epidermis, suggests that most of its sebum cholesterol comes from the epidermis, as in

man. Kandutsch (1964) speculated about the possibility of biochemical mechanisms responsible for these metabolic blocks along the biosynthetic route of cholesterol, but experimental evidence is lacking.

LIPIDS OF PREPUTIAL GLANDS OF RODENTS

Although morphologically preputial glands are true sebaceous glands, their lipid composition is entirely different from that of the sebaceous glands of the same species. Table II shows the lipid class composition of the preputial glands of the rat and the mouse. It is obvious that they differ appreciably from each other. Unlike sebum, rat and mouse preputial glands have a high triglyceride content. Nicolaides (1965) stated that the adherence of subcutaneous fat to excised rat preputial gland may account, in part at least, for the large amount of triglycerides found; from the thin-layer chromatograms of Sansone and Hamilton (1969) it appears that the glands of both species have about the same triglyceride concentration. The fatty acids of mouse preputial gland triglycerides differ from those of human sebum and vernix caseosa by having virtually no branched chains (Sansone and Hamilton, 1969; Snyder and Blank, 1969).

In addition to triglycerides, the mouse preputial gland contains glyceryl ether diesters, i.e., an approximately equal proportion of alk-1-enyl and alkyl diglycerides (Fig. 1), which are absent from the corresponding gland of the rat (Nicolaides, 1965; Sansone and Hamilton, 1969) and have not been shown to be present in skin surface lipids of any species.

The preputial gland lipid of the mouse has a high content of monoester waxes whereas the monoester wax-sterol ester fraction of the rat gland consists mainly of sterol esters (Nicolaides, 1965; Sansone and Hamilton, 1969). The alcohols of the mouse waxes differ from those of mouse sebum by possessing virtually no branched chains and by being of relatively short-chain length (from C_{14} to C_{18} with maximum content at C_{16}). The diester waxes typical of the skin surface lipids of the rodents are completely absent from the preputial glands. Instead, the preputial gland of the male mouse has a small fraction (5% of total lipids) with a thin-layer chromatographic mobility intermediate between those of type I and II diester waxes; this was shown by Spener et al. (1969) to be composed of long-chain (mainly C_{16}) alkyl acetates.

The rat preputial gland has a higher sterol content than the mouse glands. The rat gland also contains a small amount of squalene; its sterol pattern (Wilson, 1963) resembles that of the sebum (Table VII): the intermediates belong to the Kandutsch–Russel pathway and there is an apparent rate-limiting step between lathosterol and cholesterol. The sterols are actually the only constituents of preputial and sebaceous gland lipids that resemble each other.

LIPIDS OF PREEN GLANDS OF BIRDS

Although the preen glands of the birds are not true sebaceous glands, they nevertheless contain lipids which structurally resemble the lipids of mammalian sebum. The major components of the preen gland lipids of the waterfowl order (swans, ducks, geese) are monoester waxes (Haahti et al., 1964; Odham, 1967), which differ from the mammalian waxes by being completely saturated and possessing predominantly branched chains; they are either 2- or 4-methyl, 2,4-dimethyl-, 2,4,6-trimethyl-, or 2,4,6,8-tetramethyl-substituted (Odman, 1967). Moreover, other birds, like falcons and owls, have monoester waxes as major constituents of their feather lipids (Haahti et al., 1964).

Most of the preen gland lipids of fowl-like birds (hen, partridge, pheasant, turkey) have a chromatographic mobility similar to that of mammalian diester waxes and they give rise to diols and fatty acids upon saponification (Haahti et al., 1964; Nicolaides et al., 1968). The diols from chickens have been identified as alkane-2,3-diols with 23–24 carbon atoms (Haahti and Fales, 1967). Apparently the parent compound consists of a diester of the 2,3-diol with unsubstituted fatty acids and it resembles the type II diester waxes of mammalian sebum, in which, however, the diol is 1,2- rather than 2,3-.

The preen gland lipids of ring doves have been reported (Jacob and Zeman, 1972) to contain diesters of hydroxy fatty acids (maximum content at C_{10}) with straight-chain even-numbered fatty acids (maximum at C_{16}) and alcohols (maximum at C_{16}). These compounds are similar to the type I diester waxes in mammals except that they have the hydroxyl group at position 3 rather than at position 2.

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