2. A great variation in lipid content of the various organs of the sea anemone was found, according to the physiological functions they perform, the gonads containing the maximum amount of essential lipid.

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Studies of Sebum

7. THE COMPOSITION OF THE SEBUM OF SOME COMMON RODENTS*

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In order to study the mechanism by which the various components of sebum are formed a suitable laboratory animal was required. Ideally such an animal should have a sebum whose composition closely resembles that of man, but, with the exception of the sheep, little is known about the composition of the hair lipids or sebum of animals. A study was therefore made of the composition of the sebum of the common laboratory animals, namely the guinea pig, mouse, rabbit and rat. The purpose of the investigation was to find how widely the composition of the sebum differed from that of man, so that their uses and limitations as experimental animals could be assessed. A preliminary report of part of this work has already been published (Wheatley, 1953).

EXPERIMENTAL

Materials

In order to obtain a material which would compare with the sebum collected from human subjects the following method of collection was adopted. The mouth and anal regions of the freshly killed animals were cleaned with chloroform, and the body was suspended horizontally over a glass tank. A quantity of acetone (previously twice distilled in all-glass apparatus) was poured over the back of the animal in such a manner that as little as possible flowed over the ears, mouth and anal regions. The acetone which collected in the glass tank was poured back over the animal several times and the animal finally washed with a fresh quantity of acetone. With mice a slightly different procedure was adopted, owing to their smaller size and the small amount of sebum collected from each animal. These animals were simply held by the tail, after the mouth and anal regions had been cleaned with chloroform, and were immersed in a beaker containing acetone. The acetone extract from a number of animals was evaporated to dryness, and the residue dissolved in chloroform. This solution was filtered to remove cellular and other debris and then evaporated to dryness. The amount of material obtained by this procedure from a single animal was approximately as follows: guinea pig 250 mg., mouse 10 mg., rabbit 250 mg., rat 100 mg.

Analytical methods

Chemical constants. Iodine values, acid values, phosphorus and nitrogen were determined by the methods referred to in earlier work (Wheatley, 1954). Cholesterol and 'fast-acting' sterols were determined by the method described by Moore & Baumann (1952). The acrolein method (MacKenna, Wheatley & Wormall, 1950) was used to test for the presence of glycerol.

Fractionation. The fractionation into free fatty acids, combined fatty acids and unsaponifiable matter followed the procedure already described for human sebum (MacKenna *et al.* 1950).

Gas–liquid chromatography. The mixed fatty acids were esterified with methanol and sulphuric acid, and the mixed esters applied directly to the gas chromatogram with a micro-pipette. The general procedure adopted has been

* Part 6: James & Wheatley (1956).

described previously (James & Martin, 1956; James & Wheatley, 1956), unsaturated acids being removed by bromination. The column stationary phase was a high-boiling lubricating-oil extract and the temperature was maintained constant at 197° with boiling ethylene glycol. Peak areas were measured by triangulation and were corrected as described by James & Wheatley (1956).

Chromatography of the unsaponifiable matter. The unsaponifiable matter was fractionated on columns of alumina as described for human sebum (MacKenna, Wheatley & Wormall, 1952). In most cases a sample of 1 g. was fractionated on a 30 g. column of alumina (Brockmann Grade III). Blocking of the column during the initial loading proved particularly troublesome, especially with the mouse-sebum unsaponifiable matter, and was partly prevented by covering the top of the column with a thin layer of sand.

RESULTS

General composition

The general composition and properties of the sebum of these animals are shown in Table 1. It is noteworthy that in all cases only traces of glycerol were obtained on saponification; triglycerides are therefore present only in minute amounts, probably as contaminants. There are, however, marked differences in their properties and in the relative proportions of the different groups of constituents present in the individual seba.

Fatty acids

In Fig. 1 is shown the result obtained by gas–liquid chromatography of 4·8 mg. of the methyl esters of the acids present in rabbit sebum. The chromatogram is most striking in showing the presence of (*a*) large amounts of the straight-chain odd-numbered acids: peak 5, *n*-undecanoic acid; peak 8, *n*-tridecanoic acid; peak 12, *n*-pentadecanoic acid; peak 18, *n*-heptadecanoic acid; (*b*) large amounts of acids that from their position would be expected to possess a highly branched chain: peak 13, a highly branched C_{10} acid; peak 16, a highly branched C_{17} acid; peak 19, a highly branched C_{18} acid. The rabbit apparently produces a highly saturated sebum, since on bromination of the mixture the only peak to disappear is 20, which from its position can be assumed to be oleic acid.

Fig. 2 shows the result obtained with the sample of fatty acids from the rat. The chromatogram is quite distinct from that shown in Fig. 1. In particular the odd-number straight-chain fatty acids are much smaller in amount, the highly branched acids of chain length C_{16}, C_{17} and C_{18} are absent, more unsaturated C_{18} acids are present and much larger amounts of even-numbered *iso-* or *anteiso*-acids are present. Rabbit sebum appears to contain no acids of chain length greater than C_{18}, as the whole of the sample placed on the column can be accounted for in terms of the acids shown in Fig. 1. With rat sebum, however, appreciable amounts of highly branched and simply branched C_{19} and C_{20} acids are present (see Table 2), and acids of chain length up to C_{20} account for only 90% of the material applied to the column.

Mouse sebum (Table 2) contains still larger amounts of acids of chain length greater than C_{21}, only 62% being accounted for in the range C_{10}–C_{21}. This animal is noteworthy in producing larger amounts of an unsaturated C_{10} acid than of the corresponding saturated acid. The percentage of unsaturated C_{18} acids present in mouse sebum is, however, lower than that in rat sebum, except for a mono-unsaturated C_{18} acid which is present in comparable amount in the two animals. The presence of 9·2% of *n*-eicosanoic acid and 6·1% of *n*-heneicosanoic acid is an unexpected finding.

The guinea pig, too, produces a highly saturated sebum, of which only 63% can be accounted for in the range C_{10}–C_{20}. Branched-chain acids of chain length C_{15}, C_{17}, C_{19} and C_{20} are prominent. The detailed distribution of the acids is given in Table 2. These results show that the fatty acid composition of the sebum of the four animals is widely different, and taken together with the results published earlier on human sebum (James & Wheatley, 1956) suggest a marked species differentiation of such an order that all five seba could be readily distinguished on the basis of a gas–liquid chromatogram of their component fatty acids.

Table 1. *General composition and properties of the sebum of some common rodents*

	Guinea pig	Mouse	Rabbit	Rat
M.p.	34°	35°	42°	33°
Acid no.	7	14	16	14
Iodine value	33	41	9	29
Cholesterol (total) (%)	19·8	4·5	3·5	5·3
'Fast-acting' sterols (%)	2·0	8·1	0·1	4·1
Lipid P	Nil	Nil	Nil	Nil
Total N (%)	0·31	0·22	0·15	0·45
Fatty acids (free) (%)	6·0	7·5	9·0	7·4
Fatty acids (combined) (%)	49·3	36·7	43·6	51·4
Unsaponifiable matter (%)	44·8	54·6	45·9	41·4
Glycerol	Trace	Trace	Trace	Trace

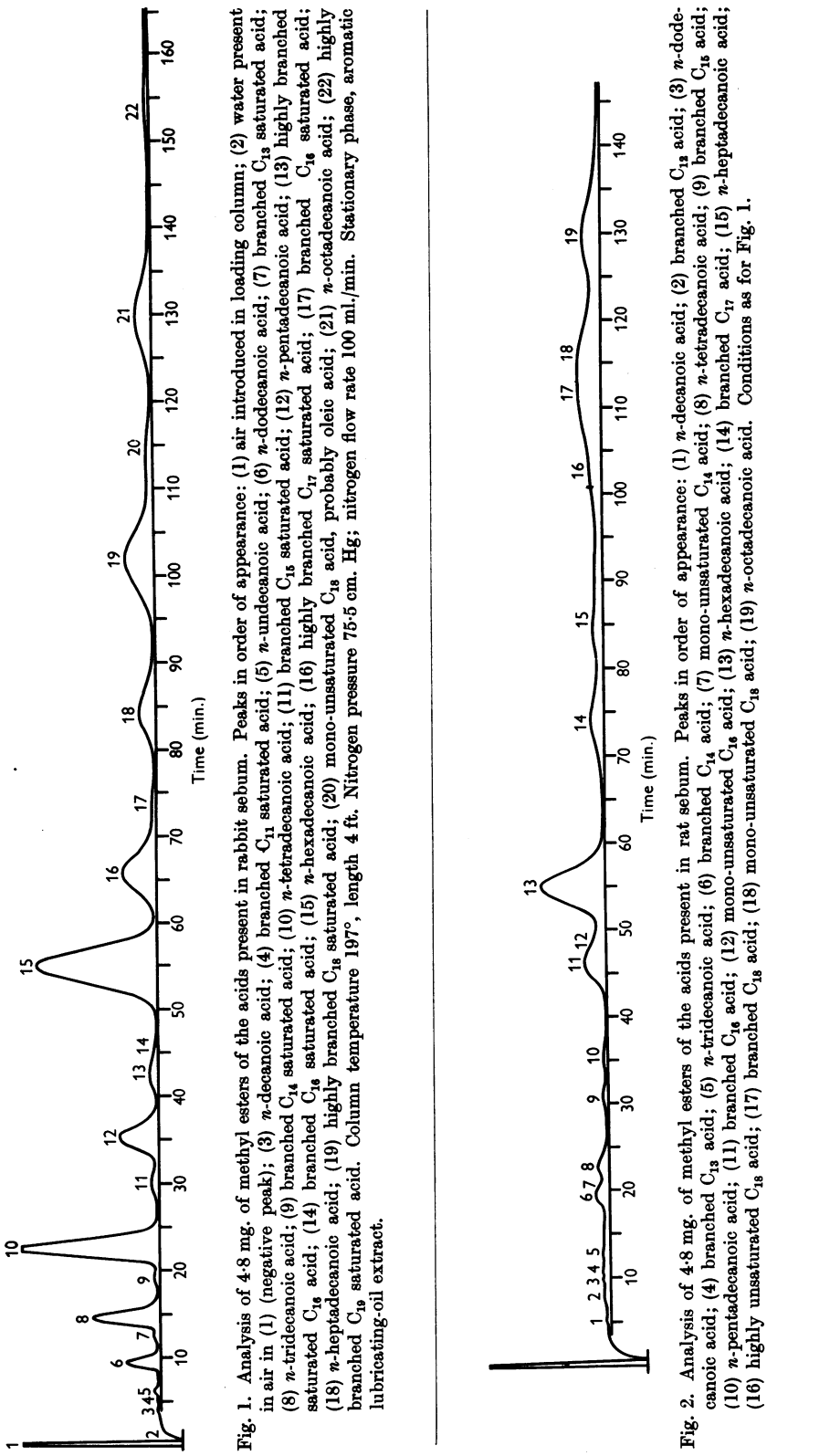

Fig. 1. Analysis of 4·8 mg. of methyl esters of the acids present in rabbit sebum. Peaks in order of appearance: (1) air introduced in loading column; (2) water present in air in (1) (negative peak); (3) *n*-decanoic acid; (4) branched C_{11} saturated acid; (5) *n*-undecanoic acid; (6) *n*-dodecanoic acid; (7) branched C_{13} saturated acid; (8) *n*-tridecanoic acid; (9) branched C_{14} saturated acid; (10) *n*-tetradecanoic acid; (11) branched C_{15} saturated acid; (12) *n*-pentadecanoic acid; (13) highly branched saturated C_{16} acid; (14) branched C_{16} saturated acid; (15) *n*-hexadecanoic acid; (16) highly branched C_{17} saturated acid; (17) branched C_{16} saturated acid; (18) *n*-heptadecanoic acid; (19) highly branched C_{18} saturated acid; (20) mono-unsaturated C_{18} acid, probably oleic acid; (21) *n*-octadecanoic acid; (22) highly branched C_{19} saturated acid. Column temperature 197°, length 4 ft. Nitrogen pressure 75·5 cm. Hg; nitrogen flow rate 100 ml./min. Stationary phase, aromatic lubricating-oil extract.

Fig. 2. Analysis of 4·8 mg. of methyl esters of the acids present in rat sebum. Peaks in order of appearance: (1) *n*-decanoic acid; (2) branched C_{12} acid; (3) *n*-dodecanoic acid; (4) branched C_{13} acid; (5) *n*-tridecanoic acid; (6) branched C_{14} acid; (7) mono-unsaturated C_{14} acid; (8) *n*-tetradecanoic acid; (9) branched C_{15} acid; (10) *n*-pentadecanoic acid; (11) branched C_{16} acid; (12) mono-unsaturated C_{16} acid; (13) *n*-hexadecanoic acid; (14) branched C_{17} acid; (15) *n*-heptadecanoic acid; (16) highly unsaturated C_{18} acid; (17) branched C_{18} acid; (18) mono-unsaturated C_{18} acid; (19) *n*-octadecanoic acid. Conditions as for Fig. 1.

Table 2. *Quantitative analysis of the acids present in the sebum of some common rodents*

The figures given represent the percentage of each acid present; 'trace' = less than 0·05 %.

Acid	Guinea pig	Mouse	Rabbit	Rat
n-Decanoic	Trace	Trace	0·12	Trace
Branched undecanoic	Trace	Trace	Trace	Trace
n-Undecanoic	Trace	Trace	0·3	Trace
Branched dodecanoic	Trace	Trace	Trace	Trace
n-Dodecanoic	Trace	Trace	2·2	Trace
Branched tridecanoic	Trace	Trace	0·6	Trace
n-Tridecanoic	Trace	Trace	6·6	Trace
Branched tetradecanoic	Trace	0·2	0·7	2·6
Mono-unsaturated C_{14}	Trace	0·4	0	0·5
n-Tetradecanoic	0·3	0·7	10·7	1·6
Branched pentadecanoic	0·7	0·3	1·3	1·1
Mono-unsaturated C_{15}	0	0·7	0	0
n-Pentadecanoic	1·6	1·1	6·0	0·8
Highly branched hexadecanoic	0	0	1·8	0
Branched hexadecanoic	0·5	1·7	0·5	4·7
Mono-unsaturated C_{16}	1·1	7·3	0	2·1
n-Hexadecanoic	15·8	5·0	24·0	17·4
Highly branched heptadecanoic	0	0	11·0	0
Branched heptadecanoic	2·4	0·9	1·7	5·0
Mono-unsaturated C_{17}	0·8	0·9	0	0
n-Heptadecanoic	5·8	1·7	6·8	3·1
Highly branched octadecanoic	3·1	0	14·3	0
Branched octadecanoic	Trace	0·7	0	11·3
Highly unsaturated C_{18}	0	0	0	1·7
Di-unsaturated C_{18}	0	0	0	3·1
Mono-unsaturated C_{18}	7·7	10·0	3·7	11·3
n-Octadecanoic	4·0	3·9	8·2	8·9
Branched nonadecanoic	9·8	1·0	0	3·1
Mono-unsaturated C_{19}	3·1	0	0	0
n-Nonadecanoic	2·2	2·2	0	0
Highly branched eicosanoic	0	0	0	4·1
Branched eicosanoic	4·3	5·1	0	6·8
n-Eicosanoic	Not measured	9·2	0	Not measured
Branched heneicosanoic	Not measured	2·5	0	Not measured
n-Heneicosanoic	Not measured	6·1	0	Not measured

Table 3. *Chromatography of the unsaponifiable matter of the sebum of common rodents*

Fraction*	Guinea pig (%)	Mouse (%)	Rabbit (%)	Rat (%)
I	3·4	2·1	7·5	3·6
II	12·8	10·2	74·5	42·8
III	42·3	23·6		24·3
IV	37·9	51·4	12·9	21·4
V	3·6	2·0	2·6	3·8
	—	10·7†	2·2†	3·1†

* See text. † Not eluted from column.

Unsaponifiable matter

The unsaponifiable matter was fractionated on columns of alumina into the five principal fractions indicated in Table 3, which were eluted with light petroleum (b.p. 40–60°), 5 % and 10 % chloroform (v/v) in light petroleum, chloroform and methanol in this order. These fractions were then examined in greater detail with the following results.

Fraction I. The materials from all four animals were soft white waxes consisting entirely of hydrocarbons. They had a relatively low iodine value (19, 20, 7 and 36 for the material from the guinea pig, mouse, rabbit and rat respectively) and it was not possible to demonstrate the presence of squalene by isolation of the hexahydrochloride. This does not exclude the possible presence of small amounts, and if all the unsaturated material present were squalene it would constitute 5·1, 5·4, 1·8 and 9·7 % of the fractions from the guinea pig, mouse, rabbit and rat respectively. This would amount to only 0·17, 0·11, 0·13 and 0·35 % respectively of the total unsaponifiable matter of the sebum from these animals.

Fractions II and III. Owing to the presence of the 'fast-acting' sterols a clear-cut separation of the sterols and wax alcohols was not achieved. It was found more convenient to pool both fractions and then remove the sterols by means of digitonin. The sterols were recovered from the precipitated digitonides by refluxing with pyridine for 2 hr., followed by treatment with ether in the usual manner. The wax alcohols were recovered from the mother liquors and washings from the digitonide precipitation after removal of the excess of digitonin. They constituted 26·1, 31·9, 92·1 and 63·3 %

of the pooled fractions from the guinea pig, mouse, rabbit and rat respectively. Urea-adduct formation (Tiedt & Truter, 1952) showed the presence of 1·6, 1·4, 66·0 and 25·6% of straight-chain alcohols respectively in the alcohols isolated from these animals, and both saturated and unsaturated alcohols were present in all cases. An attempt to examine these alcohols in greater detail by gas–liquid chromatography was unsuccessful, since the materials appeared to have a chain length greater than C_{18} and were beyond the range of the instruments at present available. They will be further examined at a later date. Recrystallization of the alcohols from the rabbit gave a white wax, m.p. 75·6° (Found: C, 80·8; H, 13·7%), indicating an average chain length of about C_{24}. Even this brief examination has, however, indicated that the alcohols from the four animals are different.

The sterols isolated from the guinea pig, mouse, rabbit and rat contained respectively 9·2, 63·9, 2·8 and 43·6% of 'fast-acting' material when examined by the method of Moore & Baumann (1952). This 'fast-acting' material has not been isolated, but, as a result of the identification by Idler & Baumann (1952) of the principal 'fast-acting' sterol in rat skin as cholest-7-en-3β-ol (lathosterol), they have been provisionally identified as this sterol. Examination of the ultraviolet spectra of these fractions showed the presence of 0·06% of 7-dehydrocholesterol in the fraction from the mouse. The fraction from the rat gave an indication of a faint trace (less than 0·01%), but those from the guinea pig and rabbit failed to show detectable amounts of 7-dehydrocholesterol.

Fraction IV. Recrystallization of the fraction from the mouse gave white feathery needles, m.p. 85°. Periodate oxidation indicated that this was a glycol, and lead tetra-acetate oxidation (Horn & Hougen, 1953) yielded formaldehyde, characterized as the dimedone complex (m.p. and mixed m.p. 189°), and long-chain fatty acids which were not further studied. The fraction from the mouse therefore consisted almost entirely of either a single alkane-1:2-diol or a mixture of several closely related diols. The average chain length appeared to be about C_{16}. (Found: C, 74·7; H, 13·5; $C_{16}H_{34}O_2$ requires C, 74·4; H, 13·2%.) No sterols were present in either the crude fraction or the material obtained from the mother liquors after removal of most of the diol, as indicated by the Liebermann–Burchardt test.

The corresponding fractions from the guinea pig, rabbit and rat, when analysed by the periodate method of Karnovsky & Rapson (1946), were found to contain 66·4, 38·2 and 33·4% respectively of diols (calculated as the C_{20} diol). Each fraction on oxidation with lead tetra-acetate yielded formaldehyde (dimedone complex), together with long-chain fatty acids, indicating that all contained alkane-1:2-diols.

In addition to these diols the fractions from the guinea pig and the rabbit contained sterols, indicated by the Liebermann–Burchardt test, which constituted 3·9 and 5% respectively of the crude fractions and were 'fast-acting'. Both materials gave positive Rosenheim and antimony trichloride tests. In both cases the colour in the Rosenheim test was an intense royal blue; with the antimony trichloride test the guinea-pig material gave a purple coloration, but the rabbit material a blue. Paper chromatography (alumina-impregnated paper, chloroform as solvent; Wheatley, 1954) indicated two components in both materials, both giving blue spots with antimony trichloride. Neither fraction gave a digitonide, nor was there any distinctive band in the ultraviolet spectrum.

The fraction from the rat gave a reddish-brown coloration with the Liebermann–Burchardt test. The absorption spectrum of the coloured material showed a broad band at about 350 mμ. The corresponding spectrum for the fraction from the guinea

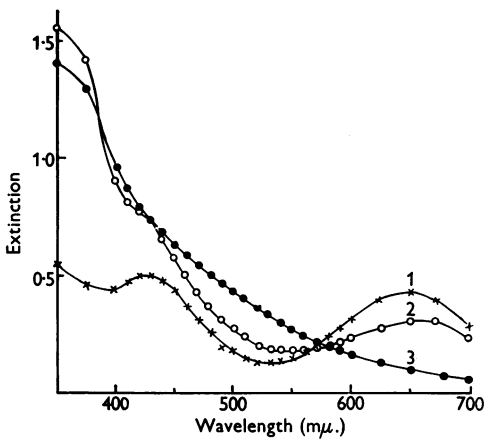

Fig. 3. Absorption spectra of the Liebermann–Burchardt colours produced by (1) cholesterol, (2) guinea-pig fraction IV, (3) rat fraction IV.

Table 4. *Estimated composition of the unsaponifiable matter of the sebum of common rodents*

	Guinea pig (%)	Mouse (%)	Rabbit (%)	Rat (%)
Hydrocarbons	3·4	2·1	7·5	3·6
Wax alcohols	11·1	10·8	68·7	42·5
Alkane-1:2-diols	25·1	50·4	4·9	7·1
Cholesterol	40·0	8·3	7·6	13·9
'Lathosterol'	4·0	14·8	0·2	10·7
Other sterols	1·5	—	0·6	0·4
Unidentified	14·9	13·7	10·5	21·8

pig showed a similar band in addition to the usual band at 560 mμ. (Fig. 3), but no indication has yet been obtained of the nature of the substance responsible for this coloration.

Fraction V. These were dirty-white or brown waxes or gums. The fractions from the guinea pig, mouse and rabbit contained little or no sterol material. That from the rat gave a strongly positive Liebermann–Burchardt test, a positive Rosenheim test but a negative antimony trichloride test. This material did not yield a digitonide, nor did it show distinctive ultraviolet absorption bands; it comprised about 5% of the crude fraction.

From the results of this detailed examination of the various fractions the composition of the unsaponifiable matter of the sebum of these animals can be calculated (Table 4).

DISCUSSION

An examination of the sebum of four common types of rodents used as laboratory animals has revealed some striking differences in composition. In general the materials differ from human sebum in that they contain little or no squalene, and differ from the sebum of the sheep and other ruminants in that they contain little or no 'isocholesterol'. Instead these rodents' sebum appears to contain lathosterol. The amount present in rabbit sebum is rather small (0·1%). This sterol gives a much more intense coloration in the Liebermann–Burchardt test than does cholesterol; consequently earlier workers have obtained erroneously high results for the cholesterol content of the hair lipids from certain animals (Eckstein, 1927).

Three classes of long-chain aliphatic compounds, namely hydrocarbons, alcohols and fatty acids, have already been shown to be present in sebum. To these may now be added a further class, the alkane-1:2-diols. These have already been shown to be present in sheep wool-wax (Horn & Hougen, 1953) and are now shown to be present in the sebum of the four animals examined here, accounting for between 5 (rabbit) and 50% (mouse) of the unsaponifiable matter. In the sheep, Horn & Hougen identified five such diols, namely *n*-hexadecane-, 16-methylheptadecane-, 18-methylnonadecane-, 20-methylheneicosane- and 22-methyltricosane-1:2-diol. In the rodents here examined it is also possible that similar homologous mixtures exist and it is intended to make a further study of the precise nature of the diols present in each animal sebum.

Examination of the fatty acids by gas–liquid chromatography has shown the nature of these acids to be characteristic for each of the animals. In many ways the acids are unusual; for instance, 27% of the acids from rabbit sebum are highly branched acids. Preliminary examination also indicates that the alcohols from each animal are different. It is also likely that both the hydrocarbons and the diols will be shown to be different for each animal.

In addition to differences in the carbon chains of these long-chain compounds there are differences in the proportions of the types of compounds present in the unsaponifiable matter. Thus the unsaponifiable matter from rabbit and rat sebum contains a high proportion of alcohols (68·7 and 42·5% respectively); that from the mouse contains large amounts of diols (50·4%), and that from the guinea pig a high proportion of cholesterol (40·0%).

In general the composition of sebum seems to be a unique property of the species from which it comes. The marked differences in the composition of the sebum from closely related animals makes the selection of experimental animals difficult, nevertheless these differences can be turned to advantage in some cases. For instance, for studies on the waxes of sebum the rabbit would be the animal of choice, while the mouse should be used for studies of the diols. The common laboratory animals can be used for a large number of metabolic studies on the formation and secretion of sebum. There are, however, many purposes for which they cannot be used, e.g. for studies on squalene, and the examination of the composition of the sebum from a number of other species will be necessary if such problems are to be studied.

SUMMARY

1. The sebum of the common rodents, the guinea pig, mouse, rabbit and rat, has been examined and the usefulness of these animals for experimental studies on sebum metabolism assessed.

2. Marked differences in the composition of the sebum of these four animals have been demonstrated, both among themselves and from that of man and the sheep. The presence of lathosterol (cholest-7-en-3β-ol), however, appears to be characteristic of the sebum of the species of this family.

3. A fourth group of long-chain aliphatic compounds, namely alkane-1:2-diols, has been established as a common constituent of sebum.

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The Separation of the C_6–C_{12} Fatty Acids by Reversed-phase Partition Chromatography

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The monoterpene (C_{10}) acids are becoming of increasing interest to biochemists as possible intermediates in the biosynthesis of cholesterol, rubber and the carotenoids. The isolation and separation of small quantities of terpene acids should be facilitated by chromatography. Since these acids are all somewhat more polar than *n*-decanoic acid a chromatographic system capable of separating the straight-chain C_6–C_{10} acids should be appropriate for their resolution.

Partition chromatography has been successfully applied to the separation of the C_5–C_{10} fatty acids (Ramsey & Patterson, 1948; Peterson & Johnson, 1948; Boldingh, 1950; Nijkamp, 1951; Vandenheuvel & Hayes, 1952; Zbinovsky, 1955), and to the acids in the lower part of this range, C_5–C_8 (Moyle, Baldwin & Scarisbrick, 1948; Fairbairn & Harpur, 1951; Popják & Tietz, 1954). The lower fatty acids can also be separated by displacement analysis (Holman & Hagdahl, 1950). Gas–liquid chromatography affords an analytical separation with the highest resolution (James & Martin, 1952, 1956). Nevertheless, the convenience, high resolving power and larger capacity of reversed-phase partition chromatography as developed by Howard & Martin (1950) justified extending this method to include the C_6–C_{12} acids.

Crombie, Comber & Boatman (1955) decreased the acetone concentration in the aqueous acetone–medicinal paraffin solvent system devised by Howard & Martin, and thereby extended the separations to include decanoic and octanoic acids. However, the aqueous methanol–chloroform system tentatively tested by Howard & Martin (1950) and developed by Wittenberg (1955) for another purpose was found particularly well suited to the separation of the C_6–C_{12} acids.

Columns of siliconized kieselguhr prepared with aqueous methanol–chloroform tend to lose the stationary phase during use (Howard & Martin, 1950). This difficulty may be obviated by the addition of a hydrocarbon to the chloroform phase (Bergström & Sjövall, 1951). Presumably the hydrocarbon achieves its effect by increasing the interfacial tension between the solvent phases (Howard & Martin, 1950). The stability of the column improves with increasing molecular weight of the added hydrocarbon. 2:2:4-Trimethylpentane stabilizes the columns far more than hexane or heptane, and decane affords a column which is stable to repeated use. Accordingly, a decane fraction, Skellysolve S, has been used to stabilize the columns described.

METHODS

Preparation of kieselguhr. Hyflo Super Cel (Johns-Manville Co. Ltd., New York, U.S.A.) was washed by decantation, twice with 3 vol. of 0·1 N-HCl (Sjövall, 1953) and repeatedly with water until the supernatant fluid was relatively free of fine particles, which otherwise would leak from the columns (Silk & Hahn, 1954). The washed kieselguhr was dried at 110°, and, when cool, stirred while passing through it a stream of dry nitrogen previously drawn through liquid dichlorodimethylsilane. The treated kieselguhr was washed with methanol, until the washings were no longer acid, was dried at 110° and stored for use.

Solvents. Solvents were used without special purification. Skellysolve S (boiling range 150–202°) is stated by the manufacturer (Skelly Oil Co., Kansas City, Missouri, U.S.A.) to contain some nonanes and undecanes and a considerable quantity of *n*- and *iso*-decanes.