cis- and *trans*-diol, together with indan-2-one (Loon, 1919). So far, however, there is no experimental evidence to show that epoxide formation occurs in the animal body during the metabolism of indene.

SUMMARY

1. Indene has been administered to rabbits by stomach tube, and fractionation of ether extracts of the urine has yielded optically active *cis*- and *trans*-indane-1:2-diol in a total amount corresponding to about 5% of the dose. Acid treatment of the urine, either before or after ether extraction, yielded indan-2-one in amounts corresponding to about 25% of the dose of indene.

2. The same two diols have been isolated from the urine of rabbits dosed with indene by subcutaneous injection.

3. *cis*-Indane-1:2-diol has been isolated from ether extracts of the urine of rats dosed with indene by stomach tube. Indan-2-one has been obtained by acid treatment of the extracted urine.

4. The interconversion of *cis*- and *trans*-indane-1:2-diol has been studied, and the metabolic formation of the two diols is discussed.

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Studies of Sebum

6. THE DETERMINATION OF THE COMPONENT FATTY ACIDS OF HUMAN FOREARM SEBUM BY GAS–LIQUID CHROMATOGRAPHY*

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Relatively little information is available concerning the component fatty acids of human sebum. Engman & Kooyman (1934) made a brief study, using lead-salt separation and fractionation of the bromine derivatives of the liquid acids. They concluded that the free and combined fatty acid fractions of sebum contained both oleic and linoleic acids and that the combined acid fraction also contained arachidonic acid. They also demonstrated appreciable amounts of saturated products in the liquid acid fraction and concluded that branched-chain acids were possibly present. Ricketts, Squire & Topley (1951) examined the free fatty acids of human forearm sebum and were able to isolate pure barium oleate (identified by X-ray diffraction). They also reported a chromatographic examination of the saturated fatty acids performed for them by Dr G. A. Howard. This showed the

* Part 5: Wheatley (1954).

presence of at least seven components, of which the most abundant was probably palmitic acid.

Weitkamp, Smiljanic & Rothman (1947) made a comprehensive study of the free fatty acids of human-hair lipid. They fractionated by amplified distillation the free fatty acids obtained from the lipids from 45 kg. of hair. The source of hair was barber's shop sweepings, and contamination of sebum was likely; nevertheless, they were able to show that the extracted fatty acids constituted a homologous series from C_7 to C_{22}, which included both odd and even members. Both saturated and unsaturated acids were present, and the position of the double bonds in the latter was atypical since octadec-6-, -8- and -9-enoic acids were present, whereas the C_{16} unsaturated acid was hexadec-6-enoic acid and not palmitoleic acid. This unusual character of the acids was thought to preclude the possibility of extensive contamination of the hair lipids; nevertheless, it was essential that this work should be repeated on specimens of sebum obtained as free as possible from contamination. Furthermore, it has been observed by Burtenshaw (1942) and by Ricketts *et al.* (1951) that the self-sterilizing power of human sebum appears to depend on the long-chain unsaturated fatty acids; Rothman, Smiljanic, Shapiro & Weitkamp (1947) showed that sebum possessed fungicidal properties associated with the shorter-chain saturated acids. It was, therefore, necessary to be able to determine the component fatty acids in a sample of sebum from a single subject in order to make possible the study of the role played by these acids in maintaining the health of the skin.

In view of the complex nature of the fatty acids of human sebum indicated by the work of Weitkamp *et al.* (1947) the method of choice must have a very high resolving power. Furthermore, sebum from certain animals, e.g. the sheep, contains a high proportion of branched-chain fatty acids, and it is therefore necessary for the method to be able to distinguish between straight- and branched-chain and saturated and unsaturated acids of a homologous series, each member differing by only one carbon atom. Conventional partition-chromatographic methods applied to the separation of fatty acids will usually separate acids which differ in chain length by two carbon atoms, but are useless if applied to sebum analysis. The method of gas–liquid chromatography developed by James & Martin (1952) for shorter-chain fatty acids has been extended (James & Martin, 1956*a*) to the methyl esters of higher fatty acids, and possesses the necessary high resolving power. When applied to sebum fatty acids it proved entirely suitable, and since less than 10 mg. of material is required for an analysis it can be applied to individual sebum samples.

EXPERIMENTAL

Sebum. A sample of sebum was collected from the forearms of six normal male subjects by the acetone method and was separated into free and combined fatty acids and non-saponifiable material by methods already described (MacKenna, Wheatley & Wormall, 1950). The sample contained: free fatty acids 26·3%, combined fatty acids 35·5% and unsaponifiable matter 33·8%. The fatty acid fractions were esterified with methanol and H_2SO_4 and analysed on the gas–liquid chromatogram.

Gas–liquid partition chromatography. The columns used had as stationary phase the high-boiling lubricating oil described previously (James & Martin, 1956*a*). Samples were applied to the column with a micropipette (James & Martin, 1956*b*). Column temperatures were maintained at 197° with boiling ethylene glycol. Bromination of the mixed esters was carried out as described by James & Martin (1956*a*).

The measurement of peak areas was carried out by drawing lines through the points of inflexion in the sides of each peak. The area enclosed by the triangle so formed is about 4% less than the area of the peak. provided that the latter approximates closely to the shape of the Gaussian error curve (this is usually the case).

The response of the gas-density balance (Martin & James, 1956) is proportional to the mol.wt. excess of the substance being detected (i.e. mol.wt. – mol.wt. of nitrogen, the carrier gas). The peak areas were corrected to those expected for a standard substance, in this case methyl palmitate, by the formula:

$$\text{Peak area} \times \frac{M_1 - 28}{M_2 - 28} \times \frac{M_2}{M_1},$$

where M_1 is the mol.wt. of the substance being detected and M_2 is the mol.wt. of methyl palmitate. The percentage of any component is then:

$$\frac{\text{Corrected peak area}}{\text{Total corrected area of all peaks}} \times 100.$$

RESULTS

In Fig. 1, curve A, is shown the result obtained from the gas–liquid chromatograph with a sample of the mixed esters (4·8 mg.) of fatty acids occurring free in human sebum. A total of nineteen peaks in the range from methyl *n*-decanoate to methyl *n*-octadecanoate can be seen. The main components are methyl *n*-dodecanoate, *n*-tetradecanoate and *n*-hexadecanoate and a substance moving in front of methyl *n*-octadecanoate. The saturated acids present in the mixture, determined after conversion of the unsaturated acids into the virtually non-volatile bromo acids by treatment of the mixture with bromine in ether, are shown in Fig. 1, curve *B*. Certain peaks have disappeared and others show a relative decrease, indicating an overlap of peaks due to saturated and unsaturated acids in curve *A*. Correlation of the two results suggests that at least twenty-nine components are present.

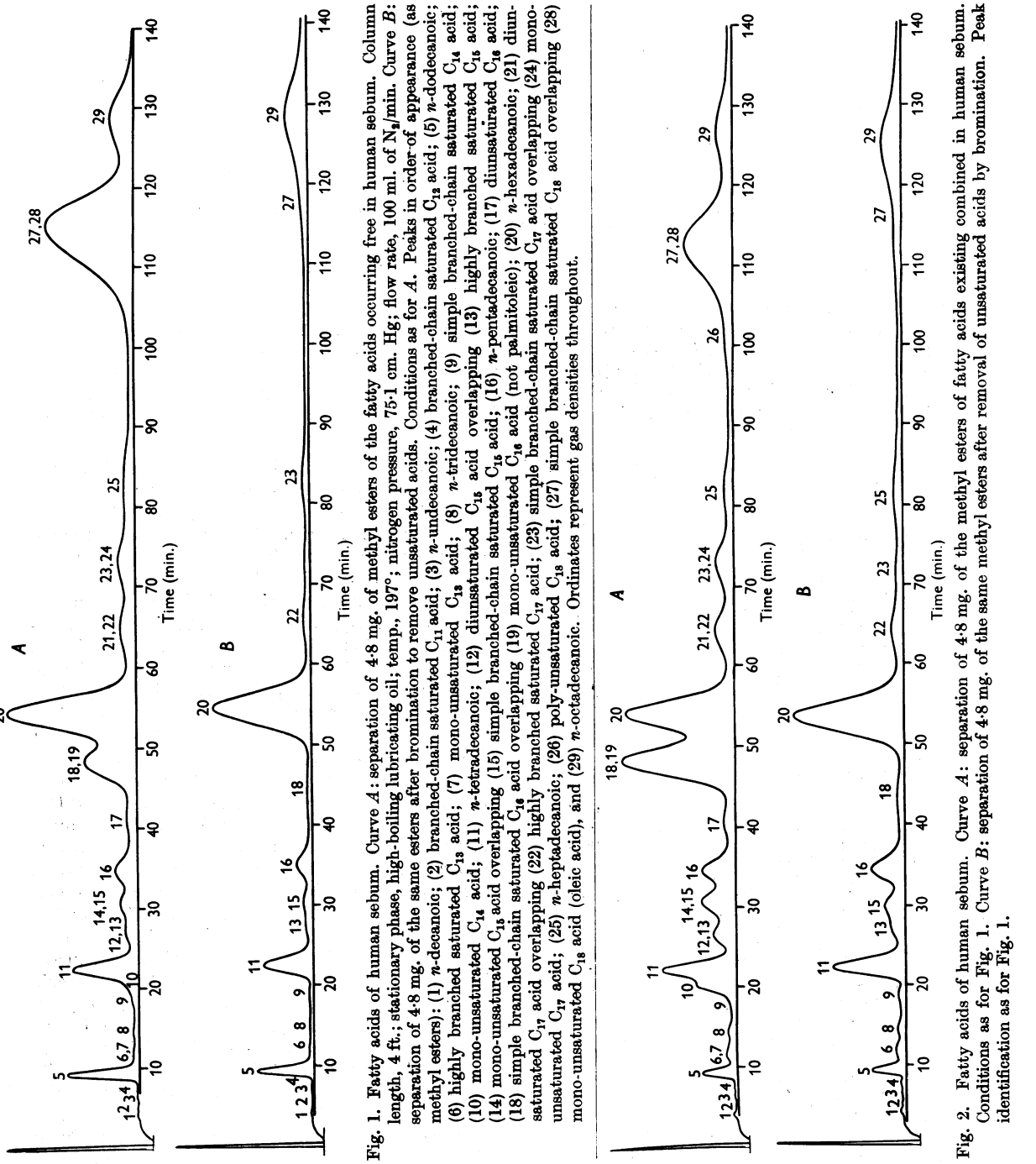

Fig. 1. Fatty acids of human sebum. Curve *A*: separation of 4·8 mg. of methyl esters of the fatty acids occurring free in human sebum. Column length, 4 ft.; stationary phase, high-boiling lubricating oil; temp., 197°; nitrogen pressure, 75·1 cm. Hg; flow rate, 100 ml. of N_2/min. Curve *B*: separation of 4·8 mg. of the same esters after bromination to remove unsaturated acids. Conditions as for *A*. Peaks in order of appearance (as methyl esters): (1) *n*-decanoic; (2) branched-chain saturated C_{11} acid; (3) *n*-undecanoic; (4) branched-chain saturated C_{12} acid; (5) *n*-dodecanoic; (6) highly branched saturated C_{13} acid; (7) mono-unsaturated C_{13} acid; (8) *n*-tridecanoic; (9) simple branched-chain saturated C_{14} acid; (10) mono-unsaturated C_{14} acid; (11) *n*-tetradecanoic; (12) diunsaturated C_{15} acid overlapping (13) highly branched saturated C_{15} acid; (14) mono-unsaturated C_{15} acid overlapping (15) simple branched-chain saturated C_{15} acid; (16) *n*-pentadecanoic; (17) diunsaturated C_{16} acid; (18) simple branched-chain saturated C_{16} acid overlapping (19) mono-unsaturated C_{16} acid (not palmitoleic); (20) *n*-hexadecanoic; (21) diunsaturated C_{17} acid overlapping (22) highly branched saturated C_{17} acid; (23) simple branched-chain saturated C_{17} acid overlapping (24) monounsaturated C_{17} acid; (25) *n*-heptadecanoic; (26) poly-unsaturated C_{18} acid; (27) simple branched-chain saturated C_{18} acid overlapping (28) mono-unsaturated C_{18} acid (oleic acid), and (29) *n*-octadecanoic. Ordinates represent gas densities throughout.

Fig. 2. Fatty acids of human sebum. Curve *A*: separation of 4·8 mg. of the methyl esters of fatty acids existing combined in human sebum. Conditions as for Fig. 1. Curve *B*: separation of 4·8 mg. of the same methyl esters after removal of unsaturated acids by bromination. Peak identification as for Fig. 1.

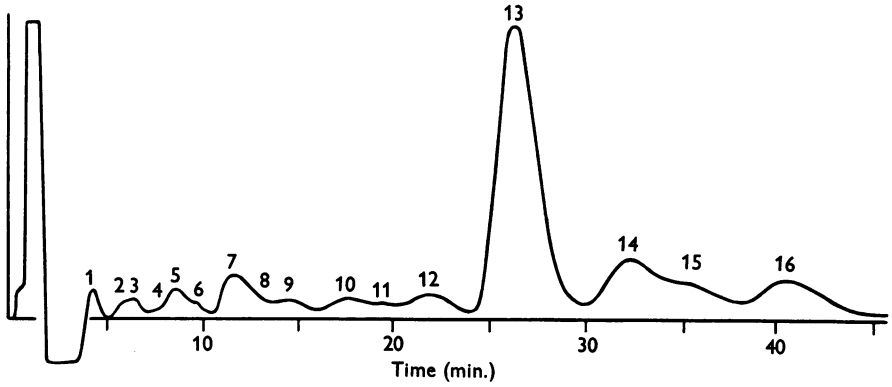

Fig. 3. Separation of methyl esters of free short-chain acids in human sebum. Total load 12 mg.; column conditions as Fig. 1, except that nitrogen pressure is 13 cm. Hg and nitrogen flow rate 14·5 ml./min. Peak identification: (1) branched-chain C_7 acid; (2) *n*-octanoic; (3) highly branched C_9 acid; (4) *n*-nonanoic; (5) highly branched C_{10} acid; (6) simple branched-chain C_{10} acid; (7) *n*-decanoic; (8) highly branched C_{11} acid; (9) simple branched C_{11} acid; (10) *n*-undecanoic; (11) highly branched C_{12} acid; (12) simple branched C_{12} acid; (13) *n*-dodecanoic; (14) highly branched C_{13} acid; (15) simple branched C_{13} acid; (16) *n*-tridecanoic. Peak 13 corresponds with peak 5 in Fig. 1.

Table 1. *Approximate analysis of the acids present in the free state in human sebum in the range* C_7–C_{18}

Acid	Percentage
Saturated *n*-C_7	Trace (i.e. <0·05%)
Branched saturated C_8	Trace
Saturated *n*-C_8	Trace
Branched saturated C_9	Trace
Saturated *n*-C_9	Trace
Branched saturated C_{10}	Trace
Saturated *n*-C_{10}	0·08
Branched saturated C_{11}	Trace
Saturated *n*-C_{11}	Trace
Branched saturated C_{12}	Trace
Saturated *n*-C_{12}	3·64
Branched saturated C_{13}	0·25
Saturated *n*-C_{13}	0·25
Mono-unsaturated C_{14}	0·76
Branched saturated C_{14}	0·32
Saturated *n*-C_{14}	6·35
Diunsaturated C_{15}	0·28
Highly branched saturated C_{15}	0·54
Mono-unsaturated C_{15}	0·64
Branched saturated C_{15}	0·67
Saturated *n*-C_{15}	2·27
Mono-unsaturated C_{16}	9·05
Branched saturated C_{16}	0·29
Saturated *n*-C_{16}	24·2
Diunsaturated C_{17}	0·69
Highly branched saturated C_{17}	1·31
Mono-unsaturated C_{17}	1·62
Branched saturated C_{17}	0·73
Saturated *n*-C_{17}	1·07
Mono-unsaturated C_{18}	35·6
Branched saturated C_{18}	1·8
Saturated *n*-C_{18}	8·0

It was pointed out in an earlier publication (James & Martin, 1956*a*) describing the application of the gas–liquid chromatogram to the long-chain fatty acids that the time of emergence of a peak can be used in structure identification. On this basis it is possible to give tentative identifications of the acids present in this natural mixture. These are as follows: peaks 1, 3, 5, 8, 11, 16, 20, 25 and 29: the homologous series of saturated straight-chain acids of chain lengths from C_{10} to C_{18}; peaks 2, 6, 13 and 22: a series of highly branched odd-numbered saturated acids of chain lengths C_{11}, C_{13}, C_{15} and C_{17}; peaks 7, 12, 17, 21 and 26: unsaturated acids containing probably two double bonds and assignable to straight-chain acids of lengths C_{13}, C_{15}, C_{16}, C_{17} and C_{18}; peaks 9, 15, 18, 23 and 27: branched-chain saturated acids (the branch being probably a single methyl group) of lengths C_{14}, C_{15}, C_{16}, C_{17} and C_{18}; peaks 10, 14, 19, 24 and 28: mono-unsaturated acids, probably straight-chain, of lengths C_{14}, C_{15}, C_{16}, C_{17} and C_{18}.

The existence of these series is shown more clearly in Fig. 2, curves *A* and *B*, which represent the result obtained from samples of the methyl esters of the combined fatty acids from human sebum. Although the relative proportions of the acids are different the general picture is the same as that obtained with the free fatty acids. Conclusive identification of the minor components must await their isolation and degradation. There is, however, no doubt that the C_{16} unsaturated acid is not palmitoleic acid (hexadec-9-enoic acid), since its peak position is different from that due to palmitoleic acid. The C_{18} unsaturated acid lies in the position expected for methyl oleate. The existence of very small amounts of shorter-chain acids in sebum was demonstrated by using a higher load and running the chromatogram more slowly. Fig. 3 gives the result obtained under these conditions; at least sixteen components are present ranging in chain length from C_7 to C_{13}. The general pattern is

very similar to that given by the higher acids. The closeness of the peaks renders identification by position more difficult, but it can be seen that in the range C_7–C_{13} a number of simply branched and more highly branched acids exist, similar to those in the range C_{12}–C_{18}.

The straight-chain saturated acids present can be assigned to the following peaks: (4) *n*-nonanoic; (7) *n*-decanoic; (10) *n*-undecanoic; (13) *n*-dodecanoic; (16) *n*-tridecanoic.

The relative amounts of fatty acids obtained by measurement of peak areas are given in Table 1 for the acids occurring free in human sebum.

DISCUSSION

The results presented here extend and corroborate those of other workers. The higher resolution of the technique has shown not only the series of acids ranging from *n*-heptanoic to *n*-octadecanoic acid demonstrated by Weitkamp *et al.*, but also two series of branched-chain acids. The first of these is assumed from its position on the chromatogram to consist of highly branched acids (though the possibility of cyclic structures should not be overlooked) and is restricted to the odd-number acids. The second series is considered to possess only a simple methyl branch and occurs with both odd and even numbers of carbon atoms. Both mono- and di-unsaturated acids of a variety of chain lengths are present.

Chemical degradation of the C_{16} mono-unsaturated acid by Weitkamp *et al.* showed it to be anomalous in that the double bond was located in the 6:7-position and not in the 9:10-position of the normally encountered palmitoleic acid. The chromatographic position of the C_{16} acid found here is not inconsistent with such an unsaturated acid. The presence of an unsaturated C_{18} acid in the position expected for methyl oleate agrees with the results of Weitkamp *et al.* (1947), Engman & Kooyman (1934) and Ricketts *et al.* (1951), all of whom identified oleic acid in human sebum.

The relative amounts of acids present as estimated by measurement of peak areas (Table 1) vary only slightly from those reported by Weitkamp *et al.* (1947). In particular, only approximately 25% of the free C_{16} acids are unsaturated, as against the 50% previously reported; such a relationship exists only in the combined fatty acid fraction (Fig. 2, curve *A*). Smaller amounts of the short-chain acids than the figures given by Weitkamp may be accounted for by losses due to solvent evaporation in the working up of the sebum samples.

No attempt has been made to extend the analysis to acids of chain length greater than C_{18}, as such acids move very slowly under the conditions described and emerge at low concentration in broad bands. However, clear indications were found of the existence of branched-chain C_{19} acids, but no conclusions were drawn as to their structure. Extension of this analytical technique to the C_{19}, C_{20} and C_{22} acids will necessitate higher column temperatures.

SUMMARY

1. The fatty acids from human forearm sebum have been analysed by gas–liquid chromatography of their methyl esters.

2. Both odd- and even-numbered straight-chain fatty acids have been shown to be present as well as two types of branched-chain odd-numbered saturated fatty acids.

3. The principal unsaturated fatty acids contain 14, 16 and 18 carbon atoms.

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