GAS CHROMATOGRAPHIC STUDY OF FATTY ACID COMPOSITION OF HUMAN SKIN SURFACE FILM LIPID

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(Received for publication December 8, 1965)

Fatty acid composition of human skin surface film lipid has been analysed in detail by many investigators.\(^1\)\(^-\)\(^6\) The difference in the amount of secreted surface lipid by sex and by age was also reported repeatedly.\(^7\)\(^-\)\(^17\) The difference of fatty acid composition of human skin surface film lipid by sex and by age was first examined by Boughton and Wheatley.\(^4\) According to these investigators, the skin surface lipids from the same subject varied little from time to time, and there were definite individual characteristics in composition; slight but not significant differences between men and women were observed; children had less unsaturated C\(_{16}\) acid and more of all three types of C\(_{18}\) acids than adults. The regional differences of the fatty acid composition of the skin surface lipid were also examined by the same investigators. The total fatty acid composition of the skin surface fat, and of the scale in health and disease in Japanese people was first reported by Nakamura.\(^18\) On the difference of fatty acid composition between the normal male and female, he noted that over twice the linoleic acid percentage was found in the female over the male, and also higher monounsaturated C\(_{16}\), C\(_{17}\) and C\(_{18}\) acids in the female. The present paper also reports changes in the total fatty acid composition of surface lipid according to sex, age and parts of the body in the Japanese people.

MATERIALS AND METHODS

Materials. Seventy-five normal men and women (from the newborn to the seventies) were swabbed on the face and neck with defatted cotton wool wetted with ether 3 to 12 hours after washing with soap and water. Possible exogenous contamination was avoided. Normal subjects without hormonal disorders or skin
diseases were carefully chosen. In some cases of the newborn and children, lipid was collected also from the scalp, upper part of the chest and of the back; in addition pooled samples were examined, because there was less surface lipid in them than in adults. In the case of one man, lipid was collected from the other parts of the body than the face and neck in order to examine the regional differences of the fatty acid composition.

Reagents. All the reagents were guaranteed pure reagent grade, and were used without redistillation. For preparing BF₃-methanol, anhydrous methanol was made by drying with metal sodium and distillation.

Methods. The lipid of human skin surface film was extracted from the swabbed cotton wool with about 50 ml of methanol-ether (3:1 v/v), hydrolysed with potassium hydroxide over a water bath for three hours. After removing nonsaponifiable substances with petroleum ether (bp. 30° to 70°C), the fatty acid, freed with concentrated hydrochloric acid, was extracted with petroleum ether (bp. 30° to 60°C), and dried over anhydrous sodium sulfate. Then the solvent was evaporated under reduced pressure of nitrogen, the residue (fatty acids) was methylated with about 2 ml of BF₃-methanol. The resulting methyl ester was introduced to gas chromatographic examination.

Gas chromatographic conditions. Chromatograph:—Hitachi, Model KGL-2A; Column dimensions:—200 × 0.4 cm o.d. stainless steel U tube; Solid support:—Diabase A (60/80 mesh) (Kotaki Co., Ltd.); Stationary phase:—poly (diethylene glycol succinate) (20%); Temperatures:—column 200°C, injection port 300°C; Carrier gas:—helium at 60 ml/min measured at outlet; Detector:—thermister detector; Analysis time:—approximately 10 minutes to oleate.

Identification of the peaks. Tentative identification of each peak was made by internal standards for myristic, palmitic and stearic acids, and by bromination in carbon tetrachloride for unsaturated acids. A procedure to divide unsaturated esters from mixtures by mercuric acetate and their hydrogenation in methanol with platinum oxide for over 6 hours was also tried.

Quantitation. A quantitation was made by cutting off the chart and weighing the peaks.

For characterization of the peaks, their relative carbon numbers were determined. The main component fatty acids of each peak are revealed in Tables 1 and 2.

RESULTS

1) Variation over different parts of the body.

Analyses were made on samples obtained from face and neck, chest and
The result (Table 1) shows that 16.00 (palmitic and branched-chain unsaturated C16), 18.00 (stearic and branched-chain unsaturated C18) and 18.44 (oleic) acids are higher, while 14.00 (myristic) and 16.50 (palmitoleic) acids are lower on the lower extremities than on the other parts of the body.

Table 1

Percentage Fatty Acid Composition of the Surface Lipid from Different Areas of Skin

<table>
<thead>
<tr>
<th>Peaks(a) (Characterization(b))</th>
<th>Area</th>
<th>Face and Neck</th>
<th>Back</th>
<th>Chest and Abdomen</th>
<th>Upper Extremities</th>
<th>Lower Extremities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 14.00</td>
<td>2.2</td>
<td>1.4</td>
<td>1.6</td>
<td>2.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>14.00 (n. sat. C16)</td>
<td>9.3</td>
<td>7.5</td>
<td>8.8</td>
<td>7.9</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>14.57 (n. unsat. C14 and br. sat. C16)</td>
<td>4.7</td>
<td>4.1</td>
<td>4.2</td>
<td>5.2</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>14.99 (n. sat. C18)</td>
<td>6.5</td>
<td>6.2</td>
<td>6.0</td>
<td>4.8</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>15.53 (n. unsat. C18 and br. sat. C16)</td>
<td>3.3</td>
<td>3.2</td>
<td>3.2</td>
<td>3.0</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>16.00 (n. sat. C18 and br. unsat. C16)</td>
<td>23.8</td>
<td>25.4</td>
<td>25.6</td>
<td>24.7</td>
<td>29.2</td>
<td></td>
</tr>
<tr>
<td>16.50 (n. unsat. C18)</td>
<td>25.6</td>
<td>25.9</td>
<td>26.2</td>
<td>24.2</td>
<td>19.4</td>
<td></td>
</tr>
<tr>
<td>Between 16.50 and 17.44</td>
<td>2.5</td>
<td>2.7</td>
<td>2.3</td>
<td>2.6</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>17.44 (n. unsat. C17)</td>
<td>4.1</td>
<td>3.7</td>
<td>3.3</td>
<td>3.6</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>18.00 (n. sat. C18 and br. unsat. C16)</td>
<td>2.8</td>
<td>4.2</td>
<td>4.1</td>
<td>5.8</td>
<td>11.4</td>
<td></td>
</tr>
<tr>
<td>18.44 (n. unsat. C18)</td>
<td>12.4</td>
<td>12.9</td>
<td>12.7</td>
<td>15.3</td>
<td>18.2</td>
<td></td>
</tr>
<tr>
<td>Further than 18.44</td>
<td>3.0</td>
<td>2.9</td>
<td>2.2</td>
<td>0.9</td>
<td>1.6</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) Peaks are denoted by their relative carbon number on a poly (diethylene-glycol succinate) column.

\(b\) n.: normal, br.: branched-chain, sat.: saturated, unsat.: unsaturated.

2) Variation by sex and age.

No less than 10 peaks were constantly observed. Computation of mean value and standard deviation of percent of each peak was made in the following five classes: newborn (below 7 days), children (below 14 years), adolescent (15 to 24 years), adult (25 to 49 years) and adult above 50 years (over 50 years). Men and women were calculated separately. The results are shown in Table 2.
Table 2  Percentage Composition of the Total Fatty Acids. Variations According to Age and Sex

<table>
<thead>
<tr>
<th>Class</th>
<th>Newborn</th>
<th>Children</th>
<th>Adolescent</th>
<th>Adult</th>
<th>Adult above 50 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Average Age (year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9.5</td>
<td>10.7</td>
<td>18.3</td>
</tr>
<tr>
<td>Number of Cases</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Peaksb (Characterizationc)</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Before 13.50d</td>
<td>2.5</td>
<td>2.7</td>
<td>2.0</td>
<td>1.9</td>
<td>1.7</td>
</tr>
<tr>
<td>13.50 (br. sat. C14)</td>
<td>2.1±0.4</td>
<td>3.1±0.7</td>
<td>2.0±0.4</td>
<td>2.2±0.8</td>
<td>1.2±0.6</td>
</tr>
<tr>
<td>14.00 (myristic)</td>
<td>9.6±0.6</td>
<td>9.3±0.7</td>
<td>6.9±0.6</td>
<td>7.0±1.0</td>
<td>9.8±1.2</td>
</tr>
<tr>
<td>14.57 (n. unsat. C14 and br. sat. C15)</td>
<td>4.4±0.4</td>
<td>4.5±0.8</td>
<td>4.4±0.8</td>
<td>4.9±0.8</td>
<td>5.4±0.4</td>
</tr>
<tr>
<td>14.99 (n. sat C15)</td>
<td>5.7±0.6</td>
<td>5.1±0.7</td>
<td>5.3±1.1</td>
<td>5.1±1.3</td>
<td>6.1±1.0</td>
</tr>
<tr>
<td>15.53 (n. unsat. C16 and br. sat. C16)</td>
<td>3.4±0.3</td>
<td>3.7±0.4</td>
<td>3.8±1.0</td>
<td>3.5±0.7</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>16.00 (palmitic and br. unsat. C16)</td>
<td>26.9±1.3</td>
<td>28.7±1.9</td>
<td>27.0±1.7</td>
<td>26.1±1.8</td>
<td>27.3±2.1</td>
</tr>
<tr>
<td>16.50 (palmitoleic)</td>
<td>20.8±0.8</td>
<td>18.7±1.8</td>
<td>18.2±3.1</td>
<td>18.4±3.5</td>
<td>24.9±3.0</td>
</tr>
<tr>
<td>Between 16.50 and 17.44d</td>
<td>1.6</td>
<td>1.9</td>
<td>2.6</td>
<td>2.5</td>
<td>1.9</td>
</tr>
<tr>
<td>17.44 (n. unsat C17)</td>
<td>2.0±0.4</td>
<td>1.2±0.7</td>
<td>2.0±0.5</td>
<td>1.9±0.5</td>
<td>2.2±0.3</td>
</tr>
<tr>
<td>18.00 (stearic and br. unsat. C18)</td>
<td>4.8±0.4</td>
<td>3.9±0.5</td>
<td>6.4±2.0</td>
<td>6.6±2.1</td>
<td>3.4±1.3</td>
</tr>
<tr>
<td>18.44 (oleic)</td>
<td>12.0±1.4</td>
<td>11.7±1.7</td>
<td>13.8±2.5</td>
<td>14.2±2.7</td>
<td>10.1±2.9</td>
</tr>
<tr>
<td>Further than 18.44d</td>
<td>4.7</td>
<td>5.2</td>
<td>6.3</td>
<td>5.8</td>
<td>2.9</td>
</tr>
</tbody>
</table>

a Mean value ± standard deviation in percent.
b Peaks are denoted by their relative carbon number on a poly (diethyleneglycol succinate) column.
c n.: normal, br.: branched-chain, sat.: saturated, unsat.: unsaturated.
d As these regions were not composed of one peak, standard deviation was not computed.
The change of some peaks against age was visualized in Figures 1 to 4.

The following differences of mean values by sex and age were evaluated using the “t” test of statistical significance.

_Difference by sex._ No remarkable sex differences of fatty acid composition were demonstrated in the newborn, childhood and adolescent stages. In the adult male much more 16.50 (palmitoleic) acid existed than in the adult female. (Pr.<0.01) In adults above 50 years, the male had less 18.00 (stearic and branched-chain unsaturated C18) acid than the female. (Pr.<0.01)

_Variation with age._ 14.00 (myristic) acid and 16.50 (palmitoleic) acid seemed to change definitely. In both sexes, the 14.00 (myristic) acid was high in the newborn, low in childhood, and recovered its high value after adolescence.
(Pr.<0.01) In the male, a significant increase of 16.50 (palmitoleic) acid from the childhood to the adolescent stage was noted. (Pr.<0.01) In the female, a similar tendency, a lower value of 16.50 (palmitoleic) acid in both childhood and in adults above 50 years, than that in the adolescent or the adult under 50 years, was revealed, but was only slightly significant. (Pr.<0.02) In the male, 18.00 (stearic and branched-chain unsaturated C18) acid decreased markedly from childhood to the adult. (Pr.<0.01) Variation of 18.00 (stearic and branched-chain unsaturated C18) and 18.44 (oleic) acids with age showed an inverse tendency to that of 14.00 (myristic) and 16.50 (palmitoleic) acids, though changes of 18.44 (oleic) acid between classes were not so significant. The other peaks did not show any remarkable changes by sex or age.

DISCUSSION

On the methods of collecting the skin surface film lipid.

According to Wheatley,24) there are 5 methods for collecting the sebum: a) from the glands, b) from underclothing, c) extraction of the intact skin surface by means of a lipid solvent (skin surface fat), d) extraction of hair, e) extraction of lipids from scrapings of horny layer or from the exfoliated scales of patients with certain skin diseases. Direct cannulation of the ducts of the glands would be expected to produce pure sebum. In this experiment, however, the skin surface was wiped with cotton wool moistened with ether, so the material can be contaminated with some epidermal lipids, though this contribution of epidermal lipids would be small.25) The cotton wool was defatted with methanol-ether (3:1 v/v) twice before use and was confirmed not to produce any noticeable peak on the chromatogram. In order to avoid exogenous contamination and to obtain surface lipid which is less oxidized, the washing of the face and neck with soap and water was performed before collection of surface lipid, but no treatment to protect the site completely from the exogenous contamination, e.g. untoward touching by hands and so on, was performed. Such pretreatment does not disturb the skin surface film.26, 27) This treatment could take off the surface lipid to some extent but not completely. The time required for restoration of the original level was found to be longer than 3 hours by Emanuel,25) but it seems to be not determined strictly.10-16, 28-31) In this experiment, samples were collected over 3 hours after washing, e.g. possibly at the saturated level. In this study it should be emphasized that the concern is with "the casual level."32) The collection of surface lipid was made on the face and neck, because there is much excretion of the sebaceous gland on the face,16, 25-27, 32-33) and rather pure lipid from the sebaceous gland was considered
to be collected. It is known that the secreted amount of surface lipid shows seasonal, and nutritional changes, even the fatty acid composition of surface lipid shows changes according to nutrition, but in the present study no attention was paid to the diet or temperature. Collection of the surface lipid was performed from April to September.

On the species of fatty acids of the skin surface film lipid and gas chromatographic conditions.

Before the advent of gas chromatography, the composition of fatty acids of surface lipid was analysed by fractional distillation, which revealed its unique nature, e.g. straight-chain fatty acids with an odd number of carbon atoms, and the predominant position of the double bond of the monoenes between the 6th and 7th carbon atoms from the carboxyl group. By application of gas chromatography for analysing fatty acid composition of human forearm sebum, James and Wheatley showed that both odd- and even-numbered straight-chain fatty acids were present as well as two types of branched-chain odd-numbered saturated fatty acids. In recent years, many other homologous series of fatty acids became known to exist in human skin surface film lipid. For analysing surface lipid, the following column conditions have been used by many investigators: a) packed or capillary column, b) polar or nonpolar stationary phase, c) isothermal or temperature programmed condition. A capillary column with a nonpolar stationary phase revealed a fine resolution. In this experiment, a polyester column was adopted, which was rather less suitable for skin surface fatty acids. So these data are not able to compare directly with the results obtained with nonpolar columns by previous investigators. By separating unsaturated fatty esters from saturated ones with mercuric acetate method, it was revealed, for example, straight-chain saturated acid methyl esters and branched-chain unsaturated esters of the same number of carbon atoms appeared as one peak with this column. This was true for straight-chain unsaturated methyl esters and branched-chain saturated ones having one more carbon than the former. In the present study, the resolution of each peak was not sufficient for such a complexity of many acids as that of skin surface lipid, and fractionation of each peak by gas chromatography and further treatment such as infrared spectroscopy were not tried, the identified peaks were considered to be mainly composed of that kind of fatty acids. Thus a peak on the chromatogram is not necessarily composed of one component, so comparison must be made not between each kind of fatty acid, but between each peak on the chromatogram (mixture of acids). As rat skin contains a fairly large amount of alpha-hydroxy acids, human skin surface lipid can also contain such kinds of acids to some extent. No treatment to exclude hydroxy acids was performed in this
study. These hydroxy acids were considered to be ignorable small because a procedure to separate them did not remarkably diminish any peak on the chromatogram up to oleic acid. The retention time of hydroxy acid methyl esters seems to be rather large, and if they actually exist, they should appear late on the chromatogram compared with nonhydroxy acid esters of the same carbon skeleton. The present chromatographic condition could not distinctly resolve fatty acids with more than 18 carbons, but these fractions seem very little: Coon suggested that the fatty acids, which had more carbons than 18, were produced in the epidermis or near the barrier zone and not in the sebaceous gland. The fatty acids with 16, 18 and 14 carbon atoms are known as the main components of the fatty acids of surface lipid, and comparable results were obtained in the present study.

On the regional differences of fatty acid composition of surface film lipid.

The regional differences of fatty acid composition of sebum were reported by Boughton and Wheatley. They showed that the saturated acids are higher and the unsaturated lower on the arms and legs than on the other areas of the body (back, chest, face and abdomen). Similar changes were observed in this experiment, (Table 1) but 14.00 (myristic) and 18.44 (oleic) acids showed a difference in change from that in their conclusion. The low percentage of 16.50 (palmitoleic) acid in the lower extremities, where low sebaceous excretion is noted, can be clearly explained by the theory of Boughton and Wheatley, that the amount of sebaceous excretion and the percentage of the unsaturated fatty acids move in parallel.

On the sex difference of fatty acid composition.

Boughton and Wheatley revealed no definite sex difference, but Nakamura reported higher value of monounsaturated C16, C17, C18 and linoleic acids in the female than in the male. The present experiment showed that the male had a higher percentage of 16.50 (palmitoleic) acid than the female adult, and aged women had more 18.00 (stearic and branched-chain unsaturated C18) acid than aged men. No significant difference in other acids by sex was noted. The discrepancy about the sex difference of 16.50 (palmitoleic) acid percentage between Nakamura's data and my experiment could be attributed to the difference in the collecting method of skin surface lipid. When the reported sex difference of the amount of skin surface fat is compared to these differences of composition of the fatty acid by sex, these changes can be almost wholly explained by the theory of Boughton and Wheatley. This will be discussed later.

On the variation of fatty acid composition with age and interpretation of the results.

Boughton and Wheatley clearly demonstrated a correlation between the
percentage of unsaturated acids in the surface lipids and sebaceous activity of the skin. In the present study, no quantitation of the skin surface lipid level was performed, but this correlation would be recognizable if the variation of the fatty acid composition with age would be compared with the reported difference of sebum level with age. It is well known that the amount of skin surface lipid is small in childhood, increasingly more from the onset of adolescence and high in adulthood. Suzuki\textsuperscript{15} stated that men secrete more sebum than women after puberty. In old age he noted a decreasing tendency in the male, and a distinct decrease of the amount of surface fat in aged females. Many other investigators\textsuperscript{7-8, 10-14, 16-17, 29-33, 41-42} noted such tendencies, too, though with some qualifications.\textsuperscript{43-44} Emanuel\textsuperscript{20} found high values, in the range of adults, in the newborn, and in those after thorough removal of vernix caseosa. It was revealed by Blecha \textit{et al}\textsuperscript{45} that the production of the total ether-extractable esterified fatty acids decreased slowly during the first year of life. In the present study, the percentage of palmitoleic acid was low in the newborn and in childhood, but high after adolescence in the male. In the aged female palmitoleic acid decreased markedly. This tendency is consistent with the above mentioned changes of the amount of surface lipid according to age with an exception of the newborn. So it would be reasonable to think that palmitoleic acid originated from the sebaceous gland as suggested by Boughton and Wheatley.\textsuperscript{41} If the high surface lipid level in the newborn is caused by high sebaceous gland activity, the percentage of the palmitoleic acid should also be high in the newborn according to this theory. This discrepancy could be explained either by a diminished contribution of the sebaceous excretion to the ether-extractable substance in the newborn, or by a decreased 16.50 (palmitoleic) acid content of the sebaceous excretion in him. This would be decided by analysing the pure sebaceous excretion. The difference by sex of 16.50 (palmitoleic) acid percentage (a higher percentage in the adult male than in the adult female) can be understood by Boughton and Wheatley's opinion, when considering Suzuki's report.\textsuperscript{15} Not all of the unsaturated acids showed this tendency. 18.44 (oleic) acid, inspite of its unsaturatedness, had an inverse tendency with age to 16.50 (palmitoleic) acid: this was a high percentage in childhood though insignificant. This tendency is possibly due to its different metabolic pathway or origin, from 16.50 (palmitoleic) acid. As the double bond of oleic acid in the surface film lipid occurs in an exceptional position as to the other unsaturated ones, Nicolaides \textit{et al}\textsuperscript{50} concluded that oleic acid had an origin other than the sebaceous gland. Therefore, it seems not necessarily correct to think that all the unsaturated acids originate from the sebaceous gland, and the saturated from epidermal cells.

Straight-chain saturated acids eluate with other kinds of acids (e.g. un-
saturated branched-chain acids of the same carbon number) at the same time by this column condition.

14.00 (myristic) acid was thought to be only slightly contaminated by other acids from the fractionation experiment with mercuric acetate. 14.00 (myristic) acid revealed a distinct variation with age. The percentage of it was low only in childhood. It can be considered to be mainly originating from the sebaceous gland, as it had a high percentage value in the newborn, and showed a comparable change according to age with the activity of the sebaceous gland as mentioned above.

16.00 (palmitic and branched-chain unsaturated C16) acid, though a main component in the skin surface fat, did not show any variation with age. This is possibly due to the actual absence of such variation in palmitic acid, but the possibility can not be denied that the variations of palmitic and branched-chain unsaturated C16 acids cancel each other.

18.00 (stearic and branched-chain unsaturated C18) acid had a similar tendency to behave with age as did oleic acid. This is consistent with the result of Boughton and Wheatley that children had more of all three types of acids with 18 carbon atoms than adults.

Reinertson and Wheatley examined the fatty acid composition of the epidermal lipid as well as that of the skin surface lipid. They noticed that the odd-numbered and branched acids, characteristic of the skin surface lipid, were almost completely absent from the epidermal lipid, where much unsaturated or branched C18 acid, much palmitic acid and little of unsaturated or branched C16 acid percentages were characteristic. On the epidermal lipid, Carruthers recently, using polar and nonpolar columns, investigated the fatty acid composition of the triglycerides and phosphatides which represented a large proportion of the total lipid of the epidermis. His study showed that the main component fatty acids were oleic, palmitic and linoleic in triglycerides, and oleic, linoleic, palmitic and stearic acids in phosphatides. Judging from these data, palmitic, oleic and linoleic acids are thought to be the main components of the fatty acids of epidermal origin. Thus the low value of palmitoleic acid and the high oleic acid in children and aged women can be explained by the prevalence of the epidermal fatty acids due to a deficient sebaceous gland secretion. However, high 18.00 (stearic and branched-chain unsaturated C18) acid in aged female can not necessarily be explained by this theory. This phenomenon needs further investigation.

It would be proper to think that the changes of fatty acid composition of surface film lipid according to age and sex are due to the varied mixture ratio of lipids from the sebaceous gland and the epidermal cells, though the newborn
and the aged female showed a slightly different kind of skin surface lipid in its composition. The regional differences of the fatty acid composition also could be explained by this idea.

The other peaks did not show any definite variations with age and sex; this is probably due to the fact that they are composed of but a few kinds of different fatty acids which change independently, or it may be due to the fact that they have an almost equal percentage in both epidermal and sebaceous fats (for example, palmitic acid). It could not be decided whether the sebaceous gland excretion differs in its fatty acid composition according to age or sex in this study.

SUMMARY

1) Human skin surface film lipid was obtained by swab method with ether from 75 normal individuals from the newborn to those in the seventies of both sexes.

2) Total fatty acid composition was analysed by gas chromatography on a polyester column.

3) Variations of composition of fatty acids according to sex, age and parts of the body were analysed.

4) Regional differences:—16.00 (palmitic and branched-chain unsaturated C₁₆) and 18.00 (stearic and branched-chain unsaturated C₁₈) acids were higher; 14.00 (myristic) and 16.50 (palmitoleic) acids were lower on the lower extremities than on the other parts of the body.

5) Variations by sex. In adults, the male had a higher 16.50 (palmitoleic) acid content, and in adults above 50 years of age, the female had higher 18.00 (stearic and branched-chain unsaturated C₁₈) acid content.

6) Variations by age. 14.00 (myristic) and 16.50 (palmitoleic) acids showed changes parallel to the amount of skin surface lipid with some exceptions: they were lower in childhood, and higher after adolescence. The percentages of 18.00 (stearic and branched-chain unsaturated C₁₈) and 18.44 (oleic) acids showed an inverse change by age: they were high in childhood.

7) These changes of fatty acid composition of skin surface film lipid can be partly explained, at least, by considering them as the result of a different mixture ratio of lipids from epidermal and sebaceous gland origin.

8) It was deduced that myristic and palmitoleic acids mainly originate from the sebaceous gland and oleic acid from the epidermal cells.
ACKNOWLEDGEMENT

The specimen was analysed by gas chromatograph of the Central Research Laboratory, School of Medicine, Keio University.

Grateful acknowledgement is made to Prof. Dr. H. Hatano, Department of Dermatology, Keio University School of Medicine, Prof. Dr. E. Hosoya, Department of Pharmacology, Keio University School of Medicine, Prof. Dr. M. Kikuno, Chemistry Department, Sophia University School of Science and Technology, and Dr. K. Yamanoto, Department of Dermatology, Keio University School of Medicine for their interest and advice in this work. The assistance of the staff of the Chemistry Department, School of Science and Technology, Sophia University, is also gratefully acknowledged.

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