A DIFFERENTIAL LIPID ANALYSIS OF BLOOD PLASMA
IN NORMAL YOUNG WOMEN BY MICRO-
OXIDATIVE METHODS

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The study of fat metabolism in human subjects has been largely
confined to a consideration of the variations in blood lipids under
different conditions. Most investigations have dealt with changes
in a single lipid, cholesterol being probably the most intensively
studied, but a few papers have appeared in which two or more
lipids were simultaneously investigated in the same subject.
Among these may be mentioned the work of Page, Pasternack,
and Burt (20) in which total fatty acids, cholesterol, phosphatides,
and iodine numbers were determined in eight subjects of both
sexes. Okey and Boyden (18) studied variations in the total
blood cholesterol, fatty acids, and phospholipid in sixteen women
during menstruation. Man and Gildea (13, 14) have reported
fatty acids and lipoid phosphorus in normal men following in-
gestion of food. Other group lipid analyses include that of de
Vass (23) on total and free cholesterol and phospholipids in blood
serum of normal subjects and cancer patients, and that of Fahrig
and Wacher (8) on lecithin, cholesterol, and neutral fat in human
blood serum.

Such composite blood lipid analyses have considerable ad-
vantage over single lipid determinations in the interpretations of
fat metabolism in relation to the blood. Recognizing this, the
author has assembled, in the investigation to be reported below,
a group of micromethods recently evolved by Bloor and his asso-
ciates (3, 4, 6, 17, 24, 25), and from these devised a procedure
whereby all the lipids known at present in plasma may be deter-
mimed on a small amount of blood.
This procedure was then applied to an analysis of the lipids present in blood plasma of normal, young, non-menstruating, non-pregnant women. Unlike men, in whom the blood lipids remain fairly constant at definite levels, it has been found that in women there is a cyclic variation of the blood lipids in relation to menstruation. Okey and Boyden (18) demonstrated that during catamenia there is a fall in whole blood cholesterol with a tendency toward alterations in the fatty acids, while the phospholipids remain relatively constant. They conclude that, "the blood cholesterol level in women is to be considered as a variable rather than a constant." These findings have been confirmed by Kaufmann and Mühlbock (10) and later by Okey and Stewart (19). The present investigation was therefore undertaken to determine the extent of this variation in blood plasma lipids of young women under controlled conditions.

EXPERIMENTAL

Subjects—Eight young women varying in age from 20 to 38, with an average of 28, acted as subjects for the investigation. In each case it was ascertained that the previous menstrual period was 1 to 3 weeks past and that menstruation was of normal flow, duration, and occurrence, so that each subject was known to be within the 2 weeks between periods. A brief history was then taken and a physical examination made, including height, weight, temperature, pulse and respiration, blood count hematocrit, urinalysis, and blood Wassermann, to eliminate conditions which are known to affect the level of blood lipids (Bloor (5)), especially infection, cancer and tumor growths, anemia, thyroid dysfunction, uterine bleeding, etc. The subject thus selected as suitable for a study of normal plasma lipids was put on a normal balanced diet for a week and kept in bed for at least 24 hours before the test to offset the lipemic effect of exercise (Stewart, Gaddie, and Dunlop (22)) and emotion (Lyons (12)).

Plasma Extracts—Following a 16 hour fast, blood was withdrawn from the arm veins between 8.30 and 9.00 a.m., shaken in a flask with dry sodium citrate, and immediately centrifuged at 2000 revolutions for \( \frac{1}{4} \) to \( \frac{3}{4} \) of an hour. The plasma was drawn off and 10 cc. added slowly and with rotation to about 125 cc. of alcohol-ether, 3:1, both redistilled, after the method of Bloor.
(3). The extract was filtered through an alcohol-extracted filter paper, the precipitate being washed several times with alcohol-ether, and the remaining solvent pressed out with a clean glass rod. After making up to 200 cc. volume, giving a 1:20 dilution of the original plasma, the extract was stored in clean bottles stoppered with alcohol-extracted corks. Iodine number estimations were made the same day the extract was prepared, but the lipids were determined later when convenient.

Methods

A number of microlipid methods were considered for this study, based on different chemical principles of analysis such as colorimetry, nephelometry, acid titration, oxidation, and microscopic counts (Bloor (5)). In general there is a fairly consistent agreement in results obtained by the several procedures where one lipid is being estimated. However, where it is desired to calculate, from experimental figures, values for lipids not directly determined, there is sufficient variation between procedures to warrant serious consideration in the choice of methods. Whereas, at the present time, it is impossible to state by which method the correct lipid values are more closely approximated, there is an obvious advantage, both theoretical as well as practical, in a procedure composed of several methods, to have each of these based on the same principle. In place of having to consider a summation of errors inherent in each of the methods used, there is but one group of errors throughout. Likewise, having the determined values for each lipid in terms of the same unit facilitates calculation of lipids not directly estimated.

With this idea in mind, it was found that the oxidative procedure was the only one which could be applied to the determination of all the lipids known at present in blood plasma. Since it had previously been found that the microoxidative method for phospholipid was satisfactory (Boyd (6)), and a preliminary investigation demonstrated, as shown below, that a similar range of experimental error existed for the remaining methods, it was decided to adopt these methods for the present analyses. Total fatty acids, total cholesterol, free cholesterol, phospholipid, iodine number of total fatty acids, and iodine number of phospholipid fatty acids were determined directly. From these values were
calculated total lipid, neutral fat, cholesterol ester, neutral fat fatty acid, cholesterol ester fatty acid, and phospholipid fatty acid. It may thus be seen that a fairly comprehensive survey of plasma lipids was obtained by this procedure.

**Total Fatty Acids**—Total fatty acids and total cholesterol were jointly determined on a 20 cc. aliquot of the alcohol-ether extract by saponification with 0.1 cc. of saturated sodium hydroxide, acidification, and petroleum ether extraction after the method of Bloor (3). Six extractions, with 3 to 5 cc. of petroleum ether (b.p. 37–60°) each time, were found to remove all the oxidizable material. As these plasma extracts were quite clear, no appreciable difference in the result was obtained by allowing the petroleum ether extract to settle overnight and filtering, an added precaution of Kimmelstiel and Becker (11) for tissue analysis. From the petroleum ether extract the subsequent evaporation and oxidation were carried out as in the Bloor procedure. The reproducibility of the method in the hands of the author was determined on known alcohol-ether solutions of fatty acids and upon stock extract of blood plasma. It was found to give a mean recovery of 99 per cent on known solutions and a reproducibility varying within 2 per cent on the stock plasma extract.

**Total Cholesterol**—The oxidative method for total cholesterol by precipitation of cholesterol as the digitonide (Okey (17)) was found to offer the most difficulty in the way of reproducibility and accuracy of results, but a procedure was finally developed which proved quite satisfactory. A number of modifications were adopted from the method of Yasuda (24) and from personal communications with Professor W. R. Bloor, Mr. L. C. Miller, and Mr. P. L. McLachlan of the Department of Biochemistry, as well as changes introduced by the author. The procedure as finally used was as follows: A 10 cc. aliquot of the alcohol-ether extract was saponified in Erlenmeyer flasks on the steam bath with 0.1 cc. of saturated sodium hydroxide. Freshly prepared sodium ethylate may also be used but this reagent on standing becomes unsatisfactory for the saponification (16). When the volume of the mixture reached 3 to 4 cc., usually in about ½ hour which, as shown by Yasuda (24), is sufficient for complete saponification, the mixture was acidified with 1 cc. of 1:3 sulfuric acid. The flask was then placed to one side on the steam bath out of contact
with active steam and heated at this relatively lower temperature until the volume reached 1 cc., after which it was completely extracted with petroleum ether as in the fatty acid procedure above, a drop of phenol red being added for convenience in separating the petroleum ether layer (15). To the combined petroleum ether extracts in a clean 125 cc. Erlenmeyer flask were added 5 cc. of a 0.2 per cent solution of Merck's digitonin (16) in 50 per cent alcohol and the mixture evaporated almost to dryness on the steam bath. A gentle stream of air proved of value in the terminal stages of this evaporation. 10 cc. of distilled water were then added, caution being observed to prevent the water running down the sides of the flask over which the flakes of precipitate would otherwise be distributed, and the contents brought to a gentle boil for 2 minutes with continuous rotation of the flask. In this manner excess digitonin was dissolved and the precipitate reduced to a fine granular suspension. Occasionally it was necessary to dislodge, with a clean glass rod, portions of the precipitate which had remained adherent to the bottom or sides of the flask.

After allowing the mixture to cool, 20 cc. of redistilled acetone were added and the flask rotated vigorously, resulting in a certain amount of agglutination of the suspension which was then filtered with suction through a sintered glass filter of the type "4G4, Schott and Gen., Jena" and washed twice with acetone and ether, as in the Yasuda (24) procedure. The rate of filtration was limited to roughly 125 drops per minute, since it has been found that a faster rate produces lower values (15). The filter was then placed within a copper coil steam-jacket, redistilled methyl alcohol added, and steam passed through the coil until the solvent began to boil, when it was drawn into a 125 cc. glass-stoppered Erlenmeyer oxidation flask through a glass suction head inserted between the filter and the flask to facilitate connections with the water vacuum pump. This arrangement was essentially similar to that used by Yasuda, but methyl alcohol has proved to be a more convenient solvent for the cholesterol digitonide than absolute alcohol (16). After washing the filter twice with methyl alcohol, the filtrate and washings were evaporated to dryness, traces of methyl alcohol removed with a current of air, and the lipid oxidized as above. Yasuda's observation that a half hour's heating in the electric oven is necessary for complete oxidation was
confirmed, less heating resulting in lower recoveries on known amounts of cholesterol. With the procedure as above outlined, it was found that 96 per cent of a known amount of cholesterol palmitate added to a plasma extract could be recovered, while determinations on a stock alcohol-ether extract of blood plasma gave figures which could be duplicated within 2 per cent.

**Phospholipid**—Phospholipid was determined on 20 cc. aliquots of the plasma alcohol-ether extract by Bloor's method (4) with the modifications as introduced by Boyd (6). The acetone mother liquor and acetone washings after precipitation of the phospholipids were quantitatively transferred to a 125 cc. Erlenmeyer flask and this fraction used for estimation of free cholesterol as described below. The experimental errors of this method have been previously reported by the author (6).

**Free Cholesterol**—Free cholesterol was estimated on 20 cc. aliquots of the alcohol-ether extract after removal of the phospholipids as described above, 5 cc. of 0.2 per cent digitonin added, and the procedure carried out as from this stage for the total cholesterol. Values obtained by this procedure checked within the experimental error with values derived when free cholesterol was directly precipitated from the alcohol-ether extract. With the acetone washings, however, time is saved in digitonin precipitation and the digitonide precipitate requires less washing, since phospholipid impurities are not present. By this procedure 96 per cent of known amounts of cholesterol could be recovered, while duplicate determinations on stock plasma extract checked within 4.5 per cent.

**Iodine Numbers of Total Fatty Acids**—Iodine numbers were determined by the method of Yasuda (25) with 50 cc. of alcohol-ether extract, the estimations being made on the same day the extract was prepared. It was found by applying the method to known amounts of cholesterol that the theoretical iodine number of cholesterol was approximated within 7.5 per cent on repeated estimations when a sufficient amount of lipid was used to produce a difference of 1 cc. or more in the final titration with 0.02 N sodium thiosulfate, while titration differences of less than 0.5 cc. were found unreliable. Duplicate determinations agreed within 3.3 per cent. Calculation of the iodine number of plasma fatty acids was based on Yasuda's formula after computing and sub-
tracting from the titration difference the cc. of 0.02 N sodium thiosulfate due to the iodine number (theoretical) of total cholesterol in the aliquot (factor, 1 mg. of cholesterol equivalent to 0.26 cc. of 0.02 N sodium thiosulfate).

Iodine Numbers of Phospholipid Fatty Acids—Phospholipids were precipitated and dissolved in moist ether from a 50 cc. aliquot of plasma extract as in the procedure by Boyd (6). 5 cc. of redistilled alcohol were added to the moist ether solution of the phospholipids in a small Erlenmeyer flask and the lipid saponified with 0.1 cc. of saturated sodium hydroxide. Direct determinations of iodine number on whole phospholipid, as described by Yasuda (25), were found to give erratic and variable results. After saponification the phospholipid fatty acids were extracted with petroleum ether, evaporated, redissolved in 1 cc. of chloroform, and the iodine number determined with the Rosenmund-Kuhnhenn reagent as used by Yasuda. On a number of extracts the phospholipid fatty acids were directly determined, but it was found that similar values for the iodine number could be obtained by computing the phospholipid fatty acids as two-thirds of the phospholipid. Duplicate determinations on stock alcohol-ether extract were found to agree within 5 per cent.

Calculated Lipids—From the experimental values obtained by the above procedures the further distribution of the plasma lipids was found by calculation. Combined cholesterol (cholesterol of cholesterol ester) levels were obtained by subtracting the value for free cholesterol from that for total cholesterol and the figure for cholesterol ester fatty acids may be computed as 0.67 times combined cholesterol (the cholesterol ester fatty acids being calculated as oleic and C₁₈ acids). The sum of the phospholipid fatty acids as above calculated plus the cholesterol ester fatty acids subtracted from the total fatty acids gives the value of the neutral fat fatty acids which constitute, on the average, 95 per cent of neutral fat, thus giving a figure from which neutral fat may be calculated. The total lipid may now be determined as the sum of the phospholipid plus the neutral fat plus the total cholesterol plus the cholesterol ester fatty acids.

Results

In Table I are recorded the results of a series of analyses of blood plasma on eight normal young women by the methods as de-
### TABLE I

**Composition of Blood Plasma Lipids in Normal Young Women**

The results are measured in mg. per 100 cc. of blood plasma.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total Lipid</th>
<th>Composition of Total Lipid</th>
<th>Iodine No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutral fat</td>
<td>Fatty acids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Phospholipid</td>
<td>Cholesterol</td>
</tr>
<tr>
<td></td>
<td>Neutral fat</td>
<td>Total</td>
<td>Phospholipid</td>
</tr>
<tr>
<td>D. E.</td>
<td>730</td>
<td>229.3</td>
<td>457</td>
</tr>
<tr>
<td>C. B.</td>
<td>546</td>
<td>159.7</td>
<td>335</td>
</tr>
<tr>
<td>J. F.</td>
<td>521</td>
<td>155.4</td>
<td>332</td>
</tr>
<tr>
<td>M. H.</td>
<td>645</td>
<td>128.6</td>
<td>370</td>
</tr>
<tr>
<td>E. J.</td>
<td>422</td>
<td>78.3</td>
<td>245</td>
</tr>
<tr>
<td>M. F.</td>
<td>590</td>
<td>146.8</td>
<td>347</td>
</tr>
<tr>
<td>I. M.</td>
<td>614</td>
<td>136</td>
<td>347</td>
</tr>
<tr>
<td>E. A.</td>
<td>642</td>
<td>166</td>
<td>391</td>
</tr>
<tr>
<td>Mean</td>
<td>589</td>
<td>153.7</td>
<td>353</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>87.2</td>
<td>42.2</td>
<td>55.8</td>
</tr>
<tr>
<td>Standard deviation, per cent</td>
<td>14.8</td>
<td>27.3</td>
<td>15.7</td>
</tr>
</tbody>
</table>
scribed above. For each lipid the mean value has been deter-
mined and from this the standard deviation calculated by the
formula \( \sigma = \sqrt{\frac{\Sigma (x)^2}{n}} \), where \( x \) represents the variation of each
value from the mean, \( n \) the total number of readings, \( \sigma \) the
standard deviation, and \( \Sigma \) a summation symbol (7). For com-
parative purposes the per cent of the standard deviation over the
mean has also been given. Since the variation of each method
has been determined above, single estimations only need be given
for the plasma lipids.

The total lipid content of the plasma varied from 0.4 to 0.7
per cent with a mean value of 0.59 mg. per cent and with \( \sigma \) (standard deviation) 0.72 or 14.8 per cent. The total lipid was com-
posed as follows:

<table>
<thead>
<tr>
<th></th>
<th>per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipid</td>
<td>33.2</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>32.7</td>
</tr>
<tr>
<td>Neutral fat</td>
<td>26.1</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Total fatty acids of plasma averaged 353 mg. per cent, varying
from 245 to 457 mg. per cent, with a standard deviation of 55.8
or 15.7 per cent of the mean. In 1921, Bloor (1) reported blood
plasma total fatty acids in normal men as 380 mg. per cent,
while Man and Gildea (14) found, in a review of the results by
various authors, an average value of 330 mg. per cent by nephelo-
metric methods. Using a modified Stoddard and Drury acid
titration technique (13), the latter investigators determined
total fatty acids in human blood serum as 11.9 milli-equivalents
or 320 mg. per cent. With the Bang-Bloor oxidative method,
Page, Pasternack, and Burt (20) found the serum fatty acids of
normal fasting men and women ranged between 243 and 470 mg.
per cent. From the present investigation it was found the total
fatty acids were distributed as follows:

<table>
<thead>
<tr>
<th></th>
<th>per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral fat fatty acids</td>
<td>41.4</td>
</tr>
<tr>
<td>Phospholipid “ “</td>
<td>36.8</td>
</tr>
<tr>
<td>Cholesterol ester fatty acids</td>
<td>21.8</td>
</tr>
</tbody>
</table>

The glycerides constituted approximately one-quarter of the
total lipid with a mean value of 153.7 mg. per cent and a standard
deviation of 42.2, varying from 78.3 to 229.3 mg. per cent. Total
cholesterol showed less variation, having an average value of 162 mg. per cent with extremes of 112 and 195 mg. per cent. Bloor (1) reported 220 mg. per cent for total cholesterol in normal men and Page et al. (20) found the level varied between 137 and 245, both investigators using the Bloor-Liebermann-Burchard colorimetric method (3). The digitonin method, as employed in the present investigation, has been shown to give slightly lower values for cholesterol than the above colorimetric reaction (16). The total cholesterol was composed of 29.3 per cent free cholesterol which gave a mean value of 47.1 mg. per cent and varied between 37.2 and 58.5 mg. per cent. There was little tendency to variation in the level of this lipid, the standard deviation, 6.28, being 13.4 per cent of the mean. Phospholipid exhibited the least tendency of all the lipids to variation; the mean value obtained was 195.7 mg. per cent, the extremes 170 and 236 mg. per cent, and the standard deviation, 22.5, constituted 11.5 per cent of the mean. By nephelometric methods, Bloor (1) reported a value of 220 mg. per cent for normal men, while Page et al. (20) found a range of 238 to 309 mg. per cent; by estimating the lipoid phosphorus Man and Gildea (14) calculated the phospholipid fatty acid from which it may be shown that they obtained a mean value of 247 mg. per cent for serum phospholipid. Values for serum lipids are, in general, somewhat higher than those for plasma (13, 14).

In contrast to the relative constancy of the lipid values noted above, the iodine numbers of plasma fatty acids showed a more marked variation. The iodine numbers of the total fatty acids varied from 112 to 64.5 with an average of 88.5, a standard deviation of 40.2, and a 45.3 per cent standard deviation. On the other hand, the iodine numbers of the phospholipid fatty acids showed less variation, having an average value of 124.2, extremes of 98.5 and 167.2, a standard deviation of 22.7, and 18.3 per cent. Page et al. (20) found the iodine numbers of serum total fatty acids varied between 98 and 132, while the iodine numbers of phospholipid fatty acids ranged from 52 to 87.

DISCUSSION

A certain amount of evidence has been presented in recent years to indicate that under controlled conditions the blood lipids are maintained relatively constant at definite levels. In 1923, Bloor
(2) found that there was little tendency to variation in the distribution of plasma fatty acids in cows, sheep, pigs, and dogs, this investigation confirming earlier work. Glusker (9) was able to show that in dogs kept on a standard diet at constant weight, the total fatty acid varied by a 13 per cent standard deviation and total cholesterol, under the same conditions, varied within a 28 per cent standard deviation. Somewhat later on the same group of dogs it was demonstrated by Boyd (6) that the phospholipid level was also maintained constant within a 17.5 per cent standard deviation. Earlier in this paper it has been shown that in the human female, under controlled conditions, a similar or even greater degree of constancy exists among the plasma lipids. In women it has been shown that total fatty acids varied by 15.7 per cent $\delta$, total cholesterol by 19.8 per cent $\delta$, and phospholipid by 11.5 per cent $\delta$. In addition to the lipids mentioned above, the present report includes means and variations for the remaining known lipids of blood plasma together with similar figures for iodine numbers. Of all the lipids, neutral fat and cholesterol ester were found to be the most variable.

In the rat, Sinclair (21) found that the iodine number of the tissue phospholipid fatty acids was markedly affected by food fats and correspondingly it has been shown above that the iodine number of the phospholipid fatty acids tends to be more constant. If diet acts on plasma lipids as demonstrated on the tissues by Sinclair, one would expect, under controlled diet for 1 week, a constancy in the iodine number of the phospholipid fatty acids, a condition corroborated experimentally above. In 75 per cent of cases a phospholipid value lower than the mean was associated with an iodine number for phospholipid fatty acids higher than the mean or vice versa, suggesting that the iodine number of the phospholipid fatty acid varies inversely as the amount of phospholipid.

As stated above, Bloor (2) and others have emphasized that there tends to be a constant relation between the various constituent lipids of plasma and tissues. In Table II a series of ratios between the several lipids studied has been computed and for each ratio the mean value with the standard deviation and per cent $\delta$ determined. For the purpose of ascertaining the constancy of a ratio, it may be concluded that where the per cent standard devia-
tion of the ratio is less than the per cent $\theta$ for each of the lipids in the ratio, then the ratio is more constant than each of its constituent lipids. Thus the ratio, total lipid to total fatty acid, with a mean value of 1.67, has a 4.0 per cent $\theta$, while the total lipid has 14.8 per cent $\varphi$ and total fatty acids 15.7 per cent $\varphi$, indicating that the ratio tends to be constant. By application of the same criterion, it has been found that the ratios, total fatty acid to cholesterol and combined cholesterol to total cholesterol, also exhibit a degree of constancy, while the ratios, total fatty acid to neutral fat and total fatty acid to phospholipid, were about as variable as the lipids themselves. The ratio, phospholipid to cholesterol, was more variable than its constituent lipids. It may therefore be concluded that under the controlled conditions of these experiments the variations in the lipid ratios were identical with the variations in the lipids themselves, and that the ratios are significant for comparative purposes only where one or other lipid level is altered by changed conditions in the experiment. It is obvious, also, that little value can be attached to the so called constancy of the ratio unless a critical standard, as described

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total lipid to total fatty acid</th>
<th>Total fatty acid to neutral fat</th>
<th>Total fatty acid to phospholipid</th>
<th>Total fatty acid to cholesterol</th>
<th>Phospholipid to cholesterol</th>
<th>Combined cholesterol to total cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. E...</td>
<td>1.60</td>
<td>2.00</td>
<td>1.97</td>
<td>7.80</td>
<td>4.03</td>
<td>0.68</td>
</tr>
<tr>
<td>C. B.....</td>
<td>1.63</td>
<td>2.10</td>
<td>1.87</td>
<td>7.00</td>
<td>3.75</td>
<td>0.67</td>
</tr>
<tr>
<td>J. F...</td>
<td>1.57</td>
<td>2.14</td>
<td>1.64</td>
<td>8.52</td>
<td>5.18</td>
<td>0.66</td>
</tr>
<tr>
<td>M. H.....</td>
<td>1.74</td>
<td>2.88</td>
<td>1.62</td>
<td>7.73</td>
<td>4.78</td>
<td>0.75</td>
</tr>
<tr>
<td>E. J.....</td>
<td>1.72</td>
<td>3.13</td>
<td>1.35</td>
<td>6.58</td>
<td>4.87</td>
<td>0.67</td>
</tr>
<tr>
<td>M. F.....</td>
<td>1.70</td>
<td>2.36</td>
<td>1.91</td>
<td>7.53</td>
<td>3.95</td>
<td>0.74</td>
</tr>
<tr>
<td>I. M.....</td>
<td>1.77</td>
<td>2.55</td>
<td>1.86</td>
<td>6.75</td>
<td>3.64</td>
<td>0.74</td>
</tr>
<tr>
<td>E. A.....</td>
<td>1.64</td>
<td>2.00</td>
<td>2.30</td>
<td>8.03</td>
<td>3.49</td>
<td>0.75</td>
</tr>
<tr>
<td>Mean.....</td>
<td>1.67</td>
<td>2.39</td>
<td>1.81</td>
<td>7.49</td>
<td>4.21</td>
<td>0.707</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.067</td>
<td>0.40</td>
<td>0.26</td>
<td>0.63</td>
<td>0.60</td>
<td>0.039</td>
</tr>
<tr>
<td>Standard deviation, per cent</td>
<td>4.0</td>
<td>16.7</td>
<td>14.4</td>
<td>8.5</td>
<td>14.3</td>
<td>5.5</td>
</tr>
</tbody>
</table>
above, be applied, and the value of such ratios, as they appeared in the literature, may thus be seriously questioned.

SUMMARY

A procedure has been described for the complete lipid analysis of small amounts of blood plasma based on oxidative methods and the advantage of using similar methods in such an analysis discussed. It was found necessary to modify a number of the published methods, particularly the digitonin method for total cholesterol which has been described in detail. The experimental errors and reliability of each procedure have been reported.

By application of this differential lipid analysis to blood plasma of eight normal young women under controlled conditions of exercise, rest, and diet, the following mean values were obtained:

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipid, mg. per cent.</td>
<td>589</td>
</tr>
<tr>
<td>Neutral fat, &quot; &quot; &quot;</td>
<td>154</td>
</tr>
<tr>
<td>Total fatty acid, mg. per cent.</td>
<td>353</td>
</tr>
<tr>
<td>Phospholipid fatty acid, mg. per cent.</td>
<td>130</td>
</tr>
<tr>
<td>Cholesterol ester fatty acid, mg. per cent.</td>
<td>77</td>
</tr>
<tr>
<td>Neutral fat fatty acid, mg. per cent.</td>
<td>146</td>
</tr>
<tr>
<td>Total cholesterol, mg. per cent.</td>
<td>102</td>
</tr>
<tr>
<td>Combined cholesterol, mg. per cent.</td>
<td>115</td>
</tr>
<tr>
<td>Free cholesterol, mg. per cent.</td>
<td>47</td>
</tr>
<tr>
<td>Phospholipid, mg. per cent.</td>
<td>196</td>
</tr>
<tr>
<td>Iodine No. of total fatty acids</td>
<td>88.5</td>
</tr>
<tr>
<td>&quot; &quot; &quot; phospholipid fatty acids</td>
<td>124</td>
</tr>
</tbody>
</table>

By computing the standard deviations it has been shown that the plasma lipids and ratios between the plasma lipids are maintained constant, within certain limits, under the controlled conditions of the experiment. The values obtained have been compared with published figures and a discussion relative to the function of the various lipids included.

BIBLIOGRAPHY

Lipid Analysis of Blood Plasma

A DIFFERENTIAL LIPID ANALYSIS OF BLOOD PLASMA IN NORMAL YOUNG WOMEN BY MICROOXIDATIVE METHODS
Eldon M. Boyd


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