THE FATTY ACIDS IN HUMAN BLOOD IN NORMAL
AND PATHOLOGICAL CONDITIONS.

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INTRODUCTION.

As has been shown in previous researches the fatty acids in
human blood exist as glycerides, cholesterol esters, soaps, or as
free acids; they are also represented as a radical of lecithin and
the phosphatide groups. Their content varies in continued star-
vation, fat absorption, anesthesia, certain pathological conditions,
etc., as recently reported and confirmed in a series of papers by
Bloor.\(^1\) In normal individuals when care is taken to avoid the
above conditions the amount of fatty acids in the blood is fairly
constant. But little, if any attention has been given to the
quality of the fatty acids, and especially to the proportion of
saturated to unsaturated fatty acids as well as the degree of
unsaturation.

That there is a difference in the quality of the fatty acids of the
whole human body is shown by the fact that the fats called in-
terstitial, depot, and organ fats, each absorb iodine in different
proportions, thus signifying that the unsaturated part of the
fatty acids varies in the different tissues. Jaeckle\(^2\) found an iodine
number of 62 to 73 for human (interstitial) fat, and 70 to 80
per cent of the fatty acids present as glycerides were oleic acid.
Hartley's\(^3\) analysis of liver fat shows the presence of higher unsat-
urated fatty acids than oleic acid as indicated by the iodine
number of 165 to 175.

\(^1\) Bloor, W. R., J. Biol. Chem., 1913-14, xvi, 517; 1914, xix, 1. Bloor,
xxxii, 575.


\(^3\) Hartley, P., J. Physiol., 1909, xxxviii. 353.
From the point of view of hemolytic action, we are especially interested in the unsaturated part of the fatty acids, since it has been shown by Noguchi and Faust and Tallquist that the higher saturated acids, palmitic and stearic, are not hemolytic either as such or as their soaps. On the other hand, the unsaturated fatty acids, as oleic acid, have a strong hemolytic action, and it has been demonstrated by Faust and Tallquist that this acid is the cause of the anemia in Bothriocephalus latus infection. Further, Lamar has shown an existing relation between the degree of unsaturation and lytic action as he was able to show that the sodium soap of linoleic acid dissolved pneumococci more rapidly in higher dilutions than sodium oleate. McPhedran, working with red blood cells, was unable to demonstrate any difference in the lytic action of linoleic and oleic acids as Lamar had claimed in the case of bacteria, and concluded that there was no relationship between the lytic action and degree of unsaturation.

In my previous paper, I reported that the iodine number of fatty acids of the blood in cases of pernicious anemia was not exceptionally high; but, as I stated, the matter needed further investigation, as the material used for iodine determinations was a mixture of saturated and unsaturated fatty acids; so that this finding had no other meaning than that unsaturated fatty acids were present. The mere presence of unsaturated fatty acids cannot be considered the primary cause of toxic hemolysis, since such acids exist in normal human blood, as shown below. It is therefore necessary to look further for the causative factor.

3 The cholesterol ester of oleic acid is hemolytic but not so strongly so as oleic acid itself. Since they demonstrated in the saponified ether extract of Bothriocephalus latus both oleic acid and cholesterol and were unable to show the presence of glycerol, they concluded that the oleic acid was present as a cholesterol ester without attempting to isolate it as such.
Methods.

I.

The saponification of the alcohol-ether extract was preferred to the direct saponification of whole blood because of the inconvenience of working with such large quantities, and the large volumes required to saponify in alcoholic KOH medium in a concentration sufficient to be sure that the esters of cholesterol are also split.

Blood was taken from the vein before breakfast. A few crystals of potassium oxalate were added to prevent clotting. 75 to 125 cc. of blood were measured with a pipette and run slowly into 250 to 400 cc. of 95 per cent alcohol with constant stirring. After the protein matter had settled it was filtered through a Buchner funnel by suction. The blood proteins were put into a large extraction shell and extracted by absolute alcohol for 24 hours and again by petroleum ether for 24 hours in a Soxhlet apparatus. The filtrate from the blood protein as well as the absolute alcohol extract was evaporated on the water bath to dryness. It was redissolved in the petroleum ether extract, filtered through cotton, measured, and about an eighth of it was used for the determination of the fat and lipoid content. From the remainder the petroleum ether was distilled off, the residue saponified, and the fatty acid determined according to the method of Gephart and Csonka.11

The first step in the separation of the unsaturated from the saturated fatty acids was to convert them into their lead soaps. These were extracted by ether, according to the method of Varrentrapp, which dissolves the lead soaps of the unsaturated fatty acids. This method is not strictly quantitative, but it is the best at present devised for the separation of unsaturated from saturated fatty acid in general.12

10 I wish to thank Drs. C. C. Hartman and W. T. Mitchell, Jr., for collecting the blood samples used in this work.


12 To demonstrate how this method worked in my hands I give one of the preliminary analyses made of Merek's oleic acid labeled as highly purified. (A), 0.1700 gm. of oleic acid gave 0.1490 gm. of unsaturated fatty acids; yield 87.6 per cent. (B), 0.1080 gm. of oleic acid gave 0.0950 gm. of unsaturated fatty acids; yield 87.9 per cent. A third sample, where 0.1635 gm. of oleic acid was saponified first, gave 0.1558 gm. of unsaturated fatty acids; yield 95.3 per cent.
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<tbody>
<tr>
<td>1. W. T. M.</td>
<td>M.</td>
<td>28</td>
<td>Normal.</td>
<td>0.790</td>
<td>73.7</td>
<td>0.345</td>
<td>0.168</td>
<td>48.8</td>
<td>84.2</td>
</tr>
<tr>
<td>2. F. A. C.</td>
<td>&quot;</td>
<td>29</td>
<td>&quot;</td>
<td>0.779</td>
<td>60.8</td>
<td>0.300</td>
<td>0.146</td>
<td>48.7</td>
<td>76.3</td>
</tr>
<tr>
<td>3. C. H. G.</td>
<td>&quot;</td>
<td>30</td>
<td>&quot;</td>
<td>0.755</td>
<td>67.2</td>
<td>0.300</td>
<td>0.136</td>
<td>45.5</td>
<td>92.1</td>
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<tr>
<td>4. C. P. B.</td>
<td>&quot;</td>
<td>50</td>
<td>&quot;</td>
<td>0.615</td>
<td>68.6</td>
<td>0.260</td>
<td>0.136</td>
<td>52.4</td>
<td>77.8</td>
</tr>
<tr>
<td>5. E. C.</td>
<td>&quot;</td>
<td>21</td>
<td>&quot;</td>
<td>0.619</td>
<td>—</td>
<td>0.313</td>
<td>0.136</td>
<td>43.4</td>
<td>105.9</td>
</tr>
<tr>
<td>6. K.</td>
<td>F.</td>
<td>24</td>
<td>&quot;</td>
<td>0.627</td>
<td>59.8</td>
<td>0.263</td>
<td>0.157</td>
<td>52.0</td>
<td>88.8</td>
</tr>
<tr>
<td>7. O. S. M.</td>
<td>M.</td>
<td>28</td>
<td>Hemolytic jaundice 16 mos. after splenectomy.</td>
<td>0.839</td>
<td>62.0</td>
<td>0.240</td>
<td>0.137</td>
<td>57.3</td>
<td>142.4</td>
</tr>
<tr>
<td>8. L.</td>
<td>F.</td>
<td>41</td>
<td>Pernicious anemia.</td>
<td>—</td>
<td>—</td>
<td>0.286</td>
<td>0.160</td>
<td>55.9</td>
<td>94.8</td>
</tr>
<tr>
<td>9. S. M.</td>
<td>M.</td>
<td>46</td>
<td>&quot;</td>
<td>0.509</td>
<td>72.8</td>
<td>0.282</td>
<td>0.157</td>
<td>55.5</td>
<td>101.0</td>
</tr>
<tr>
<td>10. A. B.</td>
<td>&quot;</td>
<td>29</td>
<td>Secondary anemia, jaundice.</td>
<td>0.564</td>
<td>75.3</td>
<td>0.241</td>
<td>0.141</td>
<td>58.5</td>
<td>107.8</td>
</tr>
<tr>
<td>11. McK.</td>
<td>&quot;</td>
<td>56</td>
<td>&quot;</td>
<td>0.685</td>
<td>73.1</td>
<td>0.292</td>
<td>0.163</td>
<td>55.8</td>
<td>71.8</td>
</tr>
<tr>
<td>12. C.</td>
<td>&quot;</td>
<td>17</td>
<td>Myelogenous leukemia.</td>
<td>0.855</td>
<td>90.1</td>
<td>0.290</td>
<td>0.140</td>
<td>48.3</td>
<td>110.9</td>
</tr>
<tr>
<td>13. S. M.</td>
<td>&quot;</td>
<td>50</td>
<td>Cerebral hemorrhage.</td>
<td>0.955</td>
<td>57.9</td>
<td>0.453</td>
<td>0.318</td>
<td>70.2</td>
<td>92.8</td>
</tr>
<tr>
<td>14. O. H.</td>
<td>&quot;</td>
<td>39</td>
<td>Chronic constipation.</td>
<td>0.777</td>
<td>88.7</td>
<td>0.362</td>
<td>0.255</td>
<td>70.4</td>
<td>111.5</td>
</tr>
<tr>
<td>15. N.</td>
<td>&quot;</td>
<td>48</td>
<td>&quot; nephritis.</td>
<td>0.800</td>
<td>78.6</td>
<td>0.370</td>
<td>0.252</td>
<td>68.2</td>
<td>130.6</td>
</tr>
<tr>
<td>16. F.</td>
<td>&quot;</td>
<td>31</td>
<td>Myocardial insufficiency, chronic nephritis.</td>
<td>0.720</td>
<td>61.0</td>
<td>0.252</td>
<td>0.157</td>
<td>62.4</td>
<td>78.3</td>
</tr>
<tr>
<td>17. D.</td>
<td>&quot;</td>
<td>30</td>
<td>Chronic nephritis.</td>
<td>0.818</td>
<td>67.3</td>
<td>0.333</td>
<td>0.154</td>
<td>46.3</td>
<td>89.8</td>
</tr>
<tr>
<td>18. H.</td>
<td>&quot;</td>
<td>46</td>
<td>Diabetes mellitus.</td>
<td>0.699</td>
<td>75.9</td>
<td>0.307</td>
<td>0.173</td>
<td>56.5</td>
<td>86.0</td>
</tr>
<tr>
<td>19. S.</td>
<td>F.</td>
<td>58</td>
<td>&quot;</td>
<td>0.823</td>
<td>78.2</td>
<td>0.393</td>
<td>0.211</td>
<td>53.8</td>
<td>101.2</td>
</tr>
</tbody>
</table>
At the end of the titration of the fatty acids by the method of Gephart and Csonka, the fatty acids are present as potassium soaps in a mixture of petroleum ether and alcohol. A portion of the blood cholesterol is present in this same solution. To remove the cholesterol the solution is made alkaline, to insure separation more alcohol is added, and extracted twice with petroleum ether. The alkaline solution containing the fatty acids as soaps is transferred to a large beaker, evaporated on the water bath, the residue dissolved in warm distilled water, neutralized by acetic acid, and the fatty acids are precipitated as lead soaps by the addition of an excess of lead acetate solution. The soaps are then filtered off and dried in vacuum. The ether-soluble lead soaps are decomposed by the addition of hydrochloric acid; the free unsaturated fatty acids, after the ether is distilled off, are dried in vacuum, weighed, and saved for the determination of the iodine number according to Hübl.

Since the iodine number of the unsaturated fatty acids in several cases as shown in Table I is higher than that of oelic acid, the

<table>
<thead>
<tr>
<th>Case</th>
<th>3</th>
<th>9</th>
<th>10</th>
<th>14</th>
<th>18</th>
<th>19</th>
</tr>
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<tbody>
<tr>
<td>Iodine No.</td>
<td>117.2</td>
<td>101.1</td>
<td>129.6</td>
<td>136.9</td>
<td>96.4</td>
<td>108.5</td>
</tr>
<tr>
<td>Unsaturated fatty acids of alcohol-soluble barium soaps, gm. in 100 cc. of blood</td>
<td>0.035</td>
<td>0.136</td>
<td>0.054</td>
<td>0.165</td>
<td>0.075</td>
<td>0.084</td>
</tr>
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</table>

question of the direct evidence of the presence of other or higher unsaturated fatty acids arose in the latter part of this work. Therefore, the unsaturated fatty acids were dissolved in absolute alcohol neutralized by alcoholic KOH and the oleic acid was partly precipitated as barium oleate, placed in a refrigerator for a few hours, and then filtered. The unsaturated fatty acids of the alcohol-soluble barium soaps (Table II) were liberated by hydrochloric acid, extracted by ether, and after the ether was evaporated off the residue was dried, weighed, and used for the determination of the iodine number.

I was especially careful to avoid any alteration or destruction of the unsaturated fatty acids which may result from the use of excessive heat. The saponification and extraction flask described in the previous communication was used to advantage in this work.
A review of the literature has shown that in the determination of blood fats the extraction followed by saponification of the extract was to be preferred to the direct saponification. The former method was introduced by Shimidzu\textsuperscript{13} who tried to apply the Kumagawa-Suto direct saponification, but found lower results than when he had first extracted the blood with alcohol and then saponified the extract. Berczeler\textsuperscript{14} confirmed Shimidzu's claim and recently Rosenthal and Trowbridge\textsuperscript{15} discussing the methods of fat determination concluded also that in blood Shimidzu's alcoholic extractions should precede saponification. As a control for the blood fat determinations in the present work it was thought advisable to run duplicate determinations according to the Gephart-Csonka\textsuperscript{11} method. To saponify in alcoholic KOH medium in a concentration sufficient to be sure that also the more resistant esters such as cholesterol esters are split with certainty, only 5 cc. of blood were used.

The results in Table III verify the applicability of the Gephart-Csonka method for blood in cases where we are interested only in the absolute values, with the advantage also of avoiding the time-consuming extraction. The above results show further that the accuracy was not limited by using such small amounts of blood; the higher results were expected, as the extraction is never complete, especially in case such large amounts of blood are extracted.

The method as applied to blood is as follows: To 5 cc. of blood measured into the author's saponification and extraction flask 20 cc. of alcohol

\begin{table}[h]
\centering
\caption{Gm. of Fatty Acids in 100 Cc. of Blood.}
\begin{tabular}{lcccccc}
\hline
Case & 2 & 3 & 6 & 9 & 14 & 16 & 18 \\
\hline
Extraction and saponification & 0.300 & 0.300 & 0.263 & 0.282 & 0.362 & 0.252 & 0.307 \\
Gephart-Csonka method & 0.325 & 0.326 & 0.271 & 0.318 & 0.355 & 0.268 & 0.428 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{13} Shimidzu, Y., \textit{Biochem. Z.}, 1910, xxviii, 237.
\textsuperscript{14} Berczeler, L., \textit{Biochem. Z.}, 1912, xlv, 193.
(95 per cent) and 4 gm. of stick potassium hydroxide are added. The flask is immersed in a boiling water bath and its contents are boiled under a reflux condenser for 1 hour, it is then cooled, and 20 cc. of 20 per cent HCl are added in small portions to free the fatty acids, cooling the flask after each addition of the acid. The flask is filled to the constriction with distilled water, 50 cc. of ether are added, the flask is closed with the stopper, and shaken in a rotary manner for a few minutes. After the ether layer has separated it is blown by pressure into a separating funnel (250 cc.). The ether extraction is repeated twice with 50 cc. portions, collecting the portions in the separating funnel. After each extraction the ether layer is brought to the constriction by adding a little distilled water. The combined ether extract in the separating funnel is washed with several portions of water until the wash water is neutral. The crude ether extract, being free from hydrochloric acid as well as lower fatty acids, is transferred into an Erlenmeyer flask (200 cc.), a glass bead added, and the ether distilled off. The flask containing the residue is placed in the water bath for a few minutes, then in a vacuum desiccator over night to free the residue from moisture. 25 cc. of light, boiling petroleum ether are added, the flask is rotated at frequent intervals, and the solution filtered through a thick plug of fat-free cotton. Wash the flask and filter well with petroleum ether (3 times with 10 cc. portions) and collect the filtrate which is perfectly clear and colorless in an Erlenmeyer flask. Bring the petroleum ether solution to boiling in a water bath and titrate it immediately with 0.04 N alcoholic KOH using phenolphthalein as an indicator; each cc. of 0.04 N KOH is equal to 10.97 mg. of fatty acids. For further details see the original article.11

DISCUSSION.

The unsaturated fatty acids are of exogenous or endogenous origin, the latter being derived by synthesis from either carbohydrate or protein. A third source is by the desaturation of food fat and depot fat, which is mobilized for transport. Leathes believes that desaturation is the role of the liver in fat metabolism, so we may consider that unsaturated fatty acids occur in normal metabolism.

As Table I shows, the blood of normal individuals contains an average of 0.143 gm. of unsaturated fatty acids per 100 cc. with an average iodine number of 87.5. 48 per cent of the total fatty acids are unsaturated. The iodine number of unsaturated fatty acids varies from 76 to 105 in normal human blood, thus indi-

408 fatty acids in human blood

eating in addition to oleic acid the presence of other both higher and lower unsaturated fatty acids in small amounts.

In pathological conditions, we have found that a generally higher proportion of unsaturated fatty acids than the normal average occurs, and that the iodine absorption power of the unsaturated fatty acids as well as their absolute amount is higher. We may divide the pathological cases according to their hemoglobin content into two groups: (A) normal hemoglobin, and (B) low hemoglobin.

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<th>TABLE IV.</th>
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<td><strong>Group A.</strong></td>
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The high iodine numbers representing unsaturated fatty acids with higher degree of unsaturation than oleic acid are found in Group B, but we should not forget that in the same group we deal with an abnormally low red cell count. Munk and Friedenthal found an increase of fat in red corpuscles during fat absorption, and Bloor suggested that most, if not all, fat metabolized through lecithin, and that lecithin formation is a result of red cell activity. In anemia, where the number of red cells is lowered, probably the fat metabolism is disturbed; and as a fact we find the amount of lecithin as well as other lipid substances generally lower than that in normal human blood. It would seem that these lipid substances especially require the unsaturated fatty acids as a radical in their formation. The higher iodine value found especially in those cases where the lipoids are low suggests a resultant excess of unsaturated fatty acids and not necessarily an increased desaturation of fatty acids by stimulated liver activity.

While we know that this excess of unsaturated fatty acids has a hemolytic action, and no doubt intensifies the hemolysis, yet it cannot be the primary cause, as we found in Cases 3, 13, 14, 19 (Tables I and II) higher unsaturated fatty acids in even larger quantities than in normal blood with normal red blood count and hemoglobin content.

The iodine values of fat plus lipoid substances show small variation in pathological cases as well as in normal cases, and have neither qualitative nor quantitative significance. A higher iodine number does not mean that there are present more highly unsaturated fatty acids than oleic, or that the proportion of unsaturated to saturated is larger.

**SUMMARY.**

Unsaturated fatty acids are a product of normal metabolism, being present in normal human blood, in which they form 48.0 per cent of the total fatty acids. Judged by their iodine absorption power there are, in addition to oleic acid, other unsaturated fatty acids both higher and lower than oleic, although in small amounts.

The iodine numbers of the unsaturated fatty acids in pathological conditions are generally higher than in normal individuals, especially in cases with low hemoglobin values. As such acids exist in normal human blood as well as in pathological conditions without anemia, it is necessary to look further for the primary cause of toxic hemolysis.

The Gephart-Csonka method for fatty acid determination in feces has been applied to blood in the present work, thus avoiding the time-consuming extraction common to other methods.
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J. Biol. Chem. 1918, 33:401-409.

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