THE PLASMA AND RED BLOOD CELL LIPIDS IN PERSISTENT (DIABETIC) LIPEMIA AND IN TRANSIENT (ALIMENTARY) LIPEMIA

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The role of the red blood cells in the transport and metabolism of lipids has been difficult to evaluate. Despite a growing body of evidence which indicates that the lipids of the red cells are structural elements, the concentrations of which are unchanged in a variety of conditions, whole blood rather than plasma is still used occasionally for lipid studies. This usage apparently rests in part on the earlier work of Bloor (1915, 1916) who found a marked increase in phosphatides and glycerides in the red cells of dogs after they were fed olive oil. Knudson (1917) confirmed the observations and further reported an increase in cholesterol esters. In a later paper Bloor (1920-21) mentioned that, "Further unreported work in this laboratory has not, however, entirely borne out these findings, since certain dogs have been found, in which the increase of lipid phosphorus took place in the plasma and not in the corpuscles." M. Bodansky (1931) confirmed Knudson's work and found it advantageous to express the results in terms of molecular equivalents.

On the basis of these observations, Bloor (1932) has suggested that in persistent lipemia (e.g., of diabetes, nephrosis, chronic hemorrhage, etc.) the red cells are inert, while in acute alimentary lipemia they may participate in lipid transport.

Other studies of alimentary lipemia do not support this viewpoint, however. Iwatsuru (1926) fed olive oil, olive oil with cholesterol, and lecithin to rabbits and observed little change in corpuscular cholesterol, phosphatides, and fatty acids. The rabbit, however, is a herbivorous animal, which does not always
react to fat feeding in the same way necessarily as carnivorous animals (Sinclair, 1934). Vahlquist (1931) found no change in the corpuscular phosphorus soluble in alcohol-ether after meat and olive oil or olive oil alone was fed to dogs. It is uncertain, however, whether this fraction is composed entirely of lipoid phosphorus. Wendt (1932) found little change in corpuscular phosphatides or cholesterol of men who were fed 100 gm. of olive oil. In relation to their body weight, this dosage is much less than the amount, 6 ml. per kilo, fed to the dogs in the experiments of Bloor, Knudson, Bodansky, and Vahlquist.

Some studies made with unnatural fats, used as indicators, have yielded conflicting results. Artom (1933) and Artom and Peretti (1933), after feeding or injecting iodized fats, observed that the red cell lipids contained small amounts of iodine, rather more in the phospholipids of the red cells than in the plasma. Sinclair (1936) pointed out that these results were inconclusive because of the small amounts of iodized fatty acids which were recovered. In experiments with cats fed elaidin, Sinclair found that whereas considerable amounts of elaidic acid entered the phospholipids of the blood plasma, none could be detected in the corpuscular phospholipids.

The recent evidence therefore favors the view that the corpuscular phospholipids are not involved in lipid transport. It is still uncertain whether cholesterol and non-phospholipid fatty acids are also unchanged. It seemed desirable to reexamine the effect of fat feeding on the corpuscular lipids of the dog. The results of such a study are given and contrasted with data obtained from an unusual case of intense diabetic lipemia.

**EXPERIMENTAL**

For the fat feeding experiments, dogs weighing between 13 and 16 kilos were fed 6 ml. of olive oil per kilo, 20 hours after the preceding meal. The dogs had previously received a stock diet consisting of Valentine’s meat powder, cracker meal, skim milk powder, dried brewers’ yeast, and Mazola. This daily ration provides 1000 calories, of which 27 per cent is derived from fat, 50 per cent from carbohydrate, and 23 per cent from protein.

At each interval, 30 ml. of blood were drawn from a jugular vein
and transferred with minimal exposure to two graduated centrifuge tubes, each of which contained 2 drops of a 4 per cent solution of heparin (Connaught) in 0.9 per cent NaCl. Heparinized bloods from the diabetic patient, a 14 year-old female, were taken at random intervals, generally about 4 hours after breakfast. All the bloods were centrifuged at 3000 r.p.m. for 1 hour. After the percentage of red cells had been noted and most of the plasma removed, the remaining plasma and the layer of white cells were carefully removed with the aid of suction. Samples of the plasma were measured by volume, of the red cells by weight. Cold extraction with Bloor's mixture was used for both plasma (Boyd, 1936, a) and red cells (Boyd, 1936, b). For the intensely lipemic diabetic plasmas, the alcohol-ether to plasma ratio was increased to 100:1. For other plasma and all red cell samples, the ratios recommended by Boyd, 20:1 and 40:1, respectively, were used.

Total lipids, lipoid phosphorus, free and total cholesterol, and iodine numbers were determined, with the slight modifications detailed below, in essentially the same manner as described in a previous report on liver lipids (Rubin et al., 1937). Alcohol-ether extracts were concentrated to near dryness in vacuo, under nitrogen, at 40° or less, and then resolved in boiling petroleum ether. After saponification of an aliquot, under nitrogen, the unsaponifiable material was used for microgravimetric determination of the total cholesterol. In the liver work noted above, total cholesterol was determined in an aliquot of the alcohol ether extract.

In the determination of cholesterol, the lipid mass and precipitate of cholesterol digitonide were treated with boiling water and acetone prior to filtration. The digitonide was then filtered, washed with water, acetone, and ether, dried, and weighed.

Choline was determined by the colorimetric procedure of Beattie (1936). In order to conserve material, the determination was performed on the alcoholic residue remaining after the extraction of the unsaponifiable and fatty acid fractions. 1 ml. of concentrated HCl per 10 ml. of solution was added; the solution was refluxed for 1 hour, concentrated to about 5 ml., and then treated with saturated Reinecke solution. The reineckate was filtered through a small funnel, according to a suggestion by Shohl (1928), washed, and dissolved in acetone. The colorimetric readings
Blood Lipids in Lipemia

were taken in a Lange photoelectric colorimeter with a green filter.\textsuperscript{1} The solution obeyed Beer's law up to the highest concentration used, which was equivalent to 0.5 mg. of choline chloride per ml. of acetone.

Carotene was determined by the procedure of Stueck and Ralli (1937), chloride by the open Carius method, and water by drying to constant weight in tared beakers containing sand.

Both plasma and red cells were extracted in duplicate for lipid determinations. The results given are the means of closely agreeing duplicate or triplicate values.

\textbf{Results}

\textbf{Plasma of Diabetic}

The plasma findings in the diabetic patient\textsuperscript{2} are given in Table I. The high figures for total lipids (22.97 per cent) and for cholesterol

\textsuperscript{1} Beattie (1936) used a methyl red solution as an arbitrary standard with which the reineckate solution was compared in a visual colorimeter. We were unable to find a methyl red preparation which matched well with the reineckate, unless a green filter, such as the Wratten No. 74-\textepsilon, were used. The use of the photoelectric colorimeter is more satisfactory, however, since it obviates errors arising from the transfer of small volumes of acetone solutions.

\textsuperscript{2} The patient, a 14 year-old female, was admitted to the Third (New York University) Medical Division, Bellevue Hospital, in diabetic ketosis. She had had diabetes for 4 years and had been treated by diet and insulin, the latter averaging about 20 units daily. There had been three previous episodes of diabetic ketosis, treated in other hospitals, the last of which was in August, 1938. 2 days prior to the present admission, the patient developed an upper respiratory infection and vomited several times. She had taken very little food during the past 2 days. The vomiting did not recur. The patient was well nourished and although the breathing was deeper than normal, it was not Kussmaul in type. The tongue was dry and there was a strong acetone odor to the breath. The lungs were clear. The heart sounds were normal; there were no murmurs and the rhythm was regular. The blood pressure was 110/70. The liver could be felt extending below the umbilicus. The spleen was not palpable. The temperature was 100.2\textdegree F. The urine showed both sugar and acetone. The patient was treated with infusions of N saline and 5 per cent glucose and repeated injections of insulin. The following day the patient was placed on a diet of 240 gm. of carbohydrate, 110 gm. of protein, and 48 gm. of fat, divided into five feedings. The total amount of insulin necessary to keep the patient sugar-free varied from 110 to 125 units daily. The liver gradually became smaller and
(1.615 per cent) observed on the day of admission to the hospital are unusual, although even higher figures have occasionally been reported. The relative magnitude of the various lipid fractions on the 1st day and the relative rates of decrease in these lipids (triglycerides > phospholipids > cholesterol) are in agreement with previous descriptions (Bloor, 1921; Geelmuyden, 1928).

The values for cephalin, calculated as per cent of the total phospholipids, were derived from the atomic ratio of choline and lipoid phosphorus. They show a drop from 23 to 3 per cent between the 13th and 20th days, followed by an apparently gradual return to 29 per cent on the 88th day. This curious change may have been due to dietary causes, although it is possible that more frequent observations might have revealed a greater variation within this period.

The percentage of free in total cholesterol fell, on the 7th day, to 31 per cent, which is close to the normal range of 24 to 30 per cent found by Sperry (1936). This is noteworthy in view of the facts that the total cholesterol was still above 1 per cent and the patient's liver was greatly enlarged. Thereafter, the percentage fluctuated between 26 and 32.

The iodine number of the total fatty acids remained fixed at 75 through the 5th day, owing to the preponderance of triglycerides. It fluctuated thereafter between 79.7 and 99.8.

Comparison of the total lipids observed as the total petroleum ether-soluble material with that calculated by summation of the various lipids shows a fairly constant excess in the former, averaging 115 mg. per 100 ml., with a range of 77 to 150. Differences of the same magnitude are apparent even in the more lipemic plasmas, but are not represented in Table I because they constitute so small a percentage of the total lipids. Since these differences may include the summated errors of several determinations, their precision is necessarily low. That they are not due to systematic analytical errors, however, is indicated by the fact that the percentage ratio of calculated over observed lipids decreases regularly from an initial value of 99.6 per cent to values around 90 per cent.

was no longer palpable on the 16th day after admission. The blood plasma of this patient was rather striking in its cream-like appearance. It remained this way until the lipid content dropped below 5 per cent.
## Table I

**Plasma of 14 Year-Old Diabetic**

All values are expressed in mg. per 100 ml. of plasma, unless otherwise noted.

<table>
<thead>
<tr>
<th>Days observed</th>
<th>Total lipids</th>
<th>Cholesterol</th>
<th>Total fatty acids</th>
<th>Tri-glycerides</th>
<th>Phospholipids</th>
<th>Cholesterol Cephalin†</th>
<th>Water</th>
<th>Chloride</th>
<th>Non-protein N</th>
<th>Carotene</th>
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<tr>
<td></td>
<td>Observed</td>
<td>Calculated*</td>
<td>Difference</td>
<td>Free</td>
<td>Total</td>
<td>Free</td>
<td>Total</td>
<td>Iodine No.</td>
<td>m eq per cent</td>
<td>gm. per 100 ml</td>
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<tr>
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<td>22,970</td>
<td>22,900</td>
<td></td>
<td>950</td>
<td>1615</td>
<td>59</td>
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<td>75.3</td>
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<td>1280</td>
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<td>543</td>
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<td>42</td>
<td>6,430</td>
<td>75.6</td>
<td>5,620</td>
<td>1150</td>
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<td></td>
<td>335</td>
<td>1080</td>
<td>31</td>
<td></td>
<td></td>
<td>770</td>
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<td>2,580</td>
<td>2,460</td>
<td>120</td>
<td>208</td>
<td>754</td>
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<td>1,775</td>
<td>1,630</td>
<td>145</td>
<td>141</td>
<td>543</td>
<td>26</td>
<td>969</td>
<td>82.7</td>
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<td>1,270</td>
<td>150</td>
<td>103</td>
<td>346</td>
<td>30</td>
<td>818</td>
<td>79.7</td>
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<tr>
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<td>1,030</td>
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<td>75</td>
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<td>29</td>
<td>649</td>
<td>92.3</td>
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<td>299</td>
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<td>114</td>
<td>104</td>
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<td>29</td>
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<td>960</td>
<td>99.8</td>
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<td>364</td>
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<tr>
<td>137</td>
<td>1,154</td>
<td>1,040</td>
<td>114</td>
<td>92</td>
<td>291</td>
<td>32</td>
<td>631</td>
<td>99.8</td>
<td>287</td>
<td>325</td>
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</tbody>
</table>

* Total lipids = triglycerides + phospholipids + total cholesterol + 0.71 X esterified cholesterol.
† Cephalin calculated as per cent of total phospholipids.
‡ Chloride corrected to average water content of 95 per cent.
The recent work of Folch and Van Slyke (1939) and of Christensen (1939) shows that considerable amounts of urea and alkali halides are entrained with the lipids when these are resolved in petroleum ether.

An interesting relationship between carotene and the phospholipids is shown in Fig. 1, in which the concentrations of the various lipids are plotted against those of carotene. A linear relation obtains only between carotene and the phospholipids.
The significance of this observation is not clear at present. Barring the possibility of coincidence in this case, one might interpret it as evidence of a common depot, presumably the liver.

The presence of large amounts of fat in the plasma introduces difficulties in the interpretation of the concentrations of other substances, unless the water content is known. Examples are given in Table I for the plasma chloride values. When the total lipids are plotted against water, a straight line is obtained, indicating that the water concentration is independent of the composition of the lipid mixture.

**TABLE II**

*Red Blood Cell Lipids of Diabetic Patient*

All values are expressed in mg. per 100 gm. of red cells, unless otherwise noted.

<table>
<thead>
<tr>
<th>Day of observation</th>
<th>Total plasma lipids (mg. per 100 ml.)</th>
<th>Hematocrit (%)</th>
<th>Cholesterol (mm per cent)</th>
<th>Total fatty acids (mm per cent)</th>
<th>Phospholipids (mm per cent)</th>
<th>Choline (mm per cent)</th>
<th>Atomic ratio, choline/P</th>
<th>Cephalin (%)</th>
<th>Triglycerides (mm per cent)</th>
<th>Total lipids (mm per cent)</th>
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<tr>
<td>2§</td>
<td>15,160</td>
<td>35</td>
<td>98</td>
<td>100</td>
<td>217</td>
<td>228</td>
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<td>13</td>
<td>2,580</td>
<td>36</td>
<td>98</td>
<td>101</td>
<td>160</td>
<td>195</td>
<td>0.137</td>
<td>0.51</td>
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<tr>
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<td>35</td>
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<td>97</td>
<td>158</td>
<td>212</td>
<td>0.134</td>
<td>0.46</td>
<td>54</td>
<td>18</td>
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<tr>
<td>102</td>
<td>1,547</td>
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<td>110</td>
<td>107</td>
<td>168</td>
<td>197</td>
<td>0.144</td>
<td>0.53</td>
<td>47</td>
<td>39</td>
</tr>
</tbody>
</table>

* Lipoid P X 23.5.
† Calculated as per cent of total phospholipids.
‡ Total lipids = phospholipids + total cholesterol + triglycerides.
§ Red cells on this day were washed once with 1.1 per cent KCl.

*Red Blood Cells of Diabetic*

Data for the red blood cells of the same patient are given in Table II. These show little change in any of the lipids studied. The triglycerides present the greatest relative variation, which is due both to the low concentration and the fact that the values are derived by difference from the other lipids. The figures for free and total cholesterol, phospholipids, and total fatty acids fall within the range given by Erickson et al. (1937) for normal children but are somewhat lower than Boyd's (1936) figures for normal adults. A notable difference is that the data in Table II...
<table>
<thead>
<tr>
<th>Time after oil ingestion (hrs.)</th>
<th>Plasma lipids*</th>
<th>Red cell lipids†</th>
<th>Hemo- tocrit per cent</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Total fatty acids</td>
<td>Cholesterol</td>
<td>Tri-glycerides</td>
</tr>
<tr>
<td></td>
<td>Free</td>
<td>Total</td>
<td>Free Total</td>
</tr>
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<td>39</td>
</tr>
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<td>398</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>6</td>
<td>446</td>
<td>41</td>
<td>46</td>
</tr>
</tbody>
</table>

* Expressed as mg. per 100 ml. of plasma unless otherwise noted.
† Calculated as per cent of total phospholipids.
‡ Expressed as mg. per 100 gm. of red cells unless otherwise noted.
show the complete absence of esterified cholesterol from the red cell; Erickson et al. found an average of 34 mg. per 100 gm., while Boyd found 28 mg. per 100 ml. Cephalin (Table II) constituted $50 \pm 2$ per cent of the phospholipids, which is in fair agreement with the average values of 56 and 60 per cent found by Williams et al. (1938) and Kirk (1938), respectively, in human red cells.

**Blood Lipids of Dogs after Fat Feeding**

Plasma—Typical protocols are given in Table III of the plasma and red cell lipids of two dogs after a single feeding of olive oil, 6 ml. per kilo. The plasma lipids show moderate increases in phospholipids and triglycerides but no changes in free or total cholesterol. The cephalin figures present slight, irregular variations which may be due to analytical causes.

Red Blood Cells—Consideration of Table III shows little or no change in any of the lipid entities.

Of the alcohol-ether-extracted phosphorus, 96 ± 2 per cent was recovered in the petroleum ether solution. This observation supports the validity of Vahlquist's use of the alcohol-ether-extracted phosphorus as a measure of phospholipids. We have been unable to find any other reference to this point, except in the paper by Williams et al. (1938), who mention, without detailing the data, that, "Evaporation under nitrogen at reduced pressure and below 50° resulted in average recoveries of 80, 90, and 93 per cent of the alcohol-ether phosphorus by petroleum ether, of plasma, erythrocytes, and erythrocyte stroma respectively."

As in the case of the diabetic patient, the values for free and total cholesterol agree within the possible limits of analytical error and point to the absence of cholesterol esters from the red cell of the dog as well.

**DISCUSSION**

Both physical and chemical evidences have been adduced recently to show that the red blood cell lipids are present as structural elements. From a consideration of the optical polarization properties of the envelope, Schmitt, Bear, and Ponder (1938) concluded that the lipids are concentrated in the envelope. Erickson et al. (1937–38) found 89 to 100 per cent of the lipids of human, bovine, and sheep erythrocytes in the stroma of these cells.
It has been shown in the present paper that the concentrations of the various corpuscular lipids remain unchanged both in the persistent and in the transitory lipemia. These results are in harmony with the view that the red cell lipids are structural components which do not participate in the transport or metabolism of fat.

It is difficult to ascertain the cause of the differences between our results on dogs fed olive oil and those reported by Bloor, Knudson, and Bodansky. Aside from differences in the techniques of fat analysis, a possible cause may lie in the fact that they estimated the red cell lipids indirectly from values for whole blood and plasma. Boyd (1936, c) has shown that the values for corpuscular lipids obtained by the indirect method are more variable than those found by the direct method.

**SUMMARY**

Analyses are given of the plasma and red blood cell lipids in a case of persistent human diabetic lipemia and in alimentary lipemia of dogs. The total plasma lipids of the diabetic decreased from an initial value of 23 per cent to about 1.2 per cent over a period of 137 days. The changes in free and total cholesterol, total phospholipids, triglycerides, and the iodine number of the total fatty acids were similar to those described in the literature. A linear relation was noted between the plasma carotene and phospholipid concentrations.

After the ingestion of large doses of olive oil, dogs showed moderate increases in the plasma phospholipids and triglycerides, but no change in free or total cholesterol.

Both in the diabetic patient and in the dogs, the corpuscular lipids remained unchanged and within normal limits, from which it is concluded that the red cells are inert both in persistent and in transient lipemia.

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