Erythrocyte and Plasma Lipids in Sickle Cell Anemia

By Maxwell P. Westerman, Lawrence E. Pierce and Wallace N. Jensen

Certain abnormalities that occur in sickle cell disease are difficult to relate directly to the presence of S hemoglobin or to the sickling phenomenon. That nonhemoglobin components of the erythrocytes from patients with sickle disease may be altered is suggested by the abnormal concentration or flux of electrolytes in the sickled cell, by a qualitative difference of red cell catalase, and by an increase in stromal lipid concentration as it relates to a unit volume of cells and to the single cell. For meaningful evaluation, the localization of cell lipid to the cell surface would suggest that lipid concentration should be related to cell surface area. The necessity for comparing the lipid concentrations of young abnormal cells with concentrations of similarly aged normal cells should also be considered. Decreased levels of plasma cholesterol in sickle cell disease, and the serum lipid alterations associated with erythrocyte lipid changes which have been observed in patients with acanthocytosis, make plasma lipid measurements of interest.

In this study, concentrations of erythrocyte and plasma lipids of patients with sickle cell disease have been made and the results related to cell surface area, to the single cell and to a unit volume of cells. The lipid concentrations in the sickle disease cells have been compared with those of a normal young population of cells.

Methods

Fasting specimens of anticoagulated venous blood (disodium ethylene diaminetetraacetic acid, 1 mg./1 ml. blood) were obtained from five male and five female patients with sickle cell anemia. The patients were between 16 and 42 years of age and in a steady state uncomplicated by infection, clinical or hematologic crises. The proportion of sickle hemoglobin in the red cells was determined by paper, starch gel and agar electrophoresis at pH 8.6 and 6.2. Fetal hemoglobin was measured by the method of Jouxis and Huisman. The blood was centrifuged (RCF = 1500 g) for 10 minutes at 21 C., and the plasma and buffy coat removed. Red cells were thrice washed with isotonic NaCl and finally centrifuged (RCF = 3000 g) for 30 minutes. After removal of the supernatant fluid, aliquots of the packed red cells were taken for cell counts, reticulocyte counts, cell diameter and thickness measurements, and for lipid analyses. Red cell concentrations were estimated by the method of Dacie and reticulocyte counts by the method of Brecher. Cell diameter and thickness measurements were determined by a previously described direct microprojection technique and the surface area and volume of the erythrocyte calculated from these determinations. Analyses of erythrocyte and plasma for total lipid,
total cholesterol, total lipid phosphorus and the various phospholipid fractions were performed by previously described technics. The red cell samples were exposed to atmospheric air and 100 per cent oxygen at regular intervals. Little or no sickling of the cells was observed in aliquots which were frequently examined during the preparative and measurement procedures. Populations of normal young cells were prepared by a modification of the centrifugation technic described and verified by Borun, Figueroa and Perry, Hoffman and Prankerd. Values for cell measurements and lipid concentrations in normal young cell populations have been previously determined and reported.

RESULTS

Cell Dimensions

In table 1 the diameters, thicknesses, calculated surface areas and calculated volumes of sickle disease erythrocytes are compared with those of a normal young red cell population. The mean diameter of the sickle hemoglobin-containing erythrocytes was greater \((p = 0.001)\) and the mean thickness less \((p < 0.02)\) than that observed in a normal young erythrocyte population. The mean surface area of the sickle hemoglobin-containing cell was larger \((p < 0.01)\) than the cell surface area of a normal young red cell. There was no significant difference in cell volume determinations between the two types of cells.

Lipid Concentrations

Concentrations of lipids have been expressed with reference to the single cell, to surface area of the cell and to a volume of packed erythrocytes. Total lipid concentration (table 2) of the sickle hemoglobin-containing cell was greater than the concentration in a normal young population of erythrocytes when related to a unit volume of cells (mg./ml.), \(p < 0.01\), and to the single cell, \(p < 0.01\). The concentration of total phospholipid (table 2) of the sickle hemoglobin-containing cell as related to a unit volume of cells (mg./ml.) and to the single cell was larger than the concentration in a normal young population of cells \((p = 0.02, p > 0.01\) respectively). There were no significant differences of other lipid moieties when they were related to other cell parameters (table 2). Similarly, the percentage of various phospholipid fractions of the sickle disease cells was not significantly different than that observed in normal young populations of cells (table 3).

In table 4, the plasma lipid concentrations of normal individuals and of patients with sickle cell anemia are shown. The total plasma lipid, total phospholipid and total cholesterol concentrations were significantly lower \((p < 0.01, 0.001 \text{ and } 0.001 \text{ respectively})\) in the sickle cell patients than in a group of normal subjects.

DISCUSSION

The mean concentrations of the various lipids were greater in the non-sickled oxygenated cells of sickle disease patients than in a normal young population of erythrocytes regardless of reference to surface area, the individual cell or the unit volume of cells. Statistically significant increases were present in the total lipid and phospholipid concentrations in the erythro-
Table 1.—Cell Dimensions in Normal and Sickle Cell Disease Erythrocytes

<table>
<thead>
<tr>
<th></th>
<th>Normal Young*</th>
<th>Sickle Cell</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter ((\mu))</td>
<td>8.85 ± 0.16*</td>
<td>9.31 ± 0.32</td>
<td>0.001</td>
</tr>
<tr>
<td>Thickness ((\mu))</td>
<td>1.65 ± 0.04</td>
<td>1.53 ± 0.14</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Volume ((\mu^3))</td>
<td>91.2 ± 5.2</td>
<td>93.1 ± 8.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Surface area ((\mu^2))</td>
<td>150.0 ± 5.2</td>
<td>162.1 ± 9.7</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Mean values ± one standard deviation.

Table 2.—Lipid Concentrations in Normal Young and Sickle Cell Disease Erythrocytes

<table>
<thead>
<tr>
<th></th>
<th>Total Lipid</th>
<th>Phospholipid</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal*</td>
<td>Sickle disease</td>
<td>Normal*</td>
</tr>
<tr>
<td>mg./ml.†</td>
<td>5.30 ± 0.87*</td>
<td>6.58 ± 0.78 (&lt;0.01)</td>
<td>3.32 ± 0.55</td>
</tr>
<tr>
<td>mg./cell‡</td>
<td>479 ± 85</td>
<td>593 ± 87 (0.01)</td>
<td>300 ± 36</td>
</tr>
<tr>
<td>mg./(\mu^3)§</td>
<td>3.18 ± 0.54</td>
<td>3.87 ± 0.59 (0.06)</td>
<td>1.99 ± 0.22</td>
</tr>
</tbody>
</table>

*Mean values ± one standard deviation.
†mg./ml packed red cells.
‡mg. x 10^-12/cell.
§mg. x 10^-12/\(\mu^3\) surface area.

Cytes from patients with sickle cell anemia when these concentrations were expressed in terms of the individual cell or of a given volume of cells, while more obvious differences were limited to the total lipid measurements. There were no significant differences in the cholesterol measurements nor in any lipid determinations as they related to cell surface area. In view of the importance of the measurement relating lipid concentration to the surface area, this would further suggest that the differences between the normal young and sickle disease cell lipid concentrations are limited. The differences which are present may represent the lack of complete similarity in age distribution between the normal young population of cells and the sickle disease cell population. The measurements described in an earlier study by Erickson et al.\(^5\) in sickle disease patients revealed an increase in all erythrocyte lipids, i.e., total lipid, phospholipid and free and esterified cholesterol in the single cell and in a unit volume of cells as compared with concentrations of a randomly aged population of normal red cells. The authors felt the differences were significant, but statistical analyses were not presented. The differences between the present and the previous results may be partially explained by differences in methods and in the mode of expression of data. In the present study, the sickle cell lipid concentrations were compared to concentrations of a normal young cell population rather than to a randomly aged group of cells. Also in this study, cell lipid concentrations were related to the unit of surface area of the cell as well as to a unit volume of cells and to the individual cell. It is unlikely that differences in the age groups of the patients in the two studies, i.e., 16–42 years of age in the present study, 6–14 years of age in the earlier study, were of importance since erythrocyte lipid concentrations do not appear to vary with age.\(^15\)
Table 3.—Phosphatide Concentration in Normal and Sickle Cell Disease Erythrocytes

<table>
<thead>
<tr>
<th></th>
<th>Normal Young (%)</th>
<th>Sickle Cell (%)</th>
<th>p</th>
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<tbody>
<tr>
<td>Sphingomyelin</td>
<td>27.7 ± 3.3</td>
<td>23.7 ± 3.7</td>
<td>&gt;0.02</td>
</tr>
<tr>
<td>Lecithin</td>
<td>26.3 ± 3.3</td>
<td>25.2 ± 2.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>16.8 ± 3.2</td>
<td>14.6 ± 3.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>18.4 ± 2.9</td>
<td>17.3 ± 2.6</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The remaining 10–12 per cent was composed of approximately equal amounts of inositol phosphatide, lysolecithin and a phosphatidic acid-like compound.

*Mean values ± one standard deviation.

Table 4.—Plasma Lipid Concentration in Normal and Sickle Cell Disease Patients

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Sickle Disease</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipid†</td>
<td>705 ± 120</td>
<td>518 ± 114</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>220 ± 31</td>
<td>136 ± 29</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>227 ± 40</td>
<td>139 ± 30</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Mean values ± one standard deviation.
†mg./100 ml.

Cell measurements indicate that the nonsickled erythrocyte from patients with sickle cell anemia is flatter and has a larger surface area than the normal cell of similar age. The presence of "thinner and more discoid shapes" in patients with this disorder was observed by Erickson and coworkers. In their study, cell dimensions were derived from the measured cell diameter, and the results were compared with a normal randomly aged population of erythrocytes. The results of the present study confirm those obtained by Erickson and coworkers despite the difference in measurement technics and despite the differently aged normal red cell populations used for comparison.

Significantly decreased concentrations of all plasma lipids occur in the sickle disease patients. The decreased total lipid and phospholipid concentrations have not previously been observed. The mechanism and importance of hypocholesterolemia of patients with sickle cell disease is unclear and is not unique in sickle cell anemia since it has been described in other types of hemolytic anemia. Similarly, the decreased serum phospholipid concentrations may represent a nonspecific change found in various anemias, although to our knowledge these measurements have not been described in other anemias. The specific relationship between hypolipemia and anemia has not been determined.

SUMMARY

Measurement of various lipid moieties of the nonsickled erythrocytes of patients with sickle cell disease demonstrate an increased concentration of all lipid fractions when compared to a normal similarly aged population of erythrocytes. Highly significant increases in sickle disease cells occurred only in the total lipid fraction. The nonsickled erythrocyte of patients with sickle cell disease appears flatter and has a larger surface area than similarly...
aged normal cells. Significant decreases in plasma total lipid, phospholipid and cholesterol were present.

**SUMMARIO IN INTERLINGUA**

Le mesuration de varie lipidos in non-falciformisate erythrocytos de pacientes con morbo a cellulas falciforme revela un augmentate concentration de omne le fractiones lipidic in comparation con un population normal de erythrocytos de un simile ancianitate. Significativissime augmentos in cellulas de morbo falciforme occurreva solmente in le fraction de lipido total. Le non-falciformisate erythrocyto de patientes con morbo de cellulas falciforme ha un apparentia plus plan e un plus grande area superficial que cellulas normal de un simile ancianitate. Significative declinos esseva presente in le plasma in lipido total, phospholipido, e cholesterol.

**ACKNOWLEDGMENT**

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**REFERENCES**

RBC AND PLASMA LIPIDS IN SICKLE CELL ANEMIA


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