Lipid Synthesis in Human Leukocytes, Platelets, and Erythrocytes*†

P. A. Marks, Alfred Gellhorn, and Chev Kidson‡

From the Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, 32 New York

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The studies on lipid synthesis were performed on the blood of 16 adults in good health and 2 patients with chronic hemolytic anemias of undetermined etiology. The subjects in good health had normal values for red blood cell, reticulocyte, white blood cell, and platelet counts, and for hemoglobin concentration. The patients with the chronic hemolytic anemia had red cell populations of which 74% (in Subject A.C.) and 83% (in Subject A.S.) were reticulocytes.

**Incubation Procedure**—Venous blood was drawn into siliconized syringes which contained, as an anticoagulant, 1 ml of 5% Versene (ethylenediaminetetraacetate) in 0.9% sodium chloride for each 10 ml of whole blood. Warburg-type flasks were used as incubation vessels. To each flask was added whole blood, 0.1 ml of 0.1 M glucose per ml of whole blood, and acetate-l-C14, together with nonisotopically labeled acetate and phospholipids, and transfer these lipids into plasma lipids. On the other hand, human erythrocyte lipid synthesis is very limited and probably occurs only in the youngest circulating cells.

**EXPERIMENTAL PROCEDURE**

The studies on lipid synthesis were performed on the blood of 16 adults in good health and 2 patients with chronic hemolytic anemias of undetermined etiology. The subjects in good health had normal values for red blood cell, reticulocyte, white blood cell, and platelet counts, and for hemoglobin concentration. The patients with the chronic hemolytic anemia had red cell populations of which 74% (in Subject A.C.) and 83% (in Subject A.S.) were reticulocytes.

It has been reported that acetate-C14 incubated with human whole blood in vitro is incorporated into lipids (2, 3). Whole blood is a mixture of varying cellular elements with known differences in their morphological and biochemical characteristics. Thus, leukocytes are nucleated cells capable of incorporating precursors into proteins, deoxyribonucleic acid, and ribonucleic acid (cf. 4–6). Platelets appear to be fragments of the cytoplasm of megakaryocytes with biochemical and morphological evidence that they may contain mitochondria and microsomes (7). Mature, nonnucleated erythrocytes have no demonstrable mitochondria, nucleic acids, protein synthesis, or tricarboxylic acid cycle activity (8). These differences in biochemical potential suggest that the different types of blood cells might also vary in their capacity to synthesize lipids.

The present study demonstrates that normal human leukocytes and platelets incorporate acetate-C14 into triglycerides and phospholipids, and transfer these lipids into plasma lipids. On the other hand, human erythrocyte lipid synthesis is very limited and probably occurs only in the youngest circulating cells.
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Lipid Synthesis in Leukocytes and Erythrocytes—The rate of acetate-1-C¹⁴ incorporation into mixed lipids of normal leukocytes, on a per cell basis, is at least 1000-fold the incorporation into lipids of erythrocytes (Table I). The erythrocyte samples prepared from whole blood of normal subjects were contaminated with as little as 1 leukocyte per 10,000 red cells. Nevertheless, the radioactivity incorporated into leukocyte lipids was so much greater than into the erythrocyte lipids, that it is likely that a significant portion of the isotope detected in lipids extracted from the red cell samples reflects the newly formed lipids of contaminating white cells. If the radioactivity in the lipids of the red cell samples is corrected for the isotope content of the lipids in the leukocytes contaminating the erythrocytes, an average of 71% of the apparently newly synthesized red cell lipids is attributable to leukocyte lipid synthesis (Table I).

The data summarized in Table I were studies performed with 0.004 M sodium acetate in the incubation medium. As indicated in Fig. 1, increasing the concentrations of acetate in the incubation medium to 0.02 M was associated with a greater amount of incorporation of acetate into leukocyte lipids. However, there was no detectable increase in erythrocyte lipid synthesis at the higher acetate concentrations.

Experiments were performed with whole blood which was defibrinated by gentle agitation with glass beads for 10 minutes to prevent the blood from clotting. In these studies, there was essentially no acetate-1-C¹⁴ incorporated into red cell lipids. This indicates that Versene routinely employed as the anticoagulant did not account for the lack of red cell lipid synthesis.

In view of the very limited rate of lipid synthesis in normal mature red cell populations, in subsequent studies of leukocyte and platelet lipid synthesis, isotope incorporation into lipids of erythrocytes was not determined.

Lipid Synthesis in Platelets—The rate of incorporation of

<table>
<thead>
<tr>
<th>Table I</th>
<th>Incorporation of acetate-1-C¹⁴ into mixed lipids of leukocytes and erythrocytes of normal subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Erythrocytes</td>
</tr>
<tr>
<td>µC X 10⁻⁴</td>
<td>µC X 10⁻⁴</td>
</tr>
<tr>
<td>Range of values</td>
<td>0-0.47</td>
</tr>
<tr>
<td>Mean</td>
<td>0.17</td>
</tr>
</tbody>
</table>

* To 20 ml of whole blood in a 125-mL Warburg-type flask were added 0.1 ml of 0.8 M sodium acetate, 2.1 µc of acetate-1-C¹⁴, and 0.2 ml of 1.0 M glucose. These additions were not adjusted for the concentration of glucose initially present in the whole blood. Incubation was carried out for 24 hours at 37°C. The concentration of sodium acetate in the incubation medium in these experiments was one-fifth that employed for studies summarized in subsequent tables. This lower concentration of unlabeled acetate accounts for the higher specific activities in the leukocyte lipids observed in these experiments compared to those summarized in Table II.

² Contamination refers to the percentage of the total radioactivity in the erythrocyte sample attributable to the leukocytes in that fraction. This percentage was calculated on the basis of the number of leukocytes in the red cell samples and the specific activity of the lipids extracted from the separated leukocytes.

³ The range and mean values of 9 studies.

H. A. Eder, personal communication.
The specific activity of the acetate in the incubation medium and the total radioactivity incorporated into the lipids of leukocytes. In this calculation, the assumption is made that the incorporation of acetate-1-C\(^{14}\) is proportional to the total acetate incorporated. The isotope incorporation into the lipids of normal leukocytes, on a per cell basis, averaged 80 times greater than that into the platelet lipids (Table II). In whole blood, the ratio of the platelet counts to white cell counts had a mean value of 30. Thus, per volume of whole blood, the radioactivity incorporated into leukocyte lipids was only about 2- to 3-fold greater than that in platelet lipids. It should be noted that the range of rates of lipid synthesis by platelets was greater than that in leukocytes.

In the platelet fractions, an average of only 5% of the radioactivity incorporated into the mixed lipids could be attributed to leukocytes contaminating this fraction. On the other hand, platelet contamination of the leukocyte fraction could account for, on the average, 18% of the radioactivity of the mixed lipids of the white cell samples (Table II).

**Lipid Exchange with Plasma**—No isotope incorporation into plasma lipids was detectable after incubation of cell-free plasma with acetate-1-C\(^{14}\). The isotope incorporated into the lipids during incubation of whole blood with acetate-1-C\(^{14}\) rapidly appeared in the plasma lipids. Thus, of the total radioactivity incorporated into mixed lipids by the cells, more than 50% was recovered in the plasma lipids. Nevertheless, the specific activities of the leukocyte and platelet mixed lipids, neutral lipids, and phospholipids exceeded that of the comparable plasma lipid fractions during a 4-hour period of incubation (Fig. 2). Comparable results were obtained when leukocytes or platelets, separated from other cellular elements, were incubated with plasma and acetate-1-C\(^{14}\).

At the completion of the 24-hour period of incubation, of the total counts incorporated into the mixed lipids of whole blood, without correction for contamination of one cell type by another, about 5% was found in the erythrocyte fraction, about 15% in the platelet fraction, about 25% in the leukocyte fraction, and about 55% in the plasma.

### Table II

| Lipid Type     | Platelets | Leukocytes | Contamination
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plts/WBC</td>
<td>WBC/Plts</td>
<td>Plts/WBC</td>
</tr>
<tr>
<td>Range of values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.20</td>
<td>95</td>
<td>2.38</td>
</tr>
<tr>
<td>% radioactivity</td>
<td>18</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

* Incubation procedure as in Footnote a of Table I, save that 0.25 ml of 1.6 m sodium acetate was added to 20 ml of whole blood.

* Under the column Plts/WBC, the figures for contamination refer to the maximal percentage of the total radioactivity in the leukocyte (WBC) sample which could be attributable to the platelets (Plts) in that fraction. This percentage was calculated on the basis of the number of platelets in the leukocyte sample and the specific activity of lipids extracted from the separated platelets, not taking into account the leukocyte contamination of the platelet fraction. Under the column WBC/Plts, the figures for contamination refer to the maximal percentage of the total radioactivity in the platelet sample which could be attributable to the leukocytes in that fraction. This percentage was calculated on the basis of the number of leukocytes in the platelet sample and the specific activity of lipids extracted from the separated leukocytes, not taking into account the platelet contamination of the leukocyte fraction.

* The range and mean values of 10 studies.

**Neutral Lipid and Phospholipid Synthesis**—In the initial studies, cholesterol esters were separated from the lipids of leukocytes and plasma and found to contain less than 5% of the total radioactivity incorporated into the mixed lipids. In subsequent experiments, the mixed lipids were separated into only two components, neutral lipids and phospholipids. The isotope content of the neutral lipids of leukocytes and, to a somewhat lesser extent, of platelets exceeded that of the phospholipids (Table III). A similar pattern of isotope incorporation in neutral lipids and phospholipids was found in the studies with leukocytes and platelets separated before incubation. In plasma lipids, an even greater proportion of the radioactivity, an average of 89%, was...
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present in the neutral lipid fraction (Table III). Similar findings were obtained for the distribution of isotope in the lipid fraction of plasma incubated with the separated leukocytes or platelets.

**Lipid Synthesis in Leukocytes and Platelets Separated before Incubation**—Both leukocytes and platelets, separated from whole blood by the method of dextran flotation and then resuspended in plasma and incubated with acetate-1-C\(^{14}\), incorporated isotope into lipids. The separation of leukocytes before incubation was, however, associated with an 80% decrease in the rate of lipid synthesis, compared to that of leukocytes separated from whole blood subsequent to incubation (Table IV). In contrast to leukocytes, platelets separated before or after incubation synthesized lipids at approximately the same rate (Table IV).

**Lipid Synthesis in Reticulocytes**—Although lipid synthesis is very limited in mature erythrocytes, if it occurs at all, previous observations suggested that young red cells do synthesize lipids (14). In the present study, a significant rate of incorporation of acetate-1-C\(^{14}\) into erythrocyte lipids has been observed in blood with red cell populations of which 74 and 83% were reticulocytes (Table V).

To evaluate lipid synthesis further in young circulating red cells, the populations of erythrocytes with high reticulocyte percentages were separated into fractions of relatively younger and older cells by centrifugation. This method (15) is based on the fact that young red cells are less dense than older erythrocytes. The level of isotope incorporation into the lipids of the younger cell fraction was, in the study in which the better separation was achieved (Subject L.C.), 10-fold greater than that in the older cells. Nevertheless, the rate of lipid synthesis even in this young red cell population containing 95% reticulocytes was less than 2% of that of the average leukocyte population (Table I).

**DISCUSSION**

The study indicates that lipid synthesis in human whole blood occurs in leukocytes and platelets. The rate of lipid synthesis, on a cell basis, is approximately 70-fold greater in leukocytes than in platelets. However, owing to the greater number of platelets in a volume of whole blood, 25 to 35% of the total lipid synthesis in whole blood may be attributable to platelets. It has been demonstrated that lipid synthesis proceeds in reticulocytes and, perhaps, young circulating erythrocytes, but not in mature red cells. It is noteworthy that as red cells age in vivo, they lose the capacity to synthesize lipids. This may be correlated with the loss of tricarboxylic acid cycle activity (20) and diminished capacity for reduced pyridine nucleotide generation (21,22). It has also been reported (23) that the lipid content of red cells decreases with aging. The diminution in lipid content may reflect the inability of erythrocytes to replace the lipid of the cell membrane.

Altman and Swisher (2), James et al. (3), and Rowe (24) ob-

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**Table III**

Incorporation of acetate-1-C\(^{14}\) into neutral lipids and phospholipids of leukocytes, platelets, and plasma

<table>
<thead>
<tr>
<th>Subject</th>
<th>Fraction</th>
<th>W.E.P.</th>
<th>Younger</th>
<th>Older</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.C.</td>
<td>Reticulocyte count (%)</td>
<td>74</td>
<td>95</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Mixed lipids (μC X 10(^{-4})/10(^{9}) RBC)</td>
<td>0.96</td>
<td>3.5</td>
<td>0.37</td>
</tr>
<tr>
<td>A.S.</td>
<td>Reticulocyte count (%)</td>
<td>83</td>
<td>94</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Mixed lipids (μC X 10(^{-4})/10(^{9}) RBC)</td>
<td>6.5</td>
<td>7.9</td>
<td>3.3</td>
</tr>
</tbody>
</table>

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**Table IV**

Leukocyte and platelet lipid synthesis: effect of separation of cells before incubation

<table>
<thead>
<tr>
<th>Subject</th>
<th>Fraction</th>
<th>W.E.P.</th>
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To permit estimation of the relative lipid-synthesizing capacities of normal human leukocytes, platelets, and erythrocytes, the following figures are useful. Bird et al. (16) found that 10\(^{9}\) leukocytes weigh 1.961 g dry weight and that dry weight of leukocytes is 18% of wet weight (17). Maupin (18) reports that 10\(^{9}\) platelets weigh 28 mg dry weight and that dry weight of platelets is 12.5% of wet weight. On the basis of the data of Williams et al. (19), 10\(^{9}\) red cells weigh 330 mg and the dry weight of red cells is 36% of wet weight.
tained evidence which they interpreted as indicating that non-
nucleated red cells actively synthesize lipids. These findings
are attributable to the fact that in these earlier studies, the
contribution to lipid synthesis by leukocytes and platelets con-
taminating the erythrocyte samples was not adequately eval-
uated. The present observation that normal leukocytes actively
are attributable to the fact that in these earlier studies, the ferrcd to plasma lipids.

Evidence which they interpreted as indicating that non-
reticulocytes, but at a low rate relative to that of leukocytes or
platelets. Lipids formed by leukocytes and platelets are trans-
ferred to plasma lipids.

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Sinclair (Editor), Essential fatty acids, Butterworths Scien-

SUMMARY

Lipid synthesis in human blood cells has been studied by in-
cubation of whole blood with acetate-1-C14. The leukocytes,
platelets, erythrocytes, and plasma were replaced by the dextran
floination method. Mixed lipids were extracted from each of
these fractions and separated into neutral lipids and phospho-
lipids, and assayed for radioactivity. Acetate-1-C14 was in-
corporated into lipids of human blood by leukocytes and platelets,
but only to a very limited extent, if at all, in mature erythrocytes.
On a per cell basis, leukocytes incorporated isotope into lipids at
a rate 70-fold greater than that of platelets. However, per unit
volume of blood, 25 to 30% of the total lipid synthesis in whole
blood is attributable to platelets. Lipid synthesis does occur in

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