The Oxidation of Long-Chain Fatty Acids by the Formed Elements of Human Blood

By ARNOLD ROSENZWEIG AND PETER WAYS

LIPID SYNTHESIS by platelets and white cells is well established. White cells can incorporate fatty acids into neutral lipid and phospholipid esters. Acetate is incorporated into neutral lipids and phospholipids of human blood by leukocytes and platelets, but only to an insignificant extent, if at all, by red blood cells. However, oxidation of fatty acids by the formed elements of human blood has not been well studied. Since human leukocytes and platelets have mitochondria, they are presumably capable of oxidizing fatty acid. Circulating erythrocytes, on the other hand, would, a priori, seem incapable of this. Because of a report apparently demonstrating oxidation of fatty acid by erythrocytes, it seemed appropriate to reexamine this question. The present results show that the oxidation of C¹⁴ palmitate previously attributed to red cells was most probably due to the presence of white cells and platelets. This oxidation of fatty acid by white cells and platelets was studied in further detail.

METHODS

Preparation of White Cell and Platelet-Free Erythrocyte Suspensions

Fresh whole blood was drawn aseptically, defibrinated by swirling with glass beads, and placed in 10 ml. plastic syringes. After sealing the syringe tips with Wintrobe tube caps, they were placed in centrifuge cups, delivery end down, and centrifuged at 1650 g for 30 minutes. Cells in the lower third of the packed column were then carefully ejected from the syringe and washed 3 times in phosphate-NaCl buffer. Twenty-five to 50 per cent suspensions of red cells in the same buffer were used for the incubations. White cells and platelets were enumerated in each red cell suspension using a Spencer bright line hemocytometer and phase microscopy. Zero to 1 white cell (1 cu. mm. suspension counted) and 100–500 platelets (1 cu. mm. suspension counted) were found.

To demonstrate that the separated red cells were representative of the total circulating red cell mass with respect to age, blood was collected from two normal donors 7 and 8 days after they had received an intravenous tracer dose of Fe⁵⁹ bound to transferrin. After separation of the red cells, their radioactivity was compared to that of comparable aliquots of unseparated whole defibrinated blood. The separated red cells contained 80

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and 90 per cent of the cpm/Gm. Hgb found in the whole blood—confirming that the separated cells were only slightly depleted of young red cells.

**Preparation of White Cell and Platelet Suspensions**

A suspension enriched with white cells and platelets was obtained from whole blood anticoagulated with EDTA (1 volume 5 per cent EDTA and 9 volumes blood) by (1) centrifuging the blood at 150 g for 15 minutes followed by aspiration of the supernatant plasma; (2) sedimentation with 3 per cent dextran (2 volumes of blood to one volume of dextran) at 4 C. for one-half hour followed by recovery of the plasma-dextran layer. These enriched cell suspensions (which were maintained at approximately 4 C. during subsequent separation procedures) were then centrifuged for 10 minutes at 225 g. After aspiration, the supernatant was centrifuged again at 275 g for 10 minutes. The cells sedimenting during the first of these centrifugations were designated the “white cell preparation.” Platelets were recovered from the final supernatant by packing at 1350 g. The platelet button was washed three times with isotonic phosphate-NaCl buffer, and then resuspended in the buffer. The white cell preparations were washed three times with the same buffer—repacking each time at 225 g for 10 minutes. After washing, the cells were resuspended in the buffer. Platelet suspensions contained no white cells and ranged in platelet concentration from 72,000 to 152,000/cu. mm. White cell preparations contained from 1500 to 25,000 WBC/cu. mm. and were contaminated with both red cells and platelets (500-15,500/cu. mm.). In two experiments, reticulocytes were counted in the white cell preparation and were less than 1 per 100 WBC. Polymorphonuclear leukocytes constituted 60 to 70 per cent of the total WBC in all WBC suspensions.

**Preparation of Fatty Acid, Albumin and Buffer Solutions**

C14-palmitic acid, obtained from Nuclear-Chicago (specific activity 1.4–3.7 mc./mM) was partially purified by dissolving it in isoctane-glacial acetic acid (1/1 vol/vol.), and then adding one-tenth volume of water to achieve a 2-phase separation. The acetic acid phase was discarded. The isoctane phase containing the long-chain fatty acids was refrigerated until used. In each experiment, an aliquot was evaporated to dryness under nitrogen. The fatty acid was redissolved in ethyl alcohol, neutralized with slightly more than an equimolar amount of potassium hydroxide, and again evaporated to dryness. Finally, hot sterile distilled water or buffer was added to make a solution of the salt.

Phosphate buffer pH 7.45, ionic strength 0.106 (0.061 M), which was 0.077 M in NaCl was used in all experiments. It is isotonic with respect to plasma.

A commercially prepared fraction V (Pentex Inc.) was used as the source of human serum albumin. Solutions of albumin in buffer were prepared directly from the commercially obtained powder. The fatty acid content of the powder was found to be 0.21 μM FA/μM albumin.

**Incubation Procedure**

Sterile glassware was used throughout and random cultures to exclude bacterial contamination were sterile. In all experiments duplicate experimental and control flasks were utilized, and all incubations were carried out with gentle shaking at 37 C.

**Red Cell Experiments**

Approximately 0.1 μM palmitate 1-C14 (in aqueous or buffer solution) was added to duplicate 25 ml. Erlenmeyer flasks. Two or 3 ml. of buffer or serum and finally 2-3 ml. of red cell suspension were then added. In some experiments the buffer was replaced by an albumin solution, prepared by adding 20 ml. of a solution containing 4 Gm. of fraction V/100 ml. of buffer to 1.87 μM of solvent-free palmitate 1-C14 in an Erlenmeyer flask. The resulting mixture of fatty acid and albumin was warmed to 40 C. for 5 minutes. The FA-albumin solution was decanted to a second flask prior to pipetting. Two cc. of
OXIDATION OF LONG-CHAIN FATTY ACIDS

this palmitate-1-C14-albumin solution were pipetted into the incubation flask followed by 3 cc. red cell suspension. When used, sufficient 50 per cent glucose solution was added to give a final concentration of 5.55 mM. Control flasks contained 2–3 ml. buffer instead of RBC suspension. Flasks containing cell suspension, buffer and/or albumin solution, but no C14 palmitate, were also included. All flasks were sealed with a rubber stopper pierced by a glass rod, bearing a glass cup on its inner end. They were incubated for 5–6 hours. At the end of this period 0.5 ml of hyamine hydrochloride was injected through the stopper into the glass cup. Then 0.5 ml of 1.0 N HCl was added to the incubation mixture. To insure release of all the CO2, the flask was again shaken at 37 C. for 1 hour. The C14O2 in hyamine was counted in a Packer Scintillation Spectrometer with an efficiency of 55 per cent and a background of 20 cts/min. The solvent phosphor solution contained 0.4 per cent 2, 5 diphenyloxazole and 0.1 per cent 2, 2' P phenylene bis-(5 phenyloxamole) in toluene.

White Cell and Platelet Experiments

In the first series of studies approximately 0.1 M palmitate-1-C14 (s.a. 1.4 mc./mM), 3 cc. buffer, and 2 cc. white cell preparation or platelet suspension were added in order to duplicate flasks. Glucose was added to a final concentration of 5.55 mM. In two experiments, palmitate-1-C14-albumin solution was prepared as in the red cell study. Three cc. of this solution were incubated with 2 cc. of the white cell and platelet suspensions with and without added glucose.

A final experiment was designed to determine what effect different concentrations of albumin might have on the amount of palmitate oxidized by WBC and platelets. One cc. of WBC suspension was added to flasks containing 100,000 cpm palmitate-1-C14 (s.a. 3.7 mc./mM) in 4 ml. of a mixed solution of buffer and albumin. The final concentration of albumin was 0.08 molar in one pair of duplicate flasks and 0.58 molar in a second pair. The control flasks contained no albumin. All WBC and platelet incubations were carried out in equipment identical to that used in the RBC experiments and were for 6 hours.

RESULTS

Oxidation of Palmitate-1-C13 by RBC Suspension

In five experiments red blood cells, free of white blood cells and platelets, were incubated with phosphate-NaCl buffer, palmitate-1-C14, and glucose (5.55 mM). The flasks containing RBC showed no increment in C14O2 production over the controls (table 1). In one experiment, serum containing 300 µEq. albumin-bound fatty acid/L was substituted for the buffer, labeled palmitate and glucose were added as before. Again no significant increment in C14O2 production was obtained (table 1). In two further experiments, the palmitate 1-C14 was bound to human serum albumin and the albumin-palmitate complex added to the flasks in place of buffer. The final concentration of albumin was 23 molar. Again the presence of RBC did not result in increased evolution of C14O2. In some of the flasks in the latter series of experiments, glucose was omitted from the incubation mixture without affecting the results. Results of a representative experiment are shown in table 2.

In all experiments the control flasks containing C14 palmitate (but no red cells) gave 10 to 30 cpm above background. This phenomenon has been seen in previous studies with palmitate-1-C14. In the present studies bacterial contamination was excluded. This radioactivity is presumed to represent non-
Table 1.—*Oxidation of Palmitate-1-C<sup>14</sup> by Red Blood Cells*

<table>
<thead>
<tr>
<th></th>
<th>CPM</th>
<th>No. RBC in Flasks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RBC with Palmitate-1-C&lt;sup&gt;14&lt;/sup&gt;</td>
</tr>
<tr>
<td>A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>31</td>
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<tr>
<td></td>
<td>40</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>41</td>
</tr>
<tr>
<td>B.</td>
<td>56</td>
<td>46</td>
</tr>
</tbody>
</table>

Each flask contained approximately 150,000 cpm palmitate-1-C<sup>14</sup> (S.A., 1.4 mc/mM). In (A) the incubation mixture consisted of RBC and buffer solution (see "Methods"). Each line is the average of duplicate flasks prepared from different red cell preparations on different days. In (B) buffer was replaced by serum containing 300 μEq./L. of free fatty acid. In the control flasks, buffer replaced RBC suspension in equivalent volume.

Table 2.—*Effect of Glucose on the Oxidation of Palmitate-1-C<sup>14</sup> by Red Blood Cells*

<table>
<thead>
<tr>
<th>Incubation Mixture</th>
<th>CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 ml. unlabeled albumin solution</td>
<td>31</td>
</tr>
<tr>
<td>3 ml. unwashed red cell suspension</td>
<td>21</td>
</tr>
<tr>
<td>3 ml. unwashed red cell suspension with glucose (5.55 mM)</td>
<td>29</td>
</tr>
<tr>
<td>3 ml. washed red cell suspension</td>
<td>25</td>
</tr>
</tbody>
</table>

All flasks contained 2 ml. of palmitate-1-C<sup>14</sup> albumin solution (150,000 cpm palmitate-1-C<sup>14</sup>, S.A. 1.4 mc./mM).

Table 3.—*Oxidation of Palmitate-1-C<sup>14</sup> by WBC and Platelets*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>WBC Preparation</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cts./Min./10&lt;sup&gt;6&lt;/sup&gt; WBC/Hr.</td>
<td>Cts./Min./10&lt;sup&gt;6&lt;/sup&gt; Platelets/Hr.</td>
</tr>
<tr>
<td>1</td>
<td>2712</td>
<td>1047</td>
</tr>
<tr>
<td>2</td>
<td>5815</td>
<td>950</td>
</tr>
<tr>
<td>3</td>
<td>9697</td>
<td>319</td>
</tr>
<tr>
<td>4</td>
<td>8984</td>
<td>1425</td>
</tr>
<tr>
<td>5</td>
<td>1656</td>
<td></td>
</tr>
</tbody>
</table>

The incubation mixture consisted of buffer, glucose, palmitate-1-C<sup>14</sup>, and WBC or platelets (see text for details). In any one experiment equal amounts (approximately 150,000 cpm) of palmitate-1-C<sup>14</sup> was added to all flasks. Results represent the difference between the CPM of C<sub>14</sub>O<sub>2</sub> liberated in experimental and control flasks.

Enzymatic evolution of C<sub>14</sub>O<sub>2</sub> from palmitate-1-C<sup>14</sup> or transfer of intact palmitic-1-C<sup>14</sup> as vapor to the hyamine.

Oxidation of Palmitate-1-C<sup>14</sup> by WBC and Platelets

In buffer or albumin solutions, WBC and platelets incubated under sterile conditions oxidized radioactive palmitate to C<sub>14</sub>O<sub>2</sub> actively. Since platelet and WBC suspensions were evaluated simultaneously in some experiments, it was possible to correct for platelet activity in the white cell preparations. The results of these experiments are shown in table 3. RBC activity, as shown above, could be disregarded. As shown, 10<sup>6</sup> WBC oxidized from 3-40 times as much palmitate per cell as 10<sup>6</sup> platelets. When suspensions of WBC and
Table 4.—Effect of Glucose on the Oxidation of Palmitate-1-C\(^{14}\) by WBC and Platelets\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Without Glucose (cts./min.)</th>
<th>With Glucose (5.55 mM) (cts./min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>800</td>
<td>270</td>
</tr>
<tr>
<td>Platelets</td>
<td>368</td>
<td>130</td>
</tr>
</tbody>
</table>

\(^a\)0.027 \mu M Palmitate-1-C\(^{14}\) bound to albumin was added to each flask. All incubations were for 6 hours.

Table 5.—Effect of Albumin on the Oxidation of Palmitate-1-C\(^{14}\) by WBC

<table>
<thead>
<tr>
<th>Incubation Mixture</th>
<th>Total CPM Evolved as C(^{14})O(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer + palmitate-1-C(^{14})</td>
<td>18.130</td>
</tr>
<tr>
<td>Buffer + palmitate-1-C(^{14}) + 0.08 molar albumin</td>
<td>5,962</td>
</tr>
<tr>
<td>Buffer + palmitate-1-C(^{14}) + 0.58 molar albumin</td>
<td>885</td>
</tr>
</tbody>
</table>

100,000 CPM palmitate-1-C\(^{14}\) and equal volumes of WBC suspension were added per flask. All incubations were for 6 hours.

platelets were incubated with palmitate-1-C\(^{14}\)-albumin solutions in the presence of glucose, significantly less palmitate was oxidized to CO\(_2\) (table 4).

When compared to a buffer medium, oxidation of C\(^{14}\) palmitate by WBC in albumin solutions was also less. Table 5 gives the results of one experiment in which WBC were incubated in duplicate flasks containing albumin in final concentrations of 0.08 and 0.58 molar. Flasks were also incubated without added albumin. It can be seen that with increasing concentrations of albumin there was a corresponding decrease in the C\(^{14}\)O\(_2\) liberated. This decrease was greater than would be expected if it were only secondary to dilution of the isotope by the unlabeled fatty acid originally bound to the albumin.

**DISCUSSION**

Under the experimental conditions employed in this investigation, palmitate-1-C\(^{14}\) was not oxidized by red blood cells whether solubilized in buffer or bound to albumin. These results were not altered by relative glucose deficiency. In three experiments, linoleic acid was substituted for palmitic acid. Again, significant oxidation by red cells did not occur. Thus, long-chain fatty acids do not appear to be oxidized by human red cells. These results are in contrast to two reports of palmitate oxidation by mature rat red cells.\(^3\)\(^7\) In those experiments, the number of contaminating white cells and platelets in the red cell suspension were not reported. Since we have found that as few as 30 white cells and 600 platelets/cu. mm. can result in a tenfold increase in evolved CO\(_2\) over the control flasks, the previously reported results may have been due to contamination by other formed elements.

Human blood leukocytes and platelets, in contrast to erythrocytes, both oxidize palmitate in either a buffer or albumin medium. This finding is in accord with studies demonstrating the oxidation of palmitate, stearate, oleate, and linoleate by white cells in animal peritoneal exudates.\(^1\)\(^2\) Under the conditions used in the present experiments, white cells appear to be more
active than platelets on a per cell basis—perhaps as a consequence of their larger size and the greater number and complexity of their mitochondria. In these studies, glucose in physiologic concentrations significantly inhibited the in vitro oxidation of palmitate by both platelets and white cells. A similar response was noted by incubating rat diaphragm with C
\textsuperscript{14} palmitate and glucose in the presence of insulin, somewhat analogously, free fatty acid oxidation by adipose tissue is elevated during starvation. In liver, however, the situation is less clear. In vitro addition of glucose to isolated liver systems is without effect on fatty acid oxidation, whereas in vivo decreased fatty acid oxidation by the liver occurs in response to hyperglycemia provided insulin is available.

White cells are known to incorporate free fatty acid into both neutral and phospholipid esters—suggesting that the effect of glucose seen in the present experiments may result from an increase in the formation of alpha glycerol phosphate—a precursor of both glycerides and phospholipids. Alternatively, however, there may be some direct inhibiting effect of glycolysis on the pathways of fatty acid oxidation.

As previously reported by Hrachovic et al., oxidation of palmitate by blood cells is decreased by the addition of albumin to the medium. This may result from competition for fatty acid ions between the binding sites on albumin and those on the white cell and platelet membranes. The association constant of red cells with long-chain fatty acids has been found to be of the same order of magnitude as the secondary binding sites on albumin, and the red cells compete poorly with albumin if low molar ratios of fatty acid to albumin exist (less than 2:1). If white cell and platelet membranes have an affinity for fatty acids comparable to that of red blood cells, the amount of fatty acid they can bind and subsequently utilize may also be appreciably influenced by albumin concentration in the medium. Such competitive binding has also been demonstrated for the perfused heart, skeletal muscle preparations, and lipoproteins. In each instance higher relative concentrations of free fatty acid and/or lower concentrations of albumin were found to favor cellular uptake. An alternative suggestion to direct competition for binding sites is that the albumin molecule itself exerts a direct inhibitory influence on the oxidation of palmitate. While the available evidence does not elucidate which of these mechanisms is operative, it is clear that white cells and platelets incorporate and oxidize long-chain fatty acids bound to albumin. They can, therefore, remove the fatty acid ions from the primary binding sites of albumin readily. This further confirms Goodman's statement that cells with a large number of binding sites characterized by high turnover rates and fatty acid association constants within one or two orders of magnitude of the albumin-fatty acid association constants, can compete with albumin for unesterified fatty acids. Under normal physiologic conditions in man essentially all unesterified fatty acid is bound to the albumin molecule on its primary and secondary binding sites. Thus, successful competition in vivo presumably occurs also.

The role of unesterified fatty acids in the metabolism of white cells and platelets is not clear. Although glycolysis is apparently the major source
of energy for both, the data presented here and by others suggest that unesterified fatty acids may under certain conditions provide a significant source of energy for the oxidative metabolism of platelets and white cells, as well as a source of their membrane and cytoplasmic lipids.

**Summary**

1. In vitro, human erythrocytes free of contaminating white cells and platelets do not oxidize fatty acid in the surrounding medium, either bound to albumin or supplied in buffer as the potassium salt. These results are not influenced by glucose deficiency.

2. White cells and platelets are both capable of oxidizing fatty acid from the surrounding medium—supplied either as the potassium salt in buffer or bound to albumin. Increasing the albumin/fatty acid molar ratio results in a decrease in the quantity of fatty acid oxidized. The amount of fatty acid oxidized is greater if glucose is not added to the medium.

**Summario in Interlingua**

1. Erythrocytos human in vitro, libere de contaminante leucocytos e plachettes, non effectua le oxydation de acidos grasse in le medio ambiente sin reguardo a si illos es ligate a albumina o presente in le tampon in le forma de sal de kalium. Iste resultatos non es influentiate per carentia de glucosa.

2. Leucocytos e plachettes es ambes capace de effectuar le oxydation de acido grasse in le medio ambiente, tanto ligate a albumina como etiam presente in le tampon in le forma de sal de kalium. Quando le proportion molar de albumina a acido grasse es augmentate, il seque un declino in le quantitate de acido grasse afficite per le oxydation. Le quantitate de acido grasse oxydate es plus grande quando glucosa non es addite al medio.

**Acknowledgment**

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**References**


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