Lipid Content and In Vitro Incorporation of Free Fatty Acids Into Lipids of Human Platelets: The Effect of Storage at 4°C

By Minoru Okuma, Manfred Steiner, and Mario Baldini

Lipid content and capacity to incorporate in vitro palmitate-1-$^{14}$C and linoleate-1-$^{14}$C into lipids was investigated in fresh and stored (4°C) human platelets. Cholesterol and phospholipids decreased 30% during storage for 6 days. Molar ratio of cholesterol to phospholipids and percentage distribution of individual phospholipids were similar in fresh and stored platelets. Palmitate bound to albumin was rapidly transferred by an energy-independent mechanism into a free fatty acid fraction of platelets. From there it was incorporated into glycerides and phospholipids, a process requiring energy. More palmitate than linoleate was incorporated into fatty acids and glycerides of fresh and stored platelets but linoleate exceeded palmitate in its incorporation into phosphatidylethanolamine. Storage of platelets produced the following changes: (1) Incorporation of palmitate into total lipids was significantly reduced but not that of linoleate. (2) Both palmitate and linoleate showed increased incorporation into phosphatidylethanolamine. (3) Incorporation of linoleate into free fatty acids and triglycerides and of palmitate into phosphatidylcholine was reduced.

The physiological importance of lipids as structural components of cells is well established. It has been investigated in red cells much more than in platelets. Specifically, cholesterol was shown to play a significant role in maintaining the structural stability of red cell membranes and in determining the shape of erythrocytes. Although phospholipids participate in some of these functions they also appear to have great significance for preserving the optimal activity of certain microsomal and mitochondrial enzymes. During red cell storage there is a symmetrical loss of cholesterol and phospholipids. Contrary to that of erythrocytes, the viability of platelets,
here defined as in vivo survival upon reinfusion, is rapidly lost on storage. Lipid peroxidation is markedly enhanced under such conditions. Fresh human platelets are capable of incorporating and metabolizing long-chain free fatty acids. Renewal of phospholipids by reacylation of lysocompounds and by de novo synthesis has been reported. In view of the peculiarly rapid loss of platelet viability and its outstanding importance for cell function, we have studied the cholesterol and phospholipid content of platelets during storage and determined their ability to incorporate free saturated and unsaturated fatty acids into their lipids.

**MATERIALS AND METHODS**

**Preparation of Platelet Concentrates and Method of Storage**

Blood was collected from healthy male volunteers into plastic bags (Blood pack unit JA-2C, Fenwal Laboratories, Morton Grove, Ill.) containing acid-citrate-dextrose anticoagulant (ACD, U.S.P. formula “A”). Platelet concentrates (PC) were prepared and stored at 4°C, as described previously. Red and white cell contamination was always less than 1 per 10,000 and 1 per 80,000 platelets, respectively. For incubation with FFA, aliquots of PC containing 2 × 10^9 platelets were centrifuged at 2250 g for 30 min. The platelet pellet, unless otherwise indicated, was resuspended in 2 ml of modified Gaintner’s buffer, pH 6.8 (MG buffer). When only the lipid content of platelets was to be measured, the platelets were washed once with MG buffer and were then resuspended in the same buffer (10^9 platelets/ml). Separation and washing of platelets was done at 4°C. Siliconized glassware was used throughout. Experiments with fresh platelets were always started within 5 hr from the time of blood collection.

**Lipid Preparations**

Palmitic acid-1-14C (specific activity: 55–57.1 mCi/mM) and linoleic acid-1-14C (SA: 53.0 mCi/mM) were mixed with the respective nonradioactive fatty acids to obtain the desired specific activities and were then bound to 5% bovine serum albumin (BSA) in 0.1 M Tris-HCl buffer, pH 7.4. In case this solution was not used promptly, it was divided into 0.2-ml aliquots, sealed in ampules under nitrogen and stored at −20°C. The crystalline BSA was assayed for contaminating palmitate and linoleate by quantitative gas-liquid chromatography. Lipids were extracted from BSA-bound palmitate and linoleate. After methylation, the methyl esters were purified by TLC and eluted from the silica gel. The purified methyl esters of each lipid sample were subjected to gas-liquid chromatography. Appropriate blank areas from the silica gel were eluted and carried through the same procedures as the methyl esters. Quantitative analyses of the chromatograms proved that the contamination of crystalline BSA with palmitate and linoleate was negligible compared to the amount of the respective fatty acid bound to BSA for experimental purposes. The purity of all nonradioactive lipids used was established by TLC.

**Incubation Procedures**

To 2-ml aliquots of platelet suspension (2 × 10^9 platelets in MG buffer) were added 0.2 ml of BSA-bound fatty acid-1-14C (usually 5 μCi; final SA: 10 mCi/mM). Incubations were carried out in a metabolic shaking water bath usually at 37°C with an atmosphere of 95% O₂ and 5% CO₂. No clumping of platelets was observed during or at the end of a 2-hr incubation. After incubation the platelets were separated by centrifugation at 4°C, washed five times with 3-ml aliquots of ice cold 0.5% BSA in MG buffer and resuspended in 2 ml of MG buffer. The platelets were recounted to correct for their loss during these procedures. To determine the effect of metabolic or SH inhibitors on fatty acid incorporation into platelet lipids, platelets were preincubated for 15 min at 37°C with the respective inhibitor—that is, N-ethylmaleimide (NEM), NaF, or KCN all dissolved in MG buffer. Then 0.2 ml albumin-bound palmitate-1-14C was added to the flasks and incubation con-
LIPIDS IN HUMAN PLATELETS

continued for one more hour. The reaction was stopped and platelets were washed as described above. When the release of lipids from the platelets was determined, the platelets were initially incubated with BSA-bound palmitate-1-14C, then washed as described above. After resuspending the platelets in 2 ml of MG buffer, 0.2 ml of 5% BSA in 0.1 M Tris-HCl buffer pH 7.4 was added. Platelets were reincubated for 1 hr under conditions described above, then resuspending in 2 ml of buffer.

Lipid Assays

Extraction of Lipids: Two ml of platelet suspension or of incubation medium was extracted with 48 ml of chloroform-methanol (2:1, v/v) at room temperature for 30 min. The crude lipid extract was separated after addition of 10 ml 0.154 M NaCl. The extracts from platelets incubated with fatty acids-1-14C or from the reincubation media were washed three times with chloroform-methanol-0.154 M NaCl. The extracts were concentrated at reduced temperature in vacuo and were then evaporated to dryness under a stream of nitrogen. The residues were dissolved in small volumes of extraction solvent, which were immediately used for determination of total radioactivity, assay of PL phosphorus and of CH, and for chromatographic separations.

Determination of Phospholipid Phosphorus and of Cholesterol: PL phosphorus was assayed according to Dodge and Phillips. Triplicate determinations agreed within ±3% of their mean. Total CH was determined by a modification of Searcy's method. A small aliquot of the lipid extract was evaporated to dryness under a stream of nitrogen, the residue was dissolved in 1.5 ml of FeSO4-acetic acid reagent and mixed with 0.5 ml concentrated HSO4. Optical absorbancy was measured after 10 min at 490 nm. Triplicate determinations agreed within ±4% of their mean.

Separation of Lipids: Lipids were separated by TLC on plates coated with silica gel N-HR essentially as described before. Phosphorus was assayed in the following spots on the TLC plate: (1) each clearly identifiable major PL class; (2) an area corresponding to LPC; (3) SF, which was shown to contain PA, CL, CER, and CEB as well as neutral lipids; and (4) the origin (it always showed negligible values of phosphorus). Between 94 and 101% of the PL phosphorus applied to the TLC plates was recovered after chromatographic separation.

Assay of Radioactivity

The radioactivity of the original lipid extract and that of lipids separated by TLC was measured as described previously. Overall recoveries of radioactivity after chromatographic separation were approximately 96%.

Materials

Palmitic acid-1-14C and linoleic acid-1-14C were obtained from Amersham Searle, Des Plaines, Ill. Nonradioactive fatty acids, SM, PCH, PI, PS, PE, monopalmitin, diolein, tripalmitin, arachidic acid methyl ester, cholesteryl palmitate, CEB, and PA were all obtained from Mann Research Laboratories, New York, N.Y. CER and CL were products of Applied Science Laboratories, State College, Pa., and LPC was purchased from Analab, North Haven, Conn. BHT was obtained from Sigma Chemical Co., St. Louis, Mo. Crystalline BSA (all from one lot) was obtained from Mann Research Laboratories, New York, N.Y. Silica gel N-HR was a product of Brinkmann Instruments, Westbury, N.Y.

RESULTS

Cholesterol and Phospholipid Content of Fresh and Stored Human Platelets

Fresh human platelets from five different donors contained 90.4 ± 3.2 μg CH and 11.1 ± 0.7 μg phosphorus per 10⁹ cells (mean ± SEM). Both decreased at an almost linear rate during storage reaching 70% of their initial content in platelets after 6 days. The reduction in CH and PL became significant after
Table 1.—Lipid Phosphorus in Fresh and Stored Human Platelets

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Fresh</th>
<th>Stored at 4°C</th>
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<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>3 days</td>
</tr>
<tr>
<td>Total</td>
<td>11.05 ± 0.71</td>
<td>10.48 ± 0.49</td>
</tr>
<tr>
<td>LPC</td>
<td>0.18 ± 0.04</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>SM</td>
<td>1.86 ± 0.19</td>
<td>1.76 ± 0.08</td>
</tr>
<tr>
<td>PCH</td>
<td>4.31 ± 0.25</td>
<td>4.18 ± 0.11</td>
</tr>
<tr>
<td>PI + PS</td>
<td>1.39 ± 0.20</td>
<td>1.27 ± 0.09</td>
</tr>
<tr>
<td>PE</td>
<td>2.87 ± 0.20</td>
<td>2.62 ± 0.11 †</td>
</tr>
<tr>
<td>SF</td>
<td>0.9 ± 0.03</td>
<td>0.09 ± 0.04</td>
</tr>
</tbody>
</table>

Percentage distribution*

<table>
<thead>
<tr>
<th>Lipid</th>
<th>1 day</th>
<th>3 days</th>
<th>6 days</th>
</tr>
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<tbody>
<tr>
<td>LPC</td>
<td>1.7 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>SM</td>
<td>17.3 ± 0.8</td>
<td>17.4 ± 0.3</td>
<td>17.9 ± 0.7</td>
</tr>
<tr>
<td>PCH</td>
<td>40.3 ± 0.5</td>
<td>41.2 ± 0.4</td>
<td>40.5 ± 0.4</td>
</tr>
<tr>
<td>PI + PS</td>
<td>12.9 ± 0.7</td>
<td>12.2 ± 0.3</td>
<td>11.8 ± 0.8</td>
</tr>
<tr>
<td>PE</td>
<td>27.0 ± 0.7</td>
<td>26.5 ± 0.5</td>
<td>26.8 ± 0.9</td>
</tr>
<tr>
<td>SF</td>
<td>0.8 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>1.2 ± 0.3</td>
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</table>

* Mean ± SEM of five separate determinations.
† p < 0.05.
‡ p < 0.02.
§ p < 0.01.
p is based on comparison with the corresponding value obtained in fresh platelets.

3 days of storage (p < 0.01 for CH and p < 0.02 for PL) (Table 1). The decrease in these lipids was symmetrical and thus their molar ratio did not change. Similarly unaffected was the distribution of the various PL classes in stored as compared to fresh platelets (Table 1). The individual PL did not decrease at a completely uniform rate, however; e.g., a significant reduction in PE was present after only 1 day of storage while total PL at that time were still in the normal range. The phosphorus values of LPC and SF (CL + PA) were so small and their variations so wide that no significant changes could be observed.

In Vitro Incorporation of Fatty Acids Into Lipids of Fresh and Stored Human Platelets

Time Course of Palmitate Incorporation Into Lipids of Fresh Platelets: The rate of incorporation of palmitate into total lipids was linear during the first 60 min but decreased slightly during the second hour of incubation (Fig. 1A). Of the individual lipid classes PCH showed the steepest increase and had the greatest total accumulation of palmitate. TG incorporated the fatty acid at a fairly steady rate, but the total amount accumulated after 2 hr of incubation was only one seventh of that incorporated into PCH. The uptake of palmitate into the FFA fraction was extremely rapid and attained its maximum almost instantaneously. The rates of palmitate incorporation into PI + PS and SM were linear with time and were not plotted in the figure. The distribution of radioactivity among the various lipid classes changed rather rapidly during the first 30 min of incubation (Fig. 1B). Although FFA showed the highest
Fig. 1.—Time course of incorporation of palmitate-1-14C into lipids of fresh human platelets. (A) Absolute incorporation expressed in nmoles/10^9 platelets into total lipids (inset) and various lipid classes. (B) Relative incorporation expressed as percentages of total radioactivity. PCH, squares with solid lines; PE, closed circles with solid lines; TG, squares with dashed lines; FFA, closed circles with dashed lines; DG, open circles with dashed lines. Each point represents the mean of two experiments.

activity initially, its fraction of the total decreased rapidly with time. Of the major PL only PCH increased sharply. Since the contribution of the other PL to the total radioactivity did not change significantly, only the results obtained with PE are shown in the figure.

Release of Palmitate From Platelets: The release of fatty acids from platelets initially incubated with albumin-bound palmitate is shown in Fig. 2. For comparison the values for nonreincubated platelets are also given. Approximately 90% of the radioactivity released into the medium was recovered in the FFA fraction and about 3% each was recovered in PCH, DG, and TG. Although the sum of the radioactivities in FFA recovered from platelets and from reincubation medium exceeded that of nonreincubated platelets, the sum of radioactivities in TG extracted from these sources fell significantly below the level of nonreincubated platelets.

Effect of Palmitate Concentration on Its Incorporation Into Lipids of Fresh Platelets: Incorporation of palmitate into total platelet lipids as well as into different lipid classes was measured at concentrations of the fatty acid varying from 0.09 to 0.45 mM (Fig. 3). The incorporation into total lipids continued to rise, although at a decreasing rate, throughout this concentration range. Incorporation into PCh, which was by far the highest of that into any of the other
Fig. 2.—Exchange of platelet lipids with incubation medium. Platelets which had incorporated radioactive palmitate were washed and reincubated with BSA-containing buffer without addition of palmitate. Lipids were extracted both from the platelets and from the reincubation medium. Lightly shaded area: per cent radioactivity in various lipids of reincubated platelets; mean values of two experiments are shown. White area: per cent radioactivity in lipids recovered from reincubation medium; mean values of two experiments are shown. Heavily shaded area: per cent radioactivity in various lipids of platelets that were not reincubated; mean values ± SEM of six experiments are given.

Fig. 3.—Effect of palmitate concentration in its incorporation into lipids of fresh human platelets. Incorporation into total lipids (inset) and into various lipid classes: PCH, squares with solid lines; PE, closed circles with solid lines; TG, squares with dashed lines; FFA, closed circles with dashed lines; DG, open circles with dashed lines. Each point represents the mean of two experiments.
lipid classes, leveled off at 0.23 mM palmitate. The other PL and DG, TG as well as FFA, increased their palmitate incorporation at a linear rate over the entire concentration range tested.

*Effect of Metabolic and Sulfhydryl Inhibitors on Palmitate Uptake by Fresh Platelets:* Preincubation of platelets with either NEM or NaF produced a marked reduction in the incorporation of palmitate into total lipids and into PL, TG and DG (Fig. 4). Incubation of platelets in the cold (4°C) had a similar effect. Cyanide, on the other hand, enhanced the incorporation of palmitate into lipids. None of these inhibitors affected the uptake of palmitate into FFA of platelets. Incorporation into DG was in general less depressed than that into PL and TG. NaF-preincubated platelets showed a highly significant increase above normal in the radioactivity of this lipid fraction.

*Incorporation of Palmitate and Linoleate Into Fresh and Stored Platelets:* Although incorporation of palmitate and linoleate into total lipids of fresh platelets did not differ significantly, each fatty acid showed a very distinct pattern of incorporation into the various lipid classes (Tables 2 and 3). Although palmitate showed a significantly higher incorporation into DG \( (p < 0.05) \), FFA \( (p < 0.05) \) and TG \( (p < 0.01) \) than linoleate, the latter's distribution in PCH \( (p < 0.01) \) and PE \( (p < 0.01) \) exceeded that of palmitate. Storage of platelets progressively decreased the incorporation of fatty acids

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**Fig. 4.—**Effect of metabolic inhibitors and sulfhydryl-reactive agent on palmitate uptake by lipids of fresh human platelets. \( 2 \times 10^9 \) platelets were preincubated for 15 min at 37°C with either buffer (control), 1 mM KCN, 10 mM NaF, or 1 mM NEM. Albumin-bound palmitate-1\(^{14}\)C was then added and incubation continued for one more hour. In other experiments, platelets were incubated with albumin-bound palmitate-1\(^{14}\)C at 4°C for 1 hr without preincubation. Incorporations into total lipids (white area), total PL (heavily shaded area), DG (stippled area), FFA (dark area), and TG (lightly shaded area) are expressed as percentages of control. Mean values of two experiments are shown.
Table 2.—In Vitro Incorporation of Palmitic Acid-1-\(^{14}\)C Into Fresh and Stored Human Platelets and Distribution of Radioactivity Among Major Lipid Classes

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Fresh</th>
<th>Stored at 4°C</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No. of experiments</td>
<td>1 day</td>
</tr>
<tr>
<td></td>
<td>(nmoles/10^9 platelets)</td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>6.2 ± 0.4</td>
<td>3.37 ± 0.46</td>
</tr>
<tr>
<td>PCH</td>
<td>63.2 ± 1.2</td>
<td>62.1 ± 3.0</td>
</tr>
<tr>
<td>PI + PS</td>
<td>1.4 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>PE</td>
<td>3.6 ± 0.3</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>DG</td>
<td>3.4 ± 0.2</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>FFA</td>
<td>9.5 ± 1.0</td>
<td>9.0 ± 1.1</td>
</tr>
<tr>
<td>TG</td>
<td>11.8 ± 1.0</td>
<td>10.5 ± 0.8</td>
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</tbody>
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* Mean ± SEM.
† p < 0.05.
‡ p < 0.02.
p is based on comparison with the corresponding value obtained in fresh platelets.

into total lipids, palmitate more than linoleate. The principal changes in the fractional incorporation of these two fatty acids during platelet storage were: (1) a progressive decline of palmitate incorporation into PCH; (2) a gradual but significant increase in the incorporation of both fatty acids into PE; and (3) a significant decrease in the accumulation of linoleate in FFA and TG. The incorporation of palmitate and linoleate into LPC, MG, CH, and CHE was always less than 1% of their total incorporation into lipids and did not change during storage. These results were omitted from Tables 2 and 3. The specific activities of linoleate in PCH and PE were significantly higher than those of palmitate both for fresh and stored platelets (p < 0.05 for PCH of fresh platelets; p < 0.01 for PC of stored platelets; p < 0.05 for

Table 3.—In Vitro Incorporation of Linoleic Acid-1-\(^{14}\)C Into Fresh and Stored Human Platelets and Distribution of Radioactivity Among Major Lipid Classes

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Fresh</th>
<th>Stored at 4°C</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No. of experiments</td>
<td>1 day</td>
</tr>
<tr>
<td></td>
<td>(nmoles/10^9 platelets)</td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>3.4 ± 0.6</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>PCH</td>
<td>72.6 ± 1.3</td>
<td>73.7 ± 3.1</td>
</tr>
<tr>
<td>PI + PS</td>
<td>1.5 ± 0.3</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>PE</td>
<td>5.3 ± 0.3</td>
<td>5.6 ± 0.3</td>
</tr>
<tr>
<td>DG</td>
<td>2.2 ± 0.3</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>FFA</td>
<td>4.8 ± 0.7</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>TG</td>
<td>5.6 ± 0.9</td>
<td>5.3 ± 1.1</td>
</tr>
</tbody>
</table>

* Mean ± SE.
† p < 0.05.
p is based on comparison with the corresponding value obtained in fresh platelets.
Fig. 5.—In vitro incorporation of linoleate- and palmitate-1-14C into major PL of fresh and stored human platelets. The results shown are the mean ± SEM of six experiments for fresh platelets and of four experiments for stored platelets.

PE of both fresh and stored platelets) (Fig. 5). Storage of platelets for only 1 day decreased the specific activity of PCH with respect to palmitate (p < 0.05). This reduction became more pronounced after 3 days of storage (p < 0.02), at which time it leveled off. The specific activity of linoleate in PCH on the other hand did not change during storage. Both fatty acids had increased specific activities in PI + PS and in PE when platelets were stored for 3 to 6 days.

**Discussion**

The content of lipid phosphorus and CH in normal fresh human platelets has been measured by a number of investigators. Lipids which make up 14.7 to 23.7% of the dry weight of platelets were shown to contain 19% CH. Recalculating these fractions on basis of platelet number results in CH values ranging from 78 to 126 μg per 10⁹ platelets. Total lipid phosphorus and CH content as measured by us agree well with the results of other authors as does the distribution of lipid phosphorus among the different PL classes.

A gradual reduction in membrane lipid content has been reported to occur in red cells during in vivo aging during storage of blood at 4°C and during in vitro incubation at 37°C. Erythrocyte viability decreased concomitantly with the loss in lipids from the red cell membranes, although the latter was
not considered critical for the subsequent survival of the stored cells. The pattern of lipid loss of red cells during storage of ACD blood at 4°C was similar to what we found in platelets; i.e., there was a parallel reduction in CH and total PL, and the loss in lipid phosphorus in stored cells involved all PL classes in proportion to their original cellular concentrations. The rate of decrease in lipids during storage was, however, quite different. While a 6-wk period of storage was necessary to reduce the red cell lipids (total lipid, lipid phosphorus, and CH) by 30%, a similar loss of lipids occurred in platelets after only 6 days of storage at 4°C. Viability of platelets is also lost much more rapidly during storage than that of erythrocytes. Although the physiological meaning of the precipitous decrease in lipids in stored platelets is not clear yet, such reduction could be expected to result in an inadequate lipid skeleton of the platelet membrane and thus constitute one of the factors that participate in loss of viability.

Fresh human platelets can incorporate albumin-bound FFA into their various lipid classes. Our results confirm this capacity of platelets. Extracellular FFA equilibrated rapidly with those in the platelet. From this pool they were then incorporated into other lipid classes. The extreme rapidity of the palmitate transfer and the absence of an inhibitory effect by lowering the temperature, by NaF and KCN or by sulfhydryl-reactive compounds on magnitude and rate of fatty acid uptake indicate an energy-independent transport of FFA into platelets, which may be a physical adsorption process. Since washing of platelets with an albumin solution could not remove the fatty acids it can be concluded that the fatty acids are tightly bound to sites as yet undetermined. The fact that a relatively large proportion of the lipids eluted from reincubated platelets were FFA is probably due to their loss from TG rather than from the original FFA fraction of platelets.

The incorporation of palmitate into TG and PL was dependent upon anaerobic glycolysis. Cyanide, an effective inhibitor of cytochrome oxidase, stimulates anaerobic glycolysis in platelets two- to threefold. The increased fatty acid incorporation into PL, DG, and TG noted with cyanide-preincubated platelets could be due to an enhanced production of glycerophosphate, thus providing a greater number of potential esterification sites for fatty acids.

A distinctive pattern of incorporation into platelet lipids has been shown for various long-chain fatty acids. The incorporation of both palmitate and linoleate into lipids decreased in stored platelets. Viability of platelets stored at 4°C is lost to a major extent within 24 hr. The only significant changes in lipids found in this time interval were a decrease in the incorporation of palmitate into PCH and a reduction in the absolute amount of PE. Whether these changes are causally related to the loss in viability or simply coincidental remains to be established. Standing of bovine blood for 8 hr before incubation with fatty acids reduced their uptake in white cells by 30%, but it had little effect on their incorporation into red cells. It has been shown that platelet PL can be renewed both by acylation of lysocompounds and by de novo synthesis. Operation of these different pathways provides several possible explanations for the changed FFA incorporation into various lipids of stored platelets. The specificity of the changes observed during storage makes it vir-
tually impossible to indict as a possible cause a decrease in the supply of ATP or a general reduction in necessary cofactors, such as coenzyme A. Changes in acyl transferase or fatty acid:CoA ligase activities could have been produced by different rates of degradation of the respective enzymes or by alterations in the concentration of the various substrates. Alterations in the membrane permeability during storage could be crucial for the access of the different fatty acids from the medium to the various ligases. It is of importance in this context that cell age was found to influence the incorporation characteristics of fatty acids into different lipids. A marked increase in the uptake of palmitate and linoleate from plasma into PE of human red cells or their ghosts was demonstrated in aged erythrocytes. At the same time there was a decline of fatty acid incorporation into PCH. These findings in red cells aged in vivo are strikingly similar to our observations in stored platelets, particularly with respect to the in vitro incorporation of palmitate. Age dependency of the activities of the relevant enzymes was postulated to account for these findings in red cells. Based on these considerations the in vitro incorporation of fatty acids into platelet lipids could be utilized as an index of cell aging and, probably, as a measurement of platelet functions that are lost by aging in vivo as well as in vitro.

REFERENCES


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