The subcellular distribution of platelet lipids labeled by acetate-1-^{14}C

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ABSTRACT The lipids of intact human platelets were labeled in vitro with acetate-1-^{14}C, and the distribution of radioactivity in individual fatty acids and in lipid classes was examined in platelet subcellular fractions separated by sucrose density gradient ultracentrifugation. The distribution of newly formed fatty acids among individual lipid classes was similar in all subcellular components, and no highly unusual or characteristic lipid metabolic pool was present in either the soluble, membrane, or granule fractions.

SUPPLEMENTARY KEY WORDS blood coagulation · fatty acid synthesis

We have previously reported (1) the pattern of radioactivity in individual fatty acids and in lipid classes when intact human platelets are incubated with acetate-1-^{14}C. In buffer, no labeled lipids were released from platelets into the medium. In plasma, 40% of the newly formed lipids was recovered in the plasma. Platelet fatty acids were formed both by de novo synthesis and by chain elongation. The fatty acids formed by de novo synthesis exchanged with plasma FFA. In the present experiments we have examined the distribution of lipids formed from acetate among the subcellular fractions of platelets separated by sucrose density gradient ultracentrifugation.

METHODS

Human platelets were prepared and incubated with acetate-1-^{14}C in media containing either phosphate buffer or plasma, as previously described (1). The platelets derived from 100 ml of blood were used for each experiment. After completion of the incubations, the platelets were separated from the medium, washed twice in phosphate buffer, pH 6.5, and finally were resuspended in 0.5 ml of 0.44 M sucrose (0.001 M EDTA). The resuspended platelets were homogenized and subjected to sucrose density gradient ultracentrifugation as described by Marcus, Zucker-Franklin, Safier, and Ullman (2), except that ultracentrifugation was conducted at 50,000 rpm in a Spinco SW-50 rotor. Fractions were collected and analyzed for lipids as previously described (1, 2). Electron microscopy of subcellular fragments was performed with a Phillips 200 electron microscope.

RESULTS

The distribution along the sucrose density gradient of radioactivity in lipids newly formed from acetate is shown in Fig. 1. Three major subcellular fractions were labeled: granules, membranes, and the soluble proteins. In homogenates derived from buffer incubations, the radioactivity in the granules was divided into two peaks, one across the major granule band and a second sharp peak at the top of the granules, where there was a discrete zone of densely packed, matted granules. In homogenates derived from platelets incubated in plasma, the total lipid radioactivity applied to the gradient was diminished because of exchange of labeled intracellular lipids with their unlabeled plasma counterparts. The distribution of radioactivity among the subcellular fractions, however, was similar to that in buffer experiments.

To exclude the possibility of exchange of lipids during the homogenization and centrifugation procedure, we prepared a labeled homogenate from a buffer incubation and separated it into two fractions: granules and a combined membrane plus soluble fraction. We then prepared two unlabeled homogenates and to one we added the labeled granules (2,500 dpm/total lipid ex-

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Abbreviations: FFA, free fatty acids.

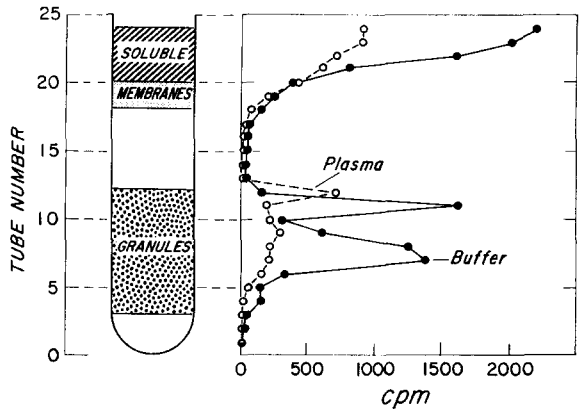

FIG. 1. Distribution of lipid radioactivity in platelet subcellular fractions separated by sucrose density ultracentrifugation. Washed human platelets were incubated with sodium acetate-1-^{14}C (final concentration 2.5 mM, specific activity 5 μCi/μmole) in phosphate buffer, pH 6.8, or in plasma dialyzed against phosphate buffer. Platelets were harvested, washed, homogenized, and subjected to sucrose density gradient ultracentrifugation (30–60% continuous gradient). 0.2-ml fractions were collected and analyzed for total lipid radioactivity.

TABLE 1 DISTRIBUTION OF RADIOACTIVITY AMONG SUBCELLULAR LIPID CLASSES

Cell Fraction	Lipid Class Distribution*		
	Lecithin	Ceramide	FFA
	(% *of total in fraction*)		
A. Buffer incubations (n = 5)			
Whole homogenate	13.1 ± 1.8†	47.8 ± 2.3	12.8 ± 1.5
Soluble	12.4 ± 0.9	56.9 ± 1.1	12.7 ± 2.6
Membranes	2.4 ± 1.2	58.5 ± 4.7	21.2 ± 2.6
Granules, upper	9.2 ± 2.4	43.0 ± 1.6	24.4 ± 3.5
Granules, lower	16.0 ± 2.5	37.2 ± 1.2	19.8 ± 3.4
B. Plasma incubations (n = 3)			
Plasma medium	0	0	88.8 ± 1.0
Whole homogenate	13.4 ± 2.9	58.9 ± 3.5	6.9 ± 0.4
Soluble	14.6 ± 1.5	62.8 ± 2.0	5.8 ± 0.8
Membranes	9.8 ± 1.5	65.7 ± 2.2	7.2 ± 1.7
Granules, upper	18.3 ± 0.7	44.7 ± 4.5	13.2 ± 1.3
Granules, lower	16.2 ± 2.4	46.8 ± 5.2	12.7 ± 1.0

*The sum of each cell fraction = 100%; minor components are not listed.
† Mean ± SEM.

tract) and to the other we added the labeled membrane and soluble fraction (7,500 dpm/total lipid extract). We then separated each by sucrose density ultracentrifugation and examined the distribution of radioactivity. 92% of the granule counts added to the unlabeled homogenate were recovered in the granule fraction after ultracentrifugation, and, similarly, of the radioactivity added as the combined soluble and membrane fraction, 90% was recovered in the appropriate fraction. Therefore, there was no redistribution of radioactivity in the whole homogenate nor during the centrifugation process.

Because of the appearance of a second peak of radioactivity at the top of the granule fraction, we reexamined the ultrastructure of the granule band. The lower main granule fraction contained small, densely staining granules, larger alpha granules, and mitochondria. The upper matted granule layer was enriched with the smaller dense granules, and mitochondria were absent from this zone.

In buffer experiments, of the total lipid radioactivity applied to the gradient 53.7% was recovered in the soluble fraction, 4.0% in the membranes, 7.7% in the upper granule band, and 34.6% in the lower granule band. The distribution of radioactivity in the major lipid classes in each subcellular fraction is shown in Table 1A. The distribution in each of the subcellular compartments was similar to that of the whole homogenate except that radioactivity in membrane lecithin was disproportionately decreased, and the percentage of radioactivity in the membrane and granule FFA was higher than in the whole homogenate.

In plasma experiments, half of the total lipid radioactivity appeared in the medium and was not available, therefore, for application to the gradient tube. Of the lipid radioactivity applied to the gradient, 60.1% appeared in the soluble fraction, 5.4% in membranes, 9.9% in the upper granule band, and 24.7% in the lower granule band. The distribution of radioactivity in the lipid classes of the subcellular fractions (Table 1B) was similar to that in buffer experiments. In accord with our prior findings, the great bulk of the radioactivity in the plasma medium was present in the FFA fraction. Other labeled fractions, each containing less than 5% of the total radioactivity, were: sphingomyelin, phosphatidylserine, phosphatidylethanolamine, free cholesterol, and mono-, di-, and triglycerides (1). The distribution of radioactivity in each of these lipid classes in each subcellular fraction was similar to that found in the whole homogenate or in the intact platelet.

The fatty acid distribution of radioactivity is shown in Table 2. In buffer (Table 2A), in the whole homogenate, and in each of the subcellular fractions, approximately 25% of the acetate was incorporated into fatty acids formed exclusively by the de novo pathway, that is, in fatty acids of chain lengths of 16 carbon atoms or less, and approximately 75% was incorporated into fatty acids formed both by the de novo pathway and by chain elongation (1). As in the buffer experiment, however, there were no striking differences among the individual subcellular fractions.

DISCUSSION

Platelets have a characteristic biological activity. During the process of coagulation platelet phospholipids pro-

TABLE 2 FATTY ACID DISTRIBUTION OF RADIOACTIVITY

Cell Fraction	Fatty Acid Distribution					
	<16:0*	16:0	18:0	20:0	22:0	>22:0
	(% *of total in fraction*)					
A Buffer incubation						
Whole homogenate	10.3	14.1	8.2	15.5	36.8	15.2
Soluble	10.5	18.6	11.3	23.8	26.3	9.6
Membranes	7.0	17.2	11.6	20.9	29.0	14.8
Granules, upper	13.0	20.1	13.0	23.0	20.3	10.3
Granules, lower	12.0	21.0	15.0	21.8	23.1	6.4
B. Plasma incubation						
Plasma medium	30.0	42.0	6.2	12.8	6.6	2.4
Whole homogenate	1.6	3.2	10.1	40.4	31.8	12.9
Soluble	1.4	3.2	9.4	38.8	32.2	14.2
Membranes	0	3.6	5.4	38.4	39.0	13.6
Granules, upper	1.5	3.9	9.9	44.3	26.1	14.4
Granules, lower	1.4	3.2	9.3	37.8	35.5	12.9

* Number of carbon atoms; number of double bonds.

mote the formation of a complex between activated factor X (Stuart) and factor V (proaccelerin). This complex cleaves prothrombin to form thrombin. In the absence of platelet phospholipids the rate of thrombin formation is greatly retarded. Marcus and his associates (2–4) investigated the procoagulant activity and the mass compositional differences in the lipids of the various subcellular fractions and could demonstrate no special lipid component that would correlate with platelet function.

It is well established that the de novo synthesis of fatty acids in mammalian cells occurs primarily in the soluble fraction of the cell, and that chain elongation is primarily a mitochondrial function. Our experiments demonstrate that in the platelet there is rapid equilibration of fatty acids formed by both mechanisms among all the major lipid classes throughout the different cell fractions. Our demonstration that no significant exchange occurs in the whole homogenate nor during ultracentrifugation, as well as the prior finding by Wirtz and Zilversmit (5) that phospholipid exchange among liver cell components in vitro is markedly suppressed at reduced temperatures, suggests that the exchange of labeled lipids occurs during incubation of intact platelets.

Although the total mass of lipid synthesized from acetate during the course of our 1-hr incubations was small in relation to the total mass of platelet lipid, and although the fatty acids formed were primarily saturated, the equilibration of newly formed lipids throughout the platelet and the absence of a highly characteristic pattern of labeling in any one subcellular fraction discount the probability of a localized, highly unusual lipid metabolic pool among those platelet lipids synthesized from acetate.

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REFERENCES

1. Deykin, D., and R. K. Desser. 1968. The incorporation of acetate and palmitate into lipids by human platelets. *J. Clin. Invest.* **47:** 1590–1602.
2. Marcus, A. J., D. Zucker-Franklin, L. B. Safier, and H. L. Ullman. 1966. Studies on human platelet granules and membranes. *J. Clin. Invest.* **45:** 14–28.
3. Marcus, A. J., H. L. Ullman, and L. B. Safier. 1969. Lipid composition of subcellular particles of human blood platelets. *J. Lipid Res.* **10:** 108–114.
4. Marcus, A. J. 1966. The role of lipids in blood coagulation. *In* Advances in Lipid Research. R. Paoletti and D. Kritchevsky, editors. Academic Press Inc., New York. **4:** 1–37.
5. Wirtz, K. W. A., and D. B. Zilversmit. 1968. Exchange of phospholipids between liver mitochondria and microsomes in vitro. *J. Biol. Chem.* **243:** 3596–3602.