

Lipid Metabolism in Human Platelets

I. EVIDENCE FOR A COMPLETE FATTY ACID SYNTHESIZING SYSTEM

PHILIP W. MAJERUS, M. B. SMITH, and G. H. CLAMON

From the Departments of Internal Medicine and Biochemistry, Washington University School of Medicine, St. Louis, Missouri 63110

ABSTRACT Extracts from human platelets contain the enzymes of de novo fatty acid biosynthesis. The pattern of incorporation of acetate-1-¹⁴C into fatty acids by intact platelets indicates that these enzymes function in platelets. The level of acetyl-coenzyme A (CoA) carboxylase activity in extracts of platelets from normal subjects is 0.036 ± 0.01 m μ mole of malonyl-CoA formed per min per mg of protein and that of fatty acid synthetase is 0.075 ± 0.016 m μ mole of malonyl-CoA utilized per min per mg of protein. Thus, platelets are the only formed elements of the blood capable of de novo fatty acid synthesis. The capacity of platelets to synthesize fatty acids is similar to human liver based on enzyme activity per milligram of soluble protein.

Acetyl-CoA carboxylase was purified 16-fold from platelet extracts, and this partially purified enzyme was compared to enzyme from rat liver. The two enzymes were similar with respect to requirements, substrate affinities, pH profile of activity, inhibition by malonyl-CoA, and aggregation in the presence of citrate. Thus, while fatty acid synthesis may serve a different function in platelets than in liver, the properties of acetyl-CoA carboxylase from these tissues are alike.

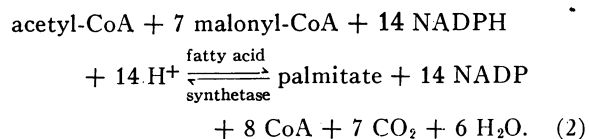
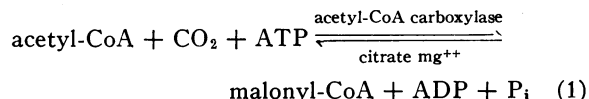
The levels of the enzymes of fatty acid synthesis were significantly higher in platelets from splenectomized subjects than in controls. Acetyl-CoA carboxylase levels were 0.086 ± 0.027 m μ mole of malonyl-CoA formed per min per mg of protein, and fatty acid synthetase levels were 0.151 ± 0.039 m μ mole of malonyl-CoA utilized per min per mg of protein. These changes in the enzymes of fatty acid synthesis occurred promptly after splenectomy with peak values being reached within 7-10 days.

Dr. Majerus is a teaching and research scholar of the American College of Physicians.

Received for publication 19 July 1968 and in revised form 13 September 1968.

INTRODUCTION

The mechanism of de novo fatty acid synthesis in mammalian systems is described by the following reactions:



Reaction 1 is catalyzed by acetyl-coenzyme A (CoA) carboxylase, a biotin containing enzyme, which catalyzes the conversion of acetyl-CoA and CO₂ to malonyl-CoA. This enzyme is rate limiting in mammalian tissues and is thought to be a regulatory enzyme (1). Thus, in the presence of citrate the enzyme is converted from an inactive-disaggregated form to an active-aggregated enzyme. All of the subsequent reactions of fatty acid synthesis in mammals are catalyzed by a "soluble" multienzyme complex which is designated fatty acid synthetase (1).

Previous studies have demonstrated that neither mature erythrocytes (2) nor leukocytes (3) are capable of de novo fatty acid synthesis because they lack acetyl-CoA carboxylase activity, although they retain fatty acid synthetase activity. Further studies suggested that a precursor hematopoietic cell may have the capacity to synthesize fatty acids and that this capability is lost as the cells mature. Thus, blast cells from leukemic patients were found to have low levels of acetyl-CoA carboxylase (3), and recently hemolysates of reticulocyte-rich blood from rabbits and humans have been found to contain acetyl-CoA carboxylase activity (4).

In contrast to erythrocytes and leukocytes, platelets contain the enzymes of fatty acid synthesis including

acetyl-CoA carboxylase. In the studies reported here, the levels of these enzymes have been measured in normal human subjects as well as patients with hematologic diseases. Acetyl-CoA carboxylase has been partially purified from platelets, and its properties have been studied. In addition, the effect of splenectomy on fatty acid synthesis in platelets has been studied.

METHODS

Blood samples were collected in plastic syringes or bags from healthy volunteers and from patients without regard to diet. The blood was anticoagulated with ACD¹; total and differential white blood cell counts, hemoglobin concentration, hematocrit value, and enumeration of erythrocytes, reticulocytes, and platelets were performed on all samples by standard techniques. All studies were carried out with either plastic or siliconized equipment, and samples were processed immediately after blood samples were drawn.

Fatty acid synthesis in platelets. 50-ml samples of blood were mixed with 5 ml of ACD and were then centrifuged at 90 *g* for 25 min at 4°C. All subsequent procedures were carried out at 4°C. The platelet-rich supernatant plasma was removed and was centrifuged at 3000 *g* for 10 min. The platelet-poor plasma supernatant solution was removed from the platelet pellet and mixed with the erythrocyte pellet obtained from the first centrifugation. This mixture was then centrifuged at 90 *g* for 25 min, and the resulting second platelet-rich plasma supernatant solution was added to the platelet pellet and was then centrifuged at 3000 *g* for 10 min. This second extraction resulted in an increase in the total platelet recovery from 50 to 85%. The final platelet pellet was then suspended in plasma and transferred into a 5 × 50 mm plastic tube and, after centrifugation at 3000 *g* for 10 min, the supernatant plasma was discarded and the platelet pellet was suspended in 1 ml of an isotonic solution containing 0.10 M potassium phosphate, pH 7.5, 0.001 M ethylenediaminetetraacetate (EDTA), 0.01 M 2-mercaptoethanol, and 0.25 M sucrose. The 2-mercaptoethanol was added to stabilize the enzymes of fatty acid synthesis, since many of these enzymes are sensitive to oxidation of key sulfhydryl groups (1). This suspension was then treated with ultrasound in a Biosonik II² sonifier with a microprobe at 10% intensity for 10 sec. Longer periods of sonication failed to release further protein. The sonicate was then centrifuged at 49,000 *g* for 1 hr, and the resulting supernatant solution was used to determine acetyl-CoA carboxylase and fatty acid synthetase activity. Similar results were also obtained with supernatant solutions after centrifugation of the sonicate at 105,000 *g* for 1 hr. The recovery of platelets by this procedure was 85 ± 10%, and the contamination of the platelet-rich plasma with leukocytes and erythrocytes was consistently less than 1/5000 platelets.

For preparative studies platelets were collected in ACD from freshly drawn blood and were pooled according to blood type and isolated by centrifugation as described above. The platelets from 10 U of blood were pooled and suspended in 15 ml of the same buffer used with small batches of platelets. While the batches of platelets used in the experiments described below were all processed immediately after collection, in other experiments it was shown that platelet pellets could be frozen in liquid nitrogen overnight without loss of enzyme activity upon subsequent study. These sus-

pensions were sonified for 10 sec at 70% intensity in a Biosonik II sonifier with the standard probe. The conditions of sonication used resulted in complete platelet rupture based upon the release of soluble protein. Sonication at higher intensity resulted in some loss of enzyme activity.

Fatty acid synthetase assay. Fatty acid synthetase activity was measured on Sephadex-treated extracts as described previously with malonyl-CoA-2-¹⁴C (1 μc/μmole) (3). This assay was linear with time for 1 hr and was proportional to enzyme concentration.

Purification of acetyl-CoA carboxylase. Acetyl-CoA carboxylase was purified as described previously (4) through the second ammonium sulfate step. In this manner enzyme was obtained which was 16.3-fold purified over the crude Sephadex-treated supernatant solution (Table I). The first ammonium sulfate step was performed at the time the platelets were collected in batches of 10 U, and the 15-30% ammonium sulfate fraction was stored in liquid nitrogen until five batches were accumulated which were then pooled for the subsequent purification shown in Table I. The yield obtained in the initial ammonium sulfate step averaged about 40%, so that the over-all yield of the 16.3-fold purified enzyme was about 11%. Acetyl-CoA carboxylase from platelets is a very unstable enzyme as has been found in other tissues. The enzyme was stable however when frozen in liquid nitrogen and stored at -140°F. This partially purified enzyme was used to study the properties of acetyl-CoA carboxylase from platelets.

Fatty acid synthesis in human liver. Specimens of histologically normal human liver were obtained from surgical biopsy specimens. The samples were immediately minced in cold buffer, and homogenates were prepared as described previously for rat liver (5) and assayed for fatty acid synthetase and acetyl-CoA carboxylase activity.

Gas-liquid chromatography was performed as described previously (3). Methyl esters were formed by heating the free fatty acids formed by platelet fatty acid synthetase in 2% methanolic sulfuric acid at 70°C for 2 hr. Standard fatty acids were added to the radioactive fatty acid product of platelet fatty acid synthesis, and the individual fatty acids were collected with a stream splitter accessory and counted in a liquid scintillation counter. Other substrates and materials were prepared as described previously (3, 5) or were obtained from commercial sources.

RESULTS

Acetyl-CoA carboxylase. Acetyl-CoA carboxylase activity was demonstrated in extracts from human platelets as shown in Table II. The requirements for this enzyme are the same as those for acetyl-CoA carboxylase from other tissues. Thus, the enzyme is dependent on acetyl CoA, adenosine triphosphate (ATP), citrate, Mg⁺⁺, and is inhibited by avidin which is a potent inhibitor of biotin-containing enzymes. About 90% of the ¹⁴CO₂ incorporated, with Sephadex-treated platelet extracts, was shown to be in malonyl-CoA, as described previously (3).

Properties of acetyl-CoA carboxylase. Acetyl-CoA carboxylase has been shown previously to be a regulatory enzyme (1). These previous studies used enzyme derived from either liver or adipose tissue. While the presumed major function of fatty acid synthesis in these

¹ ACD, acid citrate dextrose, USP formula A.

² Bronwill Scientific, Rochester, N. Y.

TABLE I
Acetyl CoA-Carboxylase Purification from Platelets

Step	Total activity	Specific activity	Fold purified
	U*	U/mg of protein	
Crude supernatant	—	0.1†	—
First ammonium sulfate	88.0	0.307	3.1
Calcium phosphate gel	21	0.37	3.7
Second ammonium sulfate	23.6	1.63	16.3

CoA, coenzyme A.

* 1 U is 1 μ mole of ^{14}C fixed into malonyl-CoA per min at 37°C.

† Average specific activity of five separate batches of platelets which were pooled for the subsequent purification.

tissues is to store energy in the form of fatty acids, fatty acid synthesis may serve some other function in platelets. Thus, acetyl-CoA carboxylase was partially purified in order to compare the properties of this enzyme to that derived from rat liver. Because of the difficulty in obtaining large quantities of platelets, only limited purification of acetyl-CoA carboxylase could be obtained (Table I). With this purified preparation, the affinities of the platelet enzyme for acetyl-CoA and ATP were determined by the method of Lineweaver and Burk (6). The Michaelis constant (K_m) for acetyl-CoA was 3×10^{-5} mole/liter while that for Mg-ATP was 2.5×10^{-4} mole/liter. In further experiments it was shown that malonyl-CoA was a competitive inhibitor of the reaction with $K_i = 2.6 \times 10^{-5}$ mole/liter. Each of these kinetic parameters agrees within 3-fold or less with the corresponding values obtained for enzyme derived from rat

TABLE II
Acetyl-CoA Carboxylase from Platelets

	Malonyl-CoA formation
	cpm
Complete system	1320
Minus MgCl_2	68
Minus citrate	36
Minus 2-mercaptoethanol	1120
Minus albumin	1030
Minus ATP	76
Minus acetyl-CoA	88
Plus 0.1 mg of avidin	210

CoA, coenzyme A; ATP, adenosine triphosphate. The complete system contained 20 μ moles of Tris-HCl, pH 7.5, 6 μ moles of MgCl_2 , 7 μ moles of potassium citrate, 0.4 μ mole of 2-mercaptoethanol, 0.3 mg of albumin, and 2.4 mg of enzyme in 0.26 ml which were preincubated at 37°C for 30 min. Then 0.7 μ mole of ATP, 0.05 μ mole of acetyl-CoA, and 6 μ moles of $\text{KH}^{14}\text{CO}_3$ ($2\mu\text{C}/\mu\text{mole}$) were added yielding a total volume of 0.33 ml. Incubations were for 5 min at 37°C, omissions as noted.

liver (5), a finding suggesting a similarity to enzyme from this source. In studies of acetyl-CoA carboxylase from rat adipose tissue, it has been shown that citrate activates the enzyme by converting it from an inactive protomeric form to an active aggregated form (7). The platelet enzyme was also dependent on citrate for activity with a K_m for citrate of 1×10^{-2} mole/liter. Sucrose gradient sedimentation of the platelet enzyme in the presence and absence of citrate is shown in Fig. 1. The platelet enzyme in the absence of citrate sedimented at about 14S, while in the presence of citrate the sedimentation constant was about 36S. This result is similar to that obtained for enzyme derived from liver or adipose tissue. The activity of the platelet acetyl-CoA carboxylase at varying pH closely paralleled that of purified acetyl-CoA carboxylase from rat liver as shown in Fig. 2 with the optimum pH at 7.5.

In further experiments, the effect of adenosine diphosphate (ADP) on acetyl-CoA carboxylase from platelets was tested, since this compound can initiate

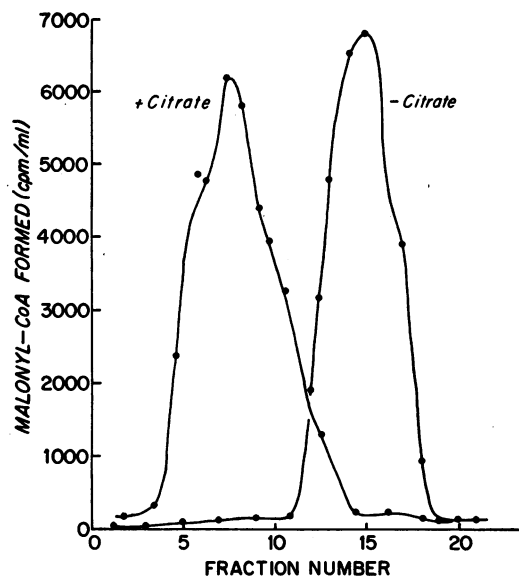


FIGURE 1 Sucrose density gradient sedimentation of acetyl-CoA carboxylase from platelets. Sucrose gradients 5–20% were prepared (8) in 0.05 M Tris-HCl, pH 7.5, containing 0.01 M 2-mercaptoethanol, 0.018 M MgCl_2 and where citrate-treated enzyme was run, 0.02 M potassium citrate. Samples containing 0.6 mg of enzyme, 2.0 μ moles of MgCl_2 , 10 μ moles of 2-mercaptoethanol, and 2 μ moles of potassium citrate were indicated in a total volume of 0.1 ml were layered on the gradients after preincubation of citrate-containing samples. Samples were sedimented at 50,000 rpm in an SW50 Spinco rotor for 200 min, and 22 fractions were collected from each tube. The fractions were numbered from the bottom of the tube. $S_{20,w}$ values were estimated from an internal standard of catalase. Aliquots of fractions were assayed for acetyl-CoA carboxylase activity as described in Methods.

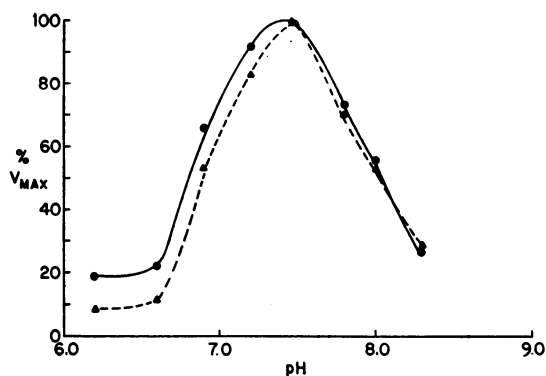


FIGURE 2 pH curve of acetyl-CoA carboxylase activity from rat liver, ●—●, and from platelets ▲—▲. Assays were carried out as described in Methods except that imidazole-HCl buffer was used for assays carried out at pH 7.2 and below. For comparison the activities are plotted as per cent of the maximal activity (V_{max}) for each enzyme.

platelet aggregation (9). ADP, which is a product of the acetyl-CoA carboxylase reaction, inhibits the enzyme from both platelets and liver in a similar manner. The kinetics of this product inhibition are complex in that only about 50% inhibition was obtainable even with very high levels of ADP. The enzymes from platelets and liver were parallel in this regard, showing maximum inhibition at about $10^{-4}M$ ADP in the presence of saturating amounts of other substrates. Thus, no clear differences between enzyme from rat liver or from platelets were uncovered.

Fatty acid synthetase. Fatty acid synthetase was also demonstrated in platelet extracts as shown in Table III. This reaction is completely dependent on both acetyl-CoA and malonyl-CoA but only partially dependent on a (NADPH) generating system. This partial activity in

TABLE III
Fatty Acid Synthetase Activity in Human Platelets

	Incorporation into fatty acids
	<i>cpm</i>
Complete system	5600
Minus acetyl-CoA	360
Minus malonyl-CoA	0
Minus NADPH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase	1850

CoA, coenzyme A; NADPH, nicotinamide adenine dinucleotide, reduced form. Reactions were carried out as described in Methods with the omissions noted. Malonyl-CoA- $2-^{14}C$ ($1 \mu C/\mu mole$) was used as the source of radioactivity except when malonyl-CoA was omitted from the reaction mixture. In this case, acetyl-CoA- $2-^{14}C$ ($5 \mu C/\mu mole$) was substituted for unlabeled acetyl-CoA.

the absence of an added source of NADPH is probably due to residual NADPH formation in the crude extracts used. The ratio of acetate to malonate incorporated in parallel incubation mixtures in which labeled acetyl-CoA and unlabeled malonyl-CoA were used in one mixture and labeled malonyl-CoA and unlabeled acetyl-CoA in the other was about 1/7-8 which is consistent with the stoichiometry of de novo fatty acid synthesis (reaction 2). The product of the platelet fatty acid synthetase was determined directly by carrying out gas-liquid chromatography of methyl esters of fatty acids formed in a large scale incubation of platelet supernatant with $2-^{14}C$ malonyl-CoA as described in Methods. In these experiments 85% of the counts incorporated into fatty acids were identified as methyl palmitate indicating that palmitate is the product of the platelet enzyme as it is for the fatty acid synthetase from other mammalian tissues. The fatty acid synthesis pathway was shown to be operative in intact platelets by the experiment recorded in Table IV. In this experiment, intact platelets were shown to incorporate acetate- $1-^{14}C$ into platelet fatty acids. Isolation of the fatty acids from these incubations followed by Schmidt degradation demonstrated that the de novo pathway was operative. This technique, which removes the carboxyl carbon of fatty acids (C-1) as CO_2 , may be used to distinguish between de novo fatty acid synthesis and chain elongation of preformed fatty acids. In de novo synthesis starting with acetate- $1-^{14}C$, alternate carbon atoms will be labeled throughout the chain starting with C-1. Therefore, palmitate formed by this pathway will contain 1/8th (12.5%) of the label in the C-1 position. The chain elongation pathway adds acetate units to preformed long-chain fatty acids. Usually only one or two acetyl groups are added so that most of the counts will be in the C-1 position. As shown in Table IV, only 20% of the counts incorporated into platelet fatty acids were in the C-1 position, a finding indicating that the de novo pathway was operative. Furthermore, Hennes, Awai, Hammarstrand, and Duboff (11) Deykin (12) have shown previously that the labeling pattern of fatty acids formed from acetate- $1-^{14}C$ is consistent with some de novo synthesis.

Fatty acid synthesis in platelets from normal subjects. The levels of the enzymes of fatty acid synthesis in extracts of platelets from normal subjects are shown in Table V. When 50 ml of blood samples was used, the mean activity of acetyl-CoA carboxylase was 0.036 ± 0.01 $m\mu mole/mg$ of protein per min in 12 samples from 9 subjects while that of fatty acid synthetase was 0.075 ± 0.016 $m\mu mole/mg$ of protein per min in 10 samples from 9 subjects.

The levels of the enzymes of fatty acid synthesis depended in part on the platelet sample size as shown in Table VI. Thus, when different sized aliquots were collected, sonicated, and assayed from a pool of 5000 ml of

TABLE IV
Incorporation of ^{14}C Acetic Acid-1- ^{14}C
into Platelet Fatty Acids

Subject	cpm incorporated	$^{14}\text{CO}_2$	cpm in C-1 position
		<i>cpm</i>	<i>%</i>
C. T.	18,300	3660	20
W. C.	17,000	3750	22

Platelet-rich plasma was obtained, and the platelets were sedimented to a final concentration of about $7 \times 10^6/\text{mm}^3$ in plasma, and 4 ml of this suspension was mixed with 1 ml of sodium acetate-1- ^{14}C to give a final concentration of 0.04 mole liter (0.5 $\mu\text{C}/\text{mole}$) in siliconized 25-ml Erlenmeyer flasks. These mixtures were incubated for 90 min at 37°C in a Dubnoff shaker. After this, total lipids were extracted and fatty acids were obtained as described previously (3). Aliquots were taken for Schmidt degradations which were performed by the methods of Brady, Bradley, and Trams (10), and the $^{14}\text{CO}_2$ obtained was counted in a liquid scintillation counter.

platelet-rich plasma, higher enzyme activities were obtained (1.7- to 2.1-fold) from large batch (equivalent to 10 U) extracts than from extracts from smaller aliquots. The reason for this difference was not determined, although it seems likely that the discrepancy is due to relatively greater inactivation of platelet enzymes when smaller volumes of platelets were subjected to ultrasonic disruption. Furthermore, there were slight differences in the levels of the enzymes when platelets were collected from 50 ml of blood (Table V) compared to the small aliquot of platelets from the large platelet-rich plasma pool (Table VI A), a finding suggesting that

TABLE V
Fatty Acid Synthesis in Platelets
from Normal Subjects

Subject	Platelet count/mm	Total recovery	Acetyl-CoA carboxylase	Fatty acid synthetase
		<i>%</i>	<i>U/mg*</i>	<i>U/mg*</i>
P. M.	219,000	75	0.036	—
	143,000	90	0.036	0.070
H. W.	130,000	95	0.026	—
	176,000	85	0.048	0.089
E. K.	137,000	95	0.037	0.075
	210,000	76	0.041	0.076
M. S.	245,000	95	0.049	0.104
	237,000	80	0.042	0.096
W. G.	163,000	81	0.050	0.081
S. K.	179,000	95	0.036	0.061
P. J.	212,000	70	0.021	0.045
P. S.	193,000	86	0.020	0.057
Mean \pm SD		84 \pm 10	0.036 \pm 0.010	0.075 \pm 0.016

CoA, coenzyme A. Samples of 50 ml of blood were used, and the total recovery of platelets from these samples is indicated. Enzymes were measured as described in Methods.

* 1 U of enzyme is 1 μmole of malonyl-CoA formed/min per mg of protein for acetyl-CoA carboxylase and is 1 μmole of malonyl-CoA incorporated into fatty acid/min per mg of protein for fatty acid synthetase.

TABLE VI
Effect of Sample Size on Levels of the Enzymes
of Fatty Acid Synthesis in Platelets

Sample No.	Aliquot	Acetyl-CoA carboxylase	Fatty acid synthetase
		<i>U/mg of protein</i>	<i>U/mg of protein</i>
A. 1	50 ml	0.052	0.045
2	50 ml	0.057	0.055
3	50 ml	0.054	0.059
Average		0.054	0.053
B. 1	5000 ml	0.112	0.104
2	5000 ml	0.099	0.089
3	5000 ml	0.135	0.082
Average		0.115	0.089

CoA, coenzyme A. Platelet-rich plasma was obtained from 10 U of blood in each experiment, and aliquots equivalent to the number of platelets contained in 50 ml of blood were removed (A), and extracts were prepared separately from this aliquot and from the remaining pooled platelet-rich plasma (B). Enzyme activities were determined on the extracts as described in Methods. Enzyme units are defined in Table V.

the size of the sample processed to obtain platelet-rich plasma had some effect on enzyme recovery. It is also possible that some of this small difference was due to greater contamination of small samples with leukocytes and erythrocytes. These differences due to sample size, were not large however, and in other experiments it was shown that when blood samples of 30–100 ml were used there were no differences in enzyme activity, and that only when samples of 500 ml or greater were used were higher activities found. Furthermore, there was no correlation between enzyme activity and platelet counts when 50-ml samples in blood was used with counts ranging from 150,000/ mm^3 to 1,500,000/ mm^3 .

Incorporation of acetate into fatty acids has been reported previously with extracts of human liver (13) and adipose (14) tissue; however, no evaluation of the levels of the enzymes of de novo fatty acid synthesis have been reported with human tissues. The levels of acetyl-CoA carboxylase and fatty acid synthetase were measured in samples of human liver obtained during surgery as shown in Table VII. The two specimens of liver obtained were histologically normal. One was from a patient who underwent cholecystectomy for chronic cholecystitis and the other patient had idiopathic thrombocytopenic purpura and underwent splenectomy. The levels of the enzymes of fatty acid synthesis in platelets from the latter patient (E.M.) are shown in Figs. 3 and 4. It is of interest that acetyl-CoA carboxylase appears to be the rate-limiting enzyme for fatty acid synthesis in liver under these conditions, with levels of 0.18 and

TABLE VII
Fatty Acid Synthesis in Human Liver

Subject	Acetyl-CoA carboxylase	Fatty acid synthetase
	U/mg	U/mg
M. M.	0.48	1.51
E. M.	0.18	1.56

CoA, coenzyme A. Assays were carried out as described under Methods. Enzyme units are defined in Table V.

0.48 $\mu\text{mole/mg}$ of malonyl-CoA protein formed per min compared to levels of 1.51 and 1.56 μmoles of malonyl-CoA utilized per min per mg of protein for fatty acid synthetase. In platelets the levels of acetyl-CoA carboxylase and fatty acid synthetase were more nearly equal (Tables V, VI), a finding thus suggesting that acetyl-CoA carboxylase may not be rate-limiting in platelets in all circumstances. The fact that the average level of acetyl-CoA carboxylase in platelets (0.115 μmole of malonyl-CoA formed per min per mg of protein Table VI B) is only slightly less than that in liver suggests that platelets are capable of significant amounts of fatty acid synthesis when compared to liver which is a major tissue for fatty acid synthesis in rats and other animals.

Fatty acid synthesis in splenectomized patients. In the course of evaluating the enzymes of fatty acid synthesis from platelets, blood samples were obtained from a number of patients with various diseases, and the only

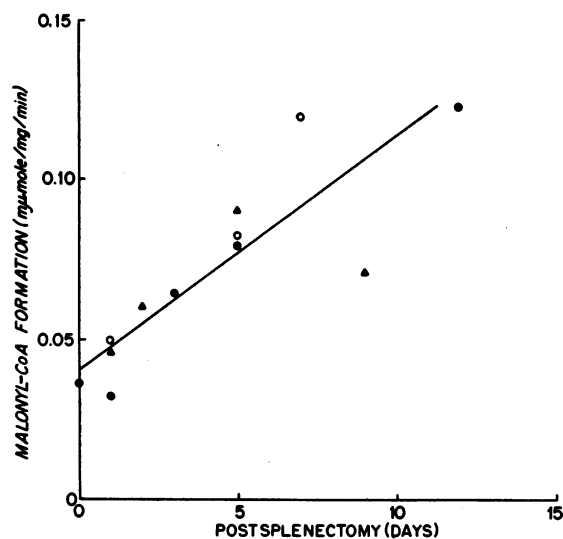


FIGURE 3 Acetyl-CoA carboxylase activity in platelets after splenectomy. Acetyl-CoA carboxylase activity was measured in Sephadex-treated platelet extracts as described in Methods at varying intervals after splenectomy in three patients; H. W. ●●●, E. M. ○○○, H. C. ▲▲▲. The results are plotted by the method of least mean squares.

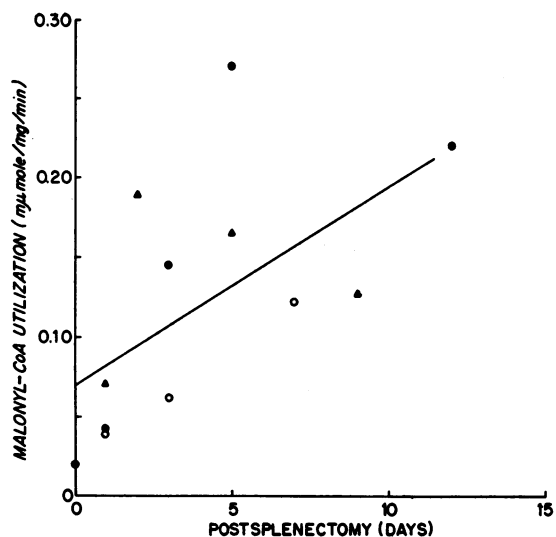


FIGURE 4 Fatty acid synthetase activity in platelets after splenectomy. Assays were carried out as described in Methods at varying intervals after splenectomy. See Fig. 3 for detailed legend.

patients noted to deviate significantly from normal subjects were patients who had undergone splenectomy. The levels of both acetyl-CoA carboxylase and fatty acid synthetase were higher in splenectomized patients than in normal subjects as shown in Table VIII. In six splenectomized subjects the mean acetyl-CoA carboxylase level was 0.086 U/mg which varied significantly from normal subjects ($P < 0.001$). Similarly, the mean fatty acid synthetase level in splenectomized subjects was 0.151 U/mg which is also significantly higher than control values ($P < 0.001$). These six patients were from 2 months to 14 yr postsplenectomy and had no common underlying disease. Patients with Idiopathic Thrombocytopenic Purpura (ITP), Idiopathic Autoimmune Hemolytic Anemia (IAHA), Lymphoma, and Hodgkin's Disease who had not had splenectomy were studied, and these patients had normal levels of platelet fatty acid synthesizing enzymes. The change in platelet enzymes was shown to occur immediately after splenectomy by measuring the levels of these enzymes at different times after splenectomy. Three patients were studied in this manner. The first had a splenectomy for IAHA and had a normal preoperative platelet count; thus, the enzyme levels were assessed both before and after splenectomy. The other two patients studied had splenectomy for ITP and, because of low preoperative platelet counts, the first samples on these patients were obtained 1 day postsplenectomy when the platelet counts were 313,000 and 190,000. The level of acetyl-CoA carboxylase after splenectomy, as shown in Fig. 3, rises progressively for about 7-10 days postsplenectomy when it levels off or falls slightly. This rise in enzyme level was significant ($r =$

TABLE VIII
Fatty Acid Synthesis in Platelets from Splenectomized Subjects

Subject	Platelets/mm ³	Time after splenectomy	Reason for splenectomy	Acetyl-CoA carboxylase	Fatty acid synthetase
				U/mg	U/mg
W. C.	146,000	2 months	Thrombocytopenia, lymphoma	0.081	0.176
R. G.	460,000	8 yr	ITP, IAHA	0.085	0.130
L. B.	215,000	14 yr	Auto accident	0.093	0.163
H. W.	1,000,000	2 months	IAHA	0.136	0.187
K. L.	505,000	18 months	Hypersplenism	0.051	0.080
M. B.	315,000	18 months	Hemolytic anemia, lymphoma	0.070	0.170
Mean ±SD				0.086 ±0.027	0.151 ±0.039

ITP, idiopathic thrombocytopenic purpura; IAHA, idiopathic autoimmune hemolytic anemia; CoA, coenzyme A. Samples of 50 ml of blood were used. Enzyme units are defined in Table V.

+ 0.86, $t = 5.38$, $P < 0.001$). Similarly, a prompt rise in fatty acid synthetase also followed splenectomy in these three patients as shown in Fig. 4. In this instance, there is more scatter to the points but the correlation is significant ($r = +0.58$, $t = 2.34$, $P < 0.05$). Glucose-6-phosphate dehydrogenase, another soluble enzyme of platelets, was also measured in these extracts, and as shown in Fig. 5, no significant change in levels of this enzyme occurred after splenectomy ($r = +0.397$, $t = 1.42$, $P > 0.2$). Similarly, lactic dehydrogenase and hexokinase activities were also measured, and neither of these enzymes was significantly increased after splenectomy. Thus, the change in the enzymes of fatty acid synthesis does not reflect a generalized increase in platelet enzymes.

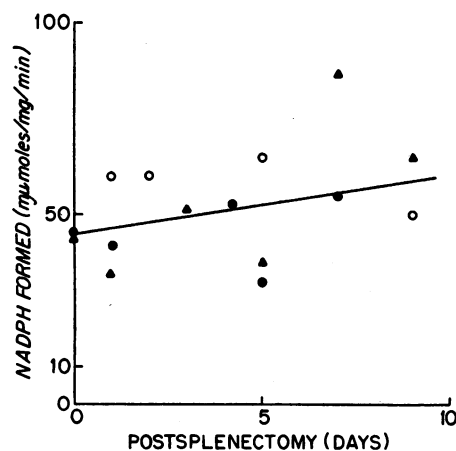


FIGURE 5 Glucose-6-phosphate dehydrogenase activity in platelets after splenectomy. Enzyme activity was measured by the formation of nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) at 340 $m\mu$ in a Gilford recording spectrophotometer. Reaction mixtures contained 120 μ moles of triethanolamine HCl, pH 7.5, 1.5 μ moles of NADP, 2 μ moles of glucose-6-phosphate, and enzyme in a final volume of 1 ml. See Fig. 3 for detailed legend.

The levels of the enzymes of fatty acid synthesis in platelets were measured pre- and postoperatively in three patients, who underwent cholecystectomy, in order to exclude the possibility that the rises in enzyme levels were nonspecific effects relating to the surgery performed. No significant changes in either acetyl-CoA carboxylase or fatty acid synthetase occurred in these patients in the 7 days postoperatively compared to preoperative levels.

DISCUSSION

These studies have demonstrated that human platelets have all of the enzymes necessary for de novo fatty acid synthesis. Furthermore, the levels of acetyl-CoA carboxylase in platelets are similar to those found in human liver, a finding suggesting that this biosynthetic pathway is relatively active in platelets. The incorporation of acetate into intact platelets shown previously (11, 12) and also in this study suggests that the pathway functions in the intact platelet. The reason why only platelets among the formed elements of the blood retain the capacity to synthesize fatty acids is obscure, and it is difficult to relate the presence of this pathway in platelets to the known role of lipids in coagulation. Furthermore, the need for complete synthesis of fatty acids in platelets is not clear since presumably platelets are able to take up preformed fatty acids from plasma (15).

While it appears unlikely that platelet fatty acid synthesis is important in coagulation, it is possible that the platelet fatty acid synthesizing system plays a role in coagulation rather than serving for caloric storage as the liver system. Our experiments suggest, however, that acetyl-CoA carboxylase from platelets is very similar to enzyme derived from rat liver. Thus, the requirements of the two enzymes are identical, the substrate affinities are similar, and both require citrate for activity, and are converted from a protomeric to polymeric form in the presence of citrate. The pH profile of activity is the

same for the two enzymes, and both are competitively inhibited by malonyl-CoA. One difference noted is the ratio of amounts of acetyl-CoA carboxylase compared to fatty acid synthetase. In both human liver (Table VII) and rat liver (15), acetyl-CoA carboxylase is the rate-limiting step for fatty acid synthesis under most conditions, while in platelets it would appear that levels of the two enzymes are more nearly equal so that changes in either might affect the over-all rate of fatty acid synthesis. Also, the product of the platelet fatty acid synthetase was shown to be palmitate, the same product formed in liver. Further support for the hypothesis that the platelet fatty acid biosynthetic enzymes are similar to those from liver are studies of Hennes et al. (11) in which the pattern of acetate incorporation into platelet fatty acids was compared in normal and diabetic subjects. From Schmidt degradation of the fatty acids formed, it was suggested that de novo fatty acid synthesis was decreased in the diabetic state, a finding previously shown in livers of diabetic rats (16).

ADP had no apparent differential effect on the platelet acetyl-CoA carboxylase compared to liver enzyme and also had no effect on platelet fatty acid synthetase, a finding thus suggesting that the ADP effect on platelets is not mediated through this pathway.

The effects of splenectomy on the enzymes of platelet fatty acid synthesis are striking and suggest that the spleen may have a regulatory influence on this biosynthetic pathway in platelets. There are several possible explanations for the rise in enzyme levels after splenectomy.

The spleen may function to remove or "depit" enzymes from platelets in a manner analogous to that proposed for inclusions within erythrocytes (17). The major difficulty with this hypothesis is that the fatty acid biosynthetic enzymes are "soluble," and thus it is difficult to understand how they would be physically removed from platelets. A second possibility is that newly formed platelets have a higher activity of fatty acid synthesis, and that normally the spleen in some way inhibits these enzymes. A third possibility is that the spleen has some regulatory effect on the amount of enzyme synthesized in the megakaryocyte which ultimately affects platelet enzyme levels. The time course of rising activity which reaches a peak after about 1 wk would be consistent with this hypothesis. Furthermore, in the ITP patients, where presumably all of the platelets circulating 1 day postsplenectomy were newly formed, the maximum levels of enzyme were not obtained for about 1 wk, a finding suggesting that the effect of the spleen might be at some step very early in platelet formation. Another possibility is that the normal pooling of a large fraction of the total platelets in the spleen (18) results in a metabolic stress to platelets leading to lower levels of the

enzymes of fatty acid synthesis in nonsplenectomized subjects. In any event, the effect on fatty acid synthesis appears to be relatively specific since several other enzymes are unchanged by splenectomy.

These studies have demonstrated that all of the enzymes required for fatty acid synthesis are present in platelets, and further studies are in progress to determine whether platelets also can synthesize phospholipids de novo. Preliminary experiments have shown that platelet extracts have an enzyme which catalyzes the formation of phosphatidic acid which is the first intermediate in phospholipid synthesis.³

ACKNOWLEDGMENTS

We wish to thank the American Red Cross, St. Louis Chapter, for providing platelets used in this study. We also acknowledge helpful discussions with Drs. Mark Halloran, Gordon Philpott, and with Elisabeth Kilburn.

This investigation was supported in part by grants from the U. S. Public Health Service, AM-10550 and HE 0022, the National Science Foundation, GB-5056, and the American Cancer Society, PRA-33.

REFERENCES

1. Majerus, P. W., and P. R. Vagelos. 1967. Fatty acid biosynthesis and the role of the acyl carrier protein I. *Advan. Lipid Res.* 5: 1.
2. Pittman, J. G., and D. B. Martin. 1966. Fatty acid biosynthesis in human erythrocytes: evidence in mature erythrocytes for an incomplete long-chain fatty acid synthesizing system. *J. Clin. Invest.* 45: 165.
3. Majerus, P. W., and R. Lastra. 1967. Fatty acid biosynthesis in human leukocytes. *J. Clin. Invest.* 46: 1596.
4. Weir, G. C., and D. B. Martin. 1968. Fatty biosynthesis in the erythrocyte: effect of red cell age on the integrity of the pathway. *Diabetes*. 17 (Suppl. 1): 305.
5. Majerus, P. W., R. Jacobs, M. B. Smith, and H. P. Morris. 1968. The regulation of fatty acid biosynthesis in rat hepatomas. *J. Biol. Chem.* 243: 3588.
6. Lineweaver, H., and D. J. Burk. 1934. The determination of enzyme dissociation constants. *J. Amer. Chem. Soc.* 56: 658.
7. Vagelos, P. R., A. W. Alberts, and D. B. Martin. 1963. Studies on the mechanism of activation of acetyl CoA carboxylase by citrate. *J. Biol. Chem.* 238: 533.
8. Martin, R. G., and B. N. Ames. 1961. A method for determining the sedimentation behavior of enzymes: application to protein mixtures. *J. Biol. Chem.* 236: 1372.
9. Marcus, A. J., and M. B. Zucker. 1965. *The Physiology of Blood Platelets*. Grune & Stratton, Inc., New York. 49.
10. Brady, R. O., R. M. Bradley, and E. G. Trams. 1960. Biosynthesis of fatty acids. I. Studies with enzymes derived from liver. *J. Biol. Chem.* 235: 3093.
11. Hennes, A. R., K. Awai, K. Hammarstrand, and G. Duboff. 1966. Carbon-14 in carboxyl carbon of fatty acids formed by platelets from normal and diabetic subjects. *Nature (London)*. 210: 839.
12. Deykin, D. 1968. The synthesis of lipids by human platelets. *Fed. Proc.* 27: 499. (Abstr.)

³ Majerus, P. W. Unpublished observations.

13. Bortz, W., S. Abraham, I. L. Chaikoff, and W. E. Dozier. 1962. Fatty acid synthesis from acetate by human liver homogenate fractions. *J. Clin. Invest.* **41**: 860.
14. Jacob, M. I. 1963. Fatty acid synthesis in cell free preparations of human adipose tissue. *Biochim. Biophys. Acta.* **70**: 231.
15. Deykin, D., and R. K. Desser. 1968. The synthesis of lipids by human platelets. *J. Clin. Invest.* **47**: 1590.
16. Numa, S., M. Matsuhashi, and F. Lynen. 1961. Zur störung der fettsauresynthese bei hunger und alloxan-diabetes. *Biochem. Z.* **344**: 203.
17. Crosby, W. H. 1959. Normal functions of the spleen relative to red blood cells. *Blood.* **14**: 399.
18. Aster, R. H. 1966. Pooling of platelets in the spleen: role in the pathogenesis of hypersplenic thrombocytopenia. *J. Clin. Invest.* **45**: 695.