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Research Article

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Both mature leukocytes and erythrocytes are unable to synthesize fatty acids because of a lack of acetyl CoA carboxylase even though both contain the other enzymes of fatty acid synthesis. It is possible that a precursor hematopoietic cell may have the capacity to synthesize fatty acids de novo. This hypothesis is supported by the finding of acetyl CoA carboxylase activity in extracts from human leukemic blast cells.

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Fatty Acid Biosynthesis in Human Leukocytes *

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Abstract. Extracts from human leukocytes have been examined for the enzymes of de novo fatty acid biosynthesis. These extracts do not catalyze the synthesis of long-chain fatty acids because they lack acetyl CoA carboxylase, the first enzyme unique to the fatty acid synthesis pathway.

Since these cells cannot form malonyl CoA, they are unable to synthesize long-chain fatty acids. This inability can be corrected by addition of either purified acetyl CoA carboxylase from rat liver or malonyl CoA to leukocyte extracts. The incorporation of acetate-1-^{14}C into fatty acids by intact leukocytes is shown to represent chain elongation of preformed fatty acids rather than de novo synthesis by the fact that 60–100% of the label incorporated resides in the carboxyl carbon of the fatty acids formed.

Both mature leukocytes and erythrocytes are unable to synthesize fatty acids because of a lack of acetyl CoA carboxylase even though both contain the other enzymes of fatty acid synthesis. It is possible that a precursor hematopoietic cell may have the capacity to synthesize fatty acids de novo. This hypothesis is supported by the finding of acetyl CoA carboxylase activity in extracts from human leukemic blast cells.

The leukocyte fatty acid synthetase activity from malonyl CoA of a number of normal volunteers and of patients with a variety of hematologic diseases is reported.

Introduction

The mechanism of fatty acid biosynthesis de novo has recently been elucidated through studies of yeast, mammals, and *E. coli* (for review see 1, 2). In the first reaction unique to this pathway, acetyl CoA is carboxylated to form malonyl CoA. This reaction is catalyzed by acetyl CoA carboxylase, a biotin enzyme, which is the rate-limiting enzyme in in vitro fatty acid biosynthesis measured in extracts from rat liver and adipose tissue. The subsequent reactions of fatty acid synthesis occur with the substrates bound to the sulfhydryl group of 4′-phosphopantethine, the prosthetic group of the acyl carrier protein which, in mammals, is a part of a "soluble" multienzyme complex which is designated fatty acid synthetase.

Examination of fatty acid synthesis in leukocytes was undertaken since these cells provide a ready source of material for studies of lipid metabolism in humans under various metabolic conditions. Previous studies have shown that mature erythrocytes are unable to synthesize fatty acids because of a lack of acetyl CoA carboxylase (3). Incubation of blood (intact cells) with acetate-^{14}C results in the incorporation of acetate-^{14}C into leukocyte fatty acids, but the enzymatic mechanism of this incorporation has not been elucidated (4–7). Therefore, extracts of leukocytes from normal volunteers and from patients with various hematologic diseases have been examined for the enzymes of fatty acid synthesis.

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Methods

Blood samples were collected from healthy volunteers and from patients, without regard to diet, in plastic syringes or bags. The blood was anticoagulated with either EDTA or ACD; total and differential white blood cell count, hemoglobin concentration, hematocrit value and enumeration of erythrocytes, reticulocytes, and platelets were performed on all samples by standard techniques. Leukocytes and platelets were separated from erythrocytes by the dextran flotation technique of Skoog and Beck (8). Plastic equipment or siliconized glassware was used for all procedures. The platelets were removed by sedimentation of the leukocytes at 100 *g* for 7 min at 4°C in a Sorvall refrigerated centrifuge. The pellet of leukocytes was then mixed with distilled water for 30 sec to lyse the few remaining erythrocytes. After restoring the suspension to isotonicity with saline, the leukocytes were recovered by sedimentation at 500 *g* for 7 min and the hemoglobin containing supernatant solution was discarded. Leukocytes at a concentration of 50,000–500,000 WBC/mm^3 were suspended in a solution containing 0.76% NaCl, 0.039% KCl, 0.15% $MgCl_2 \cdot 6\ H_2O$, 0.1% glucose, and 0.02 M Tris-HCl (pH 7.3). RBC, WBC, and platelet counts were performed on this cell suspension. These leukocyte preparations contained 0–2 RBC/10^3 WBC and 0–5 platelets/WBC. Subsequent studies with platelets and erythrocytes indicated that this level of contamination does not significantly affect the estimation of fatty acid synthesis in the leukocyte preparations. After counts were obtained, the leukocytes were again collected by sedimentation and resuspended in a solution containing 0.01 M imidazole-HCl (pH 7.2), 0.25 M sucrose, and 0.01 M 2-mercaptoethanol. The leukocytes were then ruptured by sonic oscillation in a Biosonik sonifier for 1 min at 50% intensity with a microprobe while being maintained in an ice bath. More than 99% of the cells were ruptured by this procedure. The sonicate was centrifuged at 49,000 *g* for 30 min and the supernatant solution was used for studies of fatty acid synthesis.

Fatty acid synthetase assay. Fatty acid synthetase was measured by a modification of the method of Martin, Horning, and Vagelos (9). Reaction mixtures contained 50 μmoles imidazole-HCl (pH 6.8), 10 μmoles 2-mercaptoethanol, 0.75 μmole NADP, 1.5 μmoles glucose 6-phosphate, 0.5 unit of glucose 6-phosphate dehydrogenase,[1] 0.07 μmole acetyl CoA, and 0.06 μmole malonyl CoA and extract in a final volume of 0.5 ml. Either malonyl CoA-2-^{14}C (1 μc/μmole) or acetyl CoA-1-^{14}C (5 μc/μmole) was labeled in these reactions. Reaction mixtures were incubated for 45 min at 30°C and the reactions were stopped by the addition of 1.0 ml of 3 N HCl in 50% ethanol. Next, 2.5 ml of hexane was added and, after extraction, the hexane layer was evaporated under a stream of nitrogen at 60°C. The fatty acids were again dissolved in hexane and counted in a liquid scintillation counter in Bray's solution (10). Preliminary experiments demonstrated that this assay is linear with enzyme concentration (0.02–0.6 mg protein/assay mixture) and with time for 1 hr.

Acetyl CoA carboxylase assay. The activity of this enzyme was assessed in two ways. The first is the direct measurement of malonyl CoA formation from acetyl CoA based upon the fixation of $^{14}CO_2$ into the free carboxyl group of malonyl CoA. Reaction mixtures contained 25 μmoles imidazole-HCl (pH 7.0), 1.0 μmole 2-mercaptoethanol, 4 μmoles potassium citrate and extract in a volume of 0.22 ml. After preincubation for 10 min, 0.05 μmole acetyl CoA, 3 μmoles ATP, 1 μmole $MnCl_2$, and 12 μmoles of $KH^{14}CO_3$[2] (2 μc/μmole) were added in a final volume of 0.32 ml. Reaction mixtures were incubated for 30 min at 30°C and reactions were stopped by the addition of 0.05 ml of 10% perchloric acid. After centrifugation, 0.1 ml of the supernatant solution was spotted on a planchet and, after drying, counted in a gas-flow counter. Alternatively, acetyl CoA carboxylase was measured by coupling the formation of malonyl CoA to fatty acid synthesis in the presence of an excess of fatty acid synthetase purified from rat adipose tissue.

Rat adipose tissue fatty acid synthetase and rat liver acetyl CoA carboxylase were purified by the methods of Martin, Horning, and Vagelos (9) and Matsuhashi, Matsuhashi, and Lynen (11), respectively. Total lipids were extracted from leukocytes by the method of Folch, Lees, and Sloane Stanley (12), and after hydrolysis in 2 N methanolic KOH for 2 hr at 70°C, aliquots were subjected to Schmidt degradation by the method of Brady, Bradley, and Trams (13). In these degradations, 1 μmole of palmitic acid was added as a carrier to each reaction mixture. Palmitic acid-1-^{14}C[1] was added to duplicate flasks as an internal standard and recoveries were from 80–95%. Acetyl CoA-1-^{14}C and nonradioactive acetyl CoA were prepared by the method of Simon and Shemin (14). Malonyl CoA and malonyl CoA-2-^{14}C were prepared by the method of Trams and Brady (15) and purified on DEAE cellulose columns. Sodium acetate-1-^{14}C, acetic anhydride-1-^{14}C, and malonic acid-2-^{14}C were purchased from New England Nuclear Corp.

Results

Fatty acid synthetase. Extracts of leukocytes were found to contain fatty acid synthetase activity which has the same requirements as that in other systems (Table I). The incomplete dependence of the reaction on acetyl CoA is probably due to the formation of small amounts of acetyl CoA from malonyl CoA through the action of malonyl CoA decarboxylase. The pH optimum of the leukocyte synthetase is 6.6 which is quite similar to that of rat liver or adipose tissue. The stoichiometry of the fatty acid synthetase is shown in Table II. These results indicate that palmitate is

[1] Sigma Chemical Co., St. Louis, Mo.

[2] New England Nuclear Corp., Boston, Mass.

TABLE I

Fatty acid synthetase

	Incorporation into fatty acids
	cpm
Complete system	2397
Minus acetyl CoA	363
Minus malonyl CoA	80
Minus NADP, glucose 6-phosphate, glucose 6-phosphate dehydrogenase	76

Reactions were carried out as described in Methods with the omissions noted. Malonyl CoA-2-^{14}C (1 μc/μmole) was used as the source of radioactivity except when malonyl CoA was omitted from the reaction mixture. In that case, acetyl CoA-1-^{14}C (5 μc/μmole) was substituted for unlabeled acetyl CoA.

probably the major product in the leukocyte fatty acid synthetase reaction since an average of 6.9 moles of malonate/mole of acetate are incorporated. Palmitate is also the major product of fatty acid synthesis in human liver (16).

Acetyl CoA carboxylase. Although fatty acid synthetase activity was easily demonstrable in all leukocyte extracts examined, acetyl CoA carboxylase activity could not be shown even when large amounts of leukocyte extract (up to 13.0 mg) were used. This assay could have detected levels of acetyl CoA carboxylase activity as low as 1000-fold less than that of the fatty acid synthetase which was present. As shown in Table III, Experiment 1, very little radioactivity was incorporated into fatty acids from acetyl CoA-1-^{14}C when acetyl CoA carboxylase cofactors were added in place of malonyl CoA. Attempts to demonstrate malonyl CoA formation directly from acetyl CoA and $^{14}CO_2$ also failed. Even the few counts incorporated in Experiment 1 do not represent de

TABLE II

Stoichiometry of leukocyte fatty acid synthetase

$$1\ \text{Acetyl CoA} + 7\ \text{malonyl CoA} \xrightarrow[\text{fatty acid synthetase}]{\text{NADPH}} \text{palmitic acid} + 7\ CO_2 + 8\ \text{CoASH} + 6\ H_2O$$

Experiment	Malonate incorporated	Acetate incorporated	Malonate/acetate
	mμmoles		*moles*
1	1.41	0.232	6.1
2	3.2	0.413	7.7
		Average	6.9

Incubations were performed as described in Methods. Parallel incubations were carried out in two tubes containing the same amounts of acetyl CoA and malonyl CoA with the ^{14}C label alternatively in the acetyl group in one tube and in the malonyl group in the other.

TABLE III

Fatty acid synthesis from acetyl CoA

Experiment	Acetate-1-^{14}C incorporated into fatty acids	cpm in C-1 position
	cpm	%
1. Complete	210	90
2. Plus 0.1 mg of rat liver acetyl CoA carboxylase	2,510	18
3. Plus 0.05 μmole malonyl CoA	10,600	—

Reaction mixtures were identical to those of the fatty acid synthetase assay with the addition of the following acetyl CoA carboxylase factors in place of malonyl CoA: 6 μmoles sodium citrate, 30 μmoles potassium bicarbonate, 1 μmole $MnCl_2$, and 5 μmoles ATP. Reaction mixtures containing buffer, enzyme, citrate, and 2-mercaptoethanol were preincubated for 10 min before the addition of the other components of the reaction mixture. In Experiment 1, a large excess of purified rat adipose tissue fatty acid synthetase was added to the reaction mixture. In each experiment, $\frac{1}{5}$ of the fatty acids formed were assayed for radioactivity and the remainder was used for Schmidt degradation.

novo synthesis as was shown by Schmidt degradation of the extracted fatty acids. 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 acetyl CoA-1-^{14}C alternate carbon atoms will be labeled throughout the chain starting with C-1. Therefore, palmitate formed in this manner will contain 1/8 (12.5%) of the label in the C-1 position. The chain elongation pathway utilizes acetyl CoA instead of malonyl CoA and adds C_2 units of preformed, long-chain fatty acids. Usually only one or two acetyl groups are added to the fatty acid so that starting with acetyl CoA-1-^{14}C most of the counts will be in the C-1 position. Thus, as shown in Table III, Experi-

TABLE IV

Incorporation of acetic acid-1-^{14}C into fatty acids

Experiment	Acetic acid-1-^{14}C incorporation	cpm in C-1 position
	cpm	%
1	465	97
2	9900	60

Isolated intact leukocytes (50,000–100,000/mm^3) were incubated in siliconized flasks in plasma containing 0.04 M sodium acetate (2 μc/μmole) for 90 min at 37°C. Aliquots were taken for Schmidt degradation. Total lipids were extracted and fatty acids obtained as described in Methods. The two experiments illustrated represent blood samples from different volunteers.

ment 1, 90% of the counts were in the C-1 position which suggests that ^{14}C-incorporation represents chain elongation rather than fatty acid synthesis.

Purified rat liver acetyl CoA carboxylase was added to the leukocyte extract to show that the inability to demonstrate acetyl CoA carboxylase was not due to an inhibitor of the enzyme contained in the extract (Table III, Experiment 2). There was no inhibition of the liver enzyme by the leukocyte extract and 18% of the label incorporated was in the C-1 position indicating de novo synthesis. The addition of purified acetyl CoA carboxylase or malonyl CoA (Table III, Experiment 3) to extracts of leukocytes corrects the inability of these extracts to synthesize fatty acids de novo. Another possible explanation for the failure to demonstrate acetyl CoA carboxylase is that the enzyme is inactivated by sonication. This possibility appears unlikely since sonication does not inactivate the acetyl CoA carboxylase from either rat liver or human platelets.[3] Thus, it appears that human leukocytes do not contain acetyl CoA carboxylase and, therefore, are unable to synthesize fatty acids de novo.

[3] Majerus, P. W. Unpublished observations.

TABLE V

Gas-liquid chromatography of fatty acids formed in Experiment 2, Table IV

Fatty acid chain length*		% of total injected onto column
	cpm	
14	22	1.9
16, 16:1	59	3.2
18, 18:1, 18:2	230	19.5
20–24‡	869	73.5

Samples were injected as methyl esters onto a column of 10% ethylene glycol succinate on acid washed Chromowash W at 170°C. Stream splitting was accomplished at a ratio of 10/1 and fractions were collected in Teflon tubes and counted in a liquid scintillation counter. Esterification of free fatty acids was carried out in 2% methanolic sulfuric acid at 70°C for 1 hr.

* Fatty acids designated either by number of carbon atoms or by number of carbon atoms:number of double bonds.

‡ 80% of the fatty acid in this fraction was arachadonic acid (20:4).

TABLE VI

Fatty acid synthetase

Patient		Malonate-^{14}C incorporated	
		mμmoles/mg per min	
Normal			
P.M.		0.058	
R.C.		0.077	
S.K.		0.067	
R.S.		0.154, 0.057*	Avg, 0.0674
L.I.		0.036	
R.L.		0.057	
J.P.		0.033	
	%		
Chronic lymphocytic leukemia	lymphocytes		
M.S.	88	0.025	
A.S.	93	0.044, 0.014*	Avg, 0.0208, $P < 0.01$‡
F.M.	99	0.006	
A.H.	97	0.015	
	%		
Acute leukemia	blasts		
A.H.	60	0.152	
F.B.	100	0.115	
Miscellaneous			
Reticulum cell sarcoma		0.052	
Leukemoid reaction		0.107	
Polycythemia vera		0.036	
Myeloid metaplasia		0.096	
Chronic myelocytic leukemia		0.067, 0.070*	

Assays were performed as described in methods using malonyl CoA-2-^{14}C.

* Blood samples obtained on separate days.

‡ P value was determined by comparing normals to chronic lymphocytic leukemia patients, using the Student's t test (20).

In order to confirm this conclusion, we incubated intact leukocytes with sodium acetate-1-^{14}C. Since leukocytes are unable to synthesize fatty acids, it is necessary to explain the incorporation of acetate-^{14}C obtained by Marks, Gellhorn, and Kidson and others (4–7). Recent experiments of Miras, Mantzos, and Levis (17) suggest that most of the label incorporated under these conditions is by the pathway of chain elongation of preformed fatty acids. The results of these intact cell experiments, shown in Table IV, confirm that acetate has been incorporated into fatty acids by chain elongation. The fatty acids formed in Experiment 2 were further studied by gas-liquid chromatography of the methyl esters (Table V). Over 90% of the radioactive fatty acids formed were of C_{18} or longer chain length. The product of the de novo synthetic pathway, palmitate (C_{16}), contained only 3.2% of the acetate incorporated. This labeling pattern and the fact that most of the counts incorporated are in the C-1 position indicate that acetate is being incorporated into fatty acids by the chain-elongation pathway and that the de novo synthetic pathway is inoperative.

Fatty acid synthetase activity. Fatty acid synthetase activity was easily demonstrable in the leukocytes from all of the subjects tested despite the persistent absence of acetyl CoA carboxylase activity (Table VI). There was no substantial difference in the results when expressed per cell rather than per mg soluble protein. The yield of soluble protein ranged from 1 to 2 mg per 10^8 cells. No correlation was found between the level of synthesis and contamination by blood platelets; also, no correlation was found between the percentage of granulocytes or lymphocytes and the level of synthetase. Thus, it is not clear whether there is a different amount of enzyme present in the various leukocyte cell types.

TABLE VII

*Acetyl CoA carboxylase from leukemic blast cells**

Additions or omissions	Incorporation
	cpm
1. Complete system	1850
2. Minus citrate	66
3. Minus acetyl CoA	195
4. Minus $MnCl_2$	80
5. Minus ATP	90
6. Plus 0.5 mg of avidin	60
7. Plus 0.5 mg of avidin, 5 mg of biotin	320

* Acetyl CoA carboxylase was measured as described in Methods. Incubation mixtures contained 2.0 mg of extract from leukemic blast cells. In Experiment 7, biotin and avidin were mixed together before the addition of enzyme.

No striking deviations from normal subjects are noted in the patients with various hematologic diseases although larger numbers of patients would be required to detect small differences. The level of fatty acid synthetase in four patients with chronic lymphocytic leukemia (mean = 0.0208 mμmole malonyl CoA incorporated/min per mg of protein, range 0.006–0.044) is lower than in the normal patients (mean = 0.0674 mμmole malonyl CoA incorporated/min per mg of protein, range 0.033–0.154). Whether this difference represents the level of fatty acid synthetase in lymphocytes in general or is specific to chronic lymphocytic leukemia is not clear.

Acetyl CoA carboxylase in leukemic blasts. While studying leukocyte extracts from a number of patients with various hematologic diseases, we noted that one of two patients with acute leukemia had acetyl CoA carboxylase activity. This patient (F.B.) had acute myelomonocytic leukemia which was untreated at the time that blood was obtained for study. His white blood cell count was 122,000/mm^3, his hemoglobin concentration was 11.8 g/100 ml and his platelet count was 12,000/mm^3. A differential WBC count showed 93% blast cells, 5% lymphocytes, 1% segmented neutrophil, and 1% band forms. The isolated leukocytes contained 1 platelet/250 WBC so that the acetyl CoA carboxylase measured could not have come from blood platelets.[3] The patient died shortly after admission to the hospital so that further studies could not be performed.

Acetyl CoA carboxylase was measured by the fixation of $KH^{14}CO_3$ into malonyl CoA. The requirements for this reaction are shown in Table VII. The reaction was totally dependent on the acetyl CoA carboxylase cofactors and was inhibited by avidin, a potent biotin inhibitor. The avidin inhibition was partially overcome by the prior addition of biotin.

The product of this reaction was shown to be malonyl CoA by carrying out a large scale incubation. In this incubation the reaction was stopped by the addition of Dowex-50 (hydrogen form) and 1 μmole of malonyl CoA was added as carrier. The mixture was then fractionated on DEAE

cellulose (18) and 20% of the radioactivity was found to cochromatograph with the carrier malonyl CoA. This peak was pooled and further identified as malonyl CoA by ascending paper chromatography on Whatmann 1 paper in NH_4OH: isobutyric acid: water (3.7: 57.7: 38.5). A portion of the isolated radioactive product was also hydrolyzed in 0.1 N NaOH at 37°C for 1 hr. The malonic acid formed was identified by ascending paper chromatography on Whatmann 1 paper in ethyl ether: acetone: acetic acid: water (6:6: 3:1). Thus it was clearly established that these leukemic blasts contain a low level of acetyl CoA carboxylase. Extracts from these blasts were incubated with acetyl CoA-1-^{14}C and the acetyl CoA carboxylase cofactors in the fatty acid–synthesizing system and the fatty acids formed were submitted to Schmidt degradation. In this case, 11% of the counts incorporated were in the C-1 position indicating de novo synthesis of fatty acids from acetyl CoA.

The acetyl CoA carboxylase activity in this extract was 5 $\mu\mu$moles malonyl CoA formed/mg of protein per min while the fatty acid synthetase activity was 152 $\mu\mu$moles malonyl CoA incorporated/mg of protein per min. Thus, acetyl CoA carboxylase is rate-limiting in this system as it is in fatty acid biosynthesis in rat liver and adipose tissue. Two additional patients with acute leukemia were studied, and in each case acetyl CoA carboxylase activity was present at levels similar to those noted above. In both of these patients more than 50% of the leukocytes in the blood were blast cells. Thus in three of the four patients with acute leukemia, acetyl CoA carboxylase activity was present in low levels and thus provided a complete fatty acid–synthesizing system in the blast cells.

Discussion

That mature erythrocytes are unable to synthesize fatty acids de novo is not surprising since 14 moles of NADPH are required to form 1 mole of palmitate. Since the erythrocyte cannot respire and is thus relatively deficient in reducing power, it might be suspected that active fatty acid synthesis would not occur in this cell. The leukocyte, however, is capable of active respiration, and it is somewhat surprising that this cell cannot synthesize fatty acids. The enzyme missing in both erythrocytes and leukocytes is acetyl CoA carboxylase which is the first enzyme unique to the fatty acid synthesis pathway.

Erythrocytes and leukocytes contain fatty acid synthetase activity, but in the absence of a source of malonyl CoA the fatty acid synthetase in both is presumably nonfunctional. It is possible that primitive hematopoietic cells have the capacity to form fatty acids and that this capacity is lost as acetyl CoA carboxylase activity decreases during maturation. By this hypothesis, the fatty acid synthetase activity measured in mature leukocytes and erythrocytes would be that due to vestigial enzyme which presumably turns over slowly. To test this hypothesis requires a source of primitive hematopoietic cells free of fat cells. The low levels of acetyl CoA carboxylase found in patients with acute leukemia and large numbers of primitive blast cells suggest that this hypothesis may be correct. Whether this observation reflects the enzyme content of normal stem cells or whether it is peculiar to the leukemic blast cell is unresolved.

The inability of leukocytes and erythrocytes to synthesize fatty acids raises a question as to how these cells are able to maintain the complement of complex lipids which are required for cell membranes as well as for other cell structures and functions. Possibly these cells are capable of activating fatty acids transported from plasma and thus can use preformed fatty acids for complex lipid biosynthesis. Recent experiments by Wittels and Hochstein (19) suggest that erythrocytes may be capable of transporting previously activated, long-chain fatty acids by way of palmityl carnitine transferase which is contained in erythrocyte membranes.

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