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LONG-CHAIN ACYL-CoA SYNTHETASE IN RAT LIVER

A NEW ASSAY PROCEDURE FOR THE ENZYME, AND STUDIES ON ITS INTRACELLULAR LOCALIZATION

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SUMMARY

1. Long-chain fatty acyl-CoA synthetase (acid:CoA ligase (AMP), EC 6.2.1.3; trivial name: palmityl-CoA synthetase) has been studied in rat-liver homogenate and cellular subfractions with the aid of a new assay procedure for the enzyme. The principles of the method are as follows: Rat-liver homogenate or subfractions are incubated with palmitate, CoA, ATP and DL-[*Me*-^{3}H]carnitine in Tris buffer (pH 7.5). The palmityl-CoA formed is quantitatively transformed to palmityl-DL-[*Me*-^{3}H]-carnitine by excess amounts of carnitine palmityltransferase. The palmityl-DL-[*Me*-^{3}H]carnitine is extracted from the incubation mixture by *n*-butanol. The radioactivity in the butanol phase is proportional to the palmityl-CoA synthetase activity.

2. Addition of particle-free supernatant of the cytoplasmic extract to the incubation mixture has a stimulatory effect on the palmityl-CoA synthetase activity in isolated mitochondria and microsomes. The stimulatory factor in the supernatant is likely to be a protein or a factor bound to protein.

3. Differential centrifugation of rat-liver homogenate shows that the palmityl-CoA synthetase has a bimodal intracellular localization. Approximately 70% of total activity is localized in the microsomes, and approximately 30% in the mitochondria. The total activity is about 1.4 units per g wet wt. of liver.

4. Sucrose density-gradient centrifugation confirms the bimodal intracellular localization revealed by differential centrifugation.

5. The enzyme is relatively firmly bound to membranes both in mitochondria and microsomes.

INTRODUCTION

The long-chain fatty acyl-CoA synthetase (acid:CoA ligase (AMP), EC 6.2.1.3; trivial name: palmityl-CoA synthetase) was first demonstrated in guinea-pig and rat-liver microsomes by KORNBERG AND PRICER[1]. Palmityl-CoA synthetase activity

has also been found in liver mitochondria (VAN DEN BERGH[2], YATES, SHEPHERD AND GARLAND[3]), and in the nuclear fraction of liver (CREASEY[4]). When using [1-^{14}C]stearate as substrate, CREASEY[4] found the highest specific activity in the nuclear fraction, and almost no activity in the mitochondrial fraction. YATES, SHEPHERD AND GARLAND[3] foud about the same specific activity in mitochondria and microsomes when they assayed the enzyme by using the disappearance of CoA.

No studies seem to have been made on the correlation of the cellular localization of palmityl-CoA synthetase with the localization of marker enzymes. In the present communication such studies are reported with a new assay method for palmityl-CoA synthetase. Previously, BREMER[5] had demonstrated that biosynthesis of palmitylcarnitine from externally added palmitate and carnitine took place in subcellular fractions of rat liver. We now report results which show that the palmityl-CoA synthetase can be assayed in whole homogenate and cellular subfractions of rat liver by incubation with palmitate, CoA, ATP, DL-[*Me*-^{3}H]carnitine and excess amounts of carnitine palmityltransferase. The palmityl-CoA formed is thereby trapped as L-[*Me*-^{3}H]carnitine-palmitate. With this procedure it is shown that approx. 70% of the palmityl-CoA synthetase activity is localized in the microsomes, while approx. 30% is found in the mitochondria. In both fractions the enzyme is relatively firmly bound to the membranes.

MATERIALS AND METHODS

Materials

CoA was purchased from C. F. Boehringer und Soehne, Mannheim, Germany; ATP and crystalline bovine albumin from Sigma Chemical Company, St. Louis, Mo., U.S.A.; and DL-carnitine from Fluka AG, Buchs, Switzerland.

DL-[*Me*-^{3}H]Carnitine with specific activity 75 μC/μmole was prepared as described by BREMER AND NORUM[6], and L-carnitine as described by FRIEDMAN *et al.*[7]. The radioactive carnitine was diluted with L-carnitine or DL-carnitine to a specific activity of 0.6 μC/μmole, or about 120 000 counts/min per μmole L-carnitine when counted in a Tri-Carb liquid scintillation spectrometer (Model 500D).

Palmitate–albumin solutions were prepared with approx. 6 moles of palmitate per mole of albumin.

Carnitine palmityltransferase was prepared from calf-liver mitochondria according to NORUM[8], except that $CaCl_2$, EDTA and palmityl-carnitine were omitted from the final extraction solution. The enzyme extract prepared in this way was free from palmityl-CoA synthetase and from palmityl-CoA hydrolase (EC 3.1.2.2). The final enzyme extract contained approx. 2 mg protein and about 0.4 units of enzyme per ml.

Animals

Female rats of the Wistar strain weighing about 150–200 g were used for the experiments. Usually the animals had free access to food and water until they were killed. The animals used for the sucrose density-gradient studies, however, were fasted for 24 h before they were used. The rats were killed by decapitation, and the liver was immediately taken out and cooled on ice.

Differential centrifugation

A weighed liver was homogenized in 0.25 M sucrose, and the homogenate was separated into 6 subfractions by differential centrifugation according to DE DUVE *et al.*[9] with minor modifications as described by NORUM AND BREMER[10]. By this procedure the following fractions were obtained: the N fraction or nuclear fraction; the E fraction or "cytoplasmic extract" which was further separated into the M fraction or heavy mitochondrial fraction; the L fraction or light mitochondrial fraction; the P fraction or microsomal fraction; and the S fraction or final supernatant. Free activity of acid phosphatase (EC 3.1.3.2) was assayed in the fractions immediately after the centrifugation. All other enzyme assays were performed on fractions stored at −15°. Freezing and thawing once was found to not cause any inactivation of the enzymes assayed.

Sucrose density-gradient centrifugation

To separate the different particles in the homogenate according to their density equilibrium (BEAUFAY AND BERTHET[11]), a linear sucrose density gradient was used.

A combined M + P + L fraction was prepared by centrifugation of the cytoplasmic extract at 198 000 × *g* for 30 min. The pellet was resuspended in 0.25 M sucrose to give the particles from 1 g of liver in 2 ml of suspension. 1 ml of this suspension was layered over 4.5 ml of the sucrose gradient and centrifuged in a swing-out rotor (Spinco SW 39) at 39 000 rev./min for 16 h. The contents of the tube were then carefully sucked off from the top by a bent pipette in fractions of 0.5 ml each.

Enzyme assays

We have recently established that carnitine palmityltransferase can be used as a marker enzyme for the mitochondria[10]. This enzyme was assayed by measuring the enzyme-catalyzed incorporation of labelled carnitine into palmityl-carnitine[8]. D-β-Hydroxybutyrate dehydrogenase (EC 1.1.1.30) and glutamate dehydrogenase (EC 1.4.1.2) as marker enzymes for mitochondria, acid phosphatase as marker enzyme for lysosomes, and glucose-6-phosphatase (EC 3.1.3.8) as marker enzyme for microsomes, were assayed as described by BEAUFAY *et al.*[12]. Free activity of acid phosphatase was measured in freshly prepared fractions to evaluate the degree of destruction of the cellular subfractions during the isolation procedure (DE DUVE *et al.*[9]).

The protein content in the various fractions was determined by the micro-Kjeldahl method. All counting of radioactivity was performed as previously described[13].

RESULTS

Several methods for the assay of palmityl-CoA synthetase have been worked out. The most widely used methods are based on the formation of hydroxamic acid (LIPMANN AND TUTTLE[14]), or the appearance or disappearance of ATP (KORNBERG[15]). These methods all suffer from some disadvantages. Hydroxylamine inhibits the palmityl-CoA synthetase, at least in purified enzyme preparations (MASSARO AND LENNARZ[16]). The other methods described are unsuitable for assay in whole homogenate. A new assay method was therefore worked out, based on the observation that palmityl-carnitine was formed *in vitro* from L-[*carboxy*-^{14}C]carnitine and palmitate

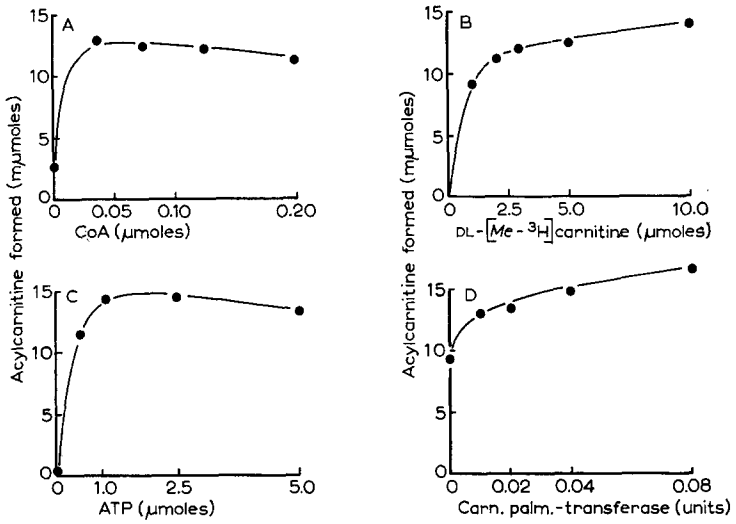

Fig. 1. The formation of palmityl-carnitine by rat-liver whole homogenate (2.5 mg of fresh tissue) shown as a function of the amount of added CoA (A), the amount of added L-carnitine (B), the amount of added ATP (C) and the amount of added carnitine palmityltransferase (D). The standard incubation mixture contained in a volume of 1.0 ml: 0.08 μmole of CoA, 5 μmoles of GSH, 10 μmoles of DL-[*Me*-^{3}H]carnitine, 1.5 μmoles of ATP, 1 μmole of palmitate, 2 μmoles of $MgCl_2$, approx. 0.04 unit of carnitine palmityltransferase and 100 μmoles of Tris buffer (pH 7.5). Incubation time was 5 min, and the temperature 30°.

in the presence of CoA and ATP (ref. 5). To obtain optimal activity, carnitine palmityltransferase had to be added.

Assay procedure for the palmityl-CoA synthetase

The conditions for the optimal activity of the palmityl-CoA synthetase are shown in Fig. 1. In these experiments 2.5 mg of liver tissue (25 μl whole homogenate) were used. The optimal concentration of CoA was approx. $8 \cdot 10^{-5}$ M. When CoA was omitted, less than 10% of the maximal activity was found. ATP was necessary for the formation of palmityl-carnitine. The optimal concentration of ATP was about $1.5 \cdot 10^{-3}$ M. Nearly maximal activity was found with a L-[*Me*-^{3}H]carnitine concentration of about $5 \cdot 10^{-3}$ M. A further increase in the L-carnitine concentration resulted only in an insignificant increase in the formation of palmityl-carnitine. The addition of exogenous carnitine palmityltransferase was necessary for optimal synthetase activity. About 0.04 unit of this enzyme increased the formation of palmityl-carnitine by 60% in whole homogenate. A further increase in the amount of added carnitine palmityltransferase had no significant effect. In the present experiments, the maximal rate of palmityl-CoA formation was less than 4 mμmoles/min (see Figs. 1–3). Under the conditions used the amount of carnitine palmityltransferase added was capable of transforming 40–50 mμmoles of palmityl-CoA per min, giving a ten-fold excess of this enzyme. The effect of added carnitine palmityltransferase was much more pronounced when isolated microsomes were used. This is reasonable as the carnitine palmityltransferase has been shown to be localized exclusively in the mitochondria.

Fig. 2 shows that the palmityl-CoA synthetase was insensitive to variations in

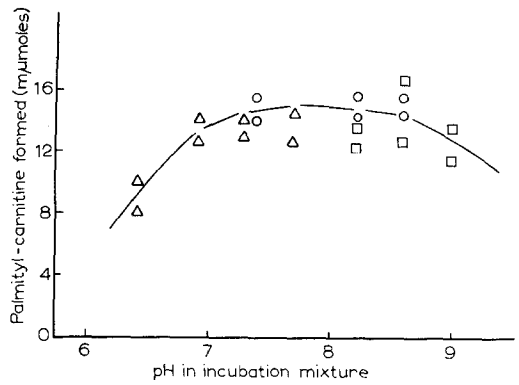

Fig. 2. Effect of pH on the formation of palmityl-carnitine. For details in the incubation mixture, see legend to Fig. 1. Triangles represent 0.1 M phosphate buffer, circles represent 0.1 M Tris buffer, and squares represent 0.1 M glycyl–glycine buffer.

the $[H^+]$ within a wide range. No obvious differences were seen in the synthetase activity between pH 7.0 and 8.5.

With freshly prepared cell subfractions the addition of free fatty acids is required for the formation of palmityl-carnitine[5]. In more aged preparations, however, it was often observed that the addition of palmitate had little effect, presumably because endogenous free fatty acids were liberated by endogenous lipases. As a routine, palmitate was added to the incubation mixture to secure availability of substrate.

Freezing and thawing once of whole homogenate or subfractions resulted in a slight increase in the synthetase activity. When freezing and thawing were repeated several times, inactivation of the enzyme occurred. Addition of a detergent (Tween 80) to solubilize the mitochondria, or KCN to inhibit possible mitochondrial oxidation of acyl-carnitines, had no significant effect on the formation of palmityl-carnitine.

On the basis of these results the palmityl-CoA synthetase was routinely assayed in the following system with an incubation volume of 1.0 ml: 0.08 μmole of CoA, 5 μmoles of GSH, 5 μmoles of L-[*Me*-^{3}H]carnitine, 1.5 μmoles of ATP, 1 μmole of

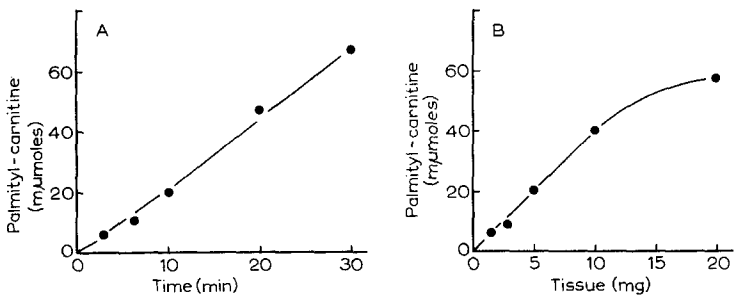

Fig. 3. The formation of palmityl-carnitine as a function of time of incubation (A), and as a function of the amount of liver incubated (B). In Expt. A the amount of tissue was 2.5 g. In B the incubation time was 5 min. For details in the incubation mixture, see legend to Fig. 1.

palmitate, 2 μmoles of $MgCl_2$ and 100 μmoles of Tris buffer (pH 7.5). Carnitine palmityltransferase was added in an amount containing 0.2 mg protein (equivalent to 0.04 enzyme unit). The reaction was started by the addition of ATP or the cellular fractions containing the palmityl-CoA synthetase. The incubation was carried out at 30° for 5 or 10 min. The reaction was stopped by the addition of 0.1 ml concentrated HCl, followed by 0.9 ml of water and 1.0 ml of *n*-butanol. The mixture was shaken, and the butanol phase was pipetted off and washed once with 1.5 ml of butanol-saturated water. With this procedure the blank contained less that 0.002% of the added radioactivity, giving blank counts of 150–200 per min.

In the chosen system the formation of palmityl-carnitine was linear with increasing amounts of tissue up to more than 10 mg of liver tissue in the incubation mixture, and also linear with increasing incubation time up to more than 20 min (Fig. 3). It should also be noted that radioactive carnitine was added in such amounts that no significant dilution of the radioactive carnitine by the endogenous, unlabelled carnitine took place.

Effect of the particle-free supernatant on the palmityl-CoA synthetase activity in isolated subcellular fractions of rat liver

Studies of the recovery of the palmityl-CoA synthetase activity in isolated subcellular fractions of rat liver revealed that a satisfactory recovery could not be obtained without the addition of particle-free supernatant to the incubation mixture. Table I shows that the addition of supernatant, from an amount of liver equivalent to that from which the mitochondria and microsomes were obtained, increased both the mitochondrial and the microsomal activity by more than 50%. When the supernatant was omitted from the incubation mixture, the recovery of the synthetase activity in the subcellular fractions was 67.5%. The recovery increased to 93% when an equivalent amount of supernatant was added to the incubation mixture (see Table II).

No stimulation was obtained when the S fraction was heated at 60° for 10 min before the incubations. This suggested that the stimulatory factor is of protein nature. Further support for this was obtained by gel filtration on a Sephadex G-25 column and by $(NH_4)_2SO_4$ precipitation both of which gave the stimulatory factor in the protein fractions. This effect of the particle-free supernatant is now the subject of further studies.

TABLE I

EFFECT OF THE S FRACTION ON PALMITYL-CoA SYNTHETASE ACTIVITY IN ISOLATED CELLULAR SUBFRACTIONS OF RAT LIVER

Mitochondria or microsomes from 2.5 mg rat liver were incubated with and without S fraction from an equivalent amount of tissue. Incubation procedure was as stated in legend to Fig. 1.

Fraction	*μmoles palmityl-carnitine formed per g tissue per min*		*% increase*
	Isolated fractions without S fraction	*Isolated fractions with S fraction*	
Mitochondria	0.242	0.383	58
Microsomes	0.569	0.880	54

TABLE II

INTRACELLULAR DISTRIBUTION OF THE PALMITYL-CoA SYNTHETASE AND SOME MARKER ENZYMES IN RAT LIVER

The absolute values for total homogenate (E + N) are given as units per g wet wt. of liver according to the International Enzyme Commission. The activity for carnitine palmityltransferase is expressed as arbitrary units. E = cytoplasmic extract, N = nuclear fraction, M = heavy mitochondrial fraction, L = light mitochondrial fraction, P = microsomal fraction, S = final supernatant. All values were corrected for the synthetase activity of the added S fraction.

Enzyme	*Absolute values total homogenate*	*Percentage values*						*% recovery*
		E + N	*N*	*M*	*L*	*P*	*S*	
Protein (mg/g wet wt.)	180	100	15.8	23.7	1.7	23.1	35.5	98.3
Acid phosphatase (total activity)	3.90	100	4.7	28.5	19.0	31.7	10.0	93.9
Acid phosphatase (free activity)	0.80	20.5	0.7	4.8	1.9	6.1	5.1	—
Carnitine palmityltransferase	5.52	100	8.0	78.8	1.7	5.8	3.1	97.9
Glucose-6-phosphatase	13.8	100	4.4	7.8	2.3	75.8	1.7	92.0
Palmityl-CoA synthetase*	1.44	100	4.9	26.2	0.4	58.0	3.7	93.2
Palmityl-CoA synthetase**	1.44	100	4.9	17.5	0.4	41.5	3.7	67.5

* The enzyme activity in the isolated particulate fractions in the presence of an equivalent amount of the S fraction.

** The enzyme activity in the particulate fractions assayed in the absence of the S fraction.

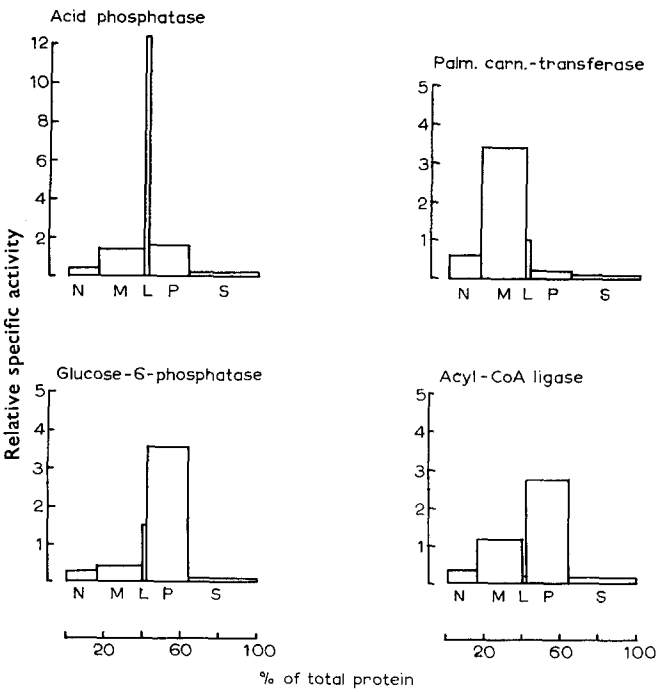

Fig. 4. The relative specific activity of the palmityl-CoA synthetase and some marker enzymes in subcellular fractions of rat liver. Ordinates: relative specific activity (percentage of total activity/percentage of total protein content) of the various fractions. Abscissa: the percentage of the total protein content in each fraction, presented in the order of isolation, from left to right, N, M, L, P and S. Standard incubation mixture for the acyl-CoA synthetase as stated in legend to Fig. 1.

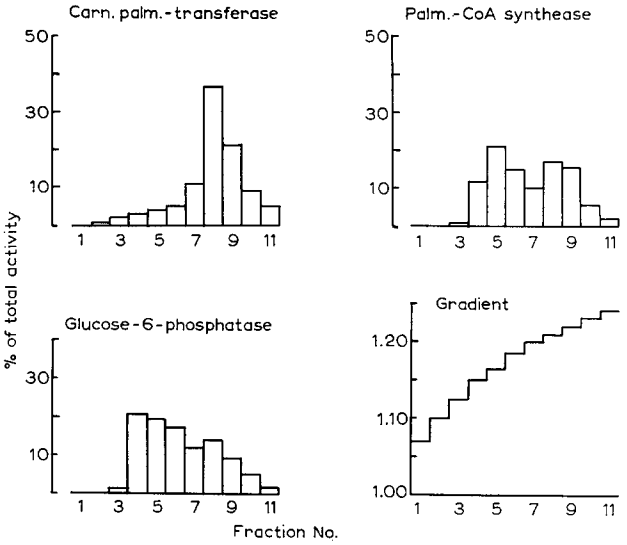

Fig. 5. The distribution of the palmityl-CoA synthetase and some marker enzymes of rat liver after centrifugation of the combined fractions M + L + P in a sucrose density gradient. Ordinates: percentage of the total activity in the fractions. Abscissa: the content in the centrifuge tube divided into eleven equal portions and presented in the order of isolation from the top (left) to the bottom (right). The specific gravity of the fractions is shown down to the right in the figure. Incubation mixture for the acyl-CoA synthetase assay as stated in legend to Fig. 1.

In the reported experiments the assays of the particulate fractions were done with an equivalent and constant amount of the S fraction present in the incubation mixture. The small synthetase activity in the isolated S fraction (see Table II) was corrected for in all figures and tables.

The subcellular localization of the palmityl-CoA synthetase

Table II shows the distribution of palmityl-CoA synthetase and of the different marker enzymes in rat-liver fractions obtained by differential centrifugation according to DE DUVE *et al.*[9]. The results are presented as these authors proposed. The recovery of protein and the different enzymes was in the range 92–98%. The free activity of acid phosphatase was 20.5% of the total activity of this enzyme. This indicates an acceptable degree of destruction of the lysosomes during the fractionation procedure[9].

The distribution of the marker enzymes—carnitine palmityltransferase for the mitochondria, acid phosphatase for the lysosomes and glucose-6-phosphatase for the microsomes—shows that the mutual contamination of the different fractions was moderate. Palmityl-CoA synthetase was found in both mitochondria and microsomes. The mitochondrial activity was far above that expected as a result of microsomal contamination, thus indicating a bimodal distribution of the enzyme. The relative specific activity for the different enzymes is shown in Fig. 4. The relative specific activity for palmityl-CoA synthetase is that expected for an enzyme with a mitochondrial as well as microsomal localization.

The results of the sucrose density-gradient studies are shown in Fig. 5. The distribution of carnitine palmityltransferase and glucose-6-phosphatase is similar to

the distribution found by NORUM AND BREMER[10]. The palmityl-CoA synthetase showed two activity maxima, one corresponding fairly well with the microsomal fraction as seen from the distribution of the glucose-6-phosphatase, and one corresponding with the mitochondrial fraction as seen from the distribution of the carnitine palmityltransferase. The difference between the distribution of glucose-6-phosphatase and palmityl-CoA synthetase in the upper part of the centrifuge tube is most likely explained by the fact that the microsomal fraction is not homogeneous, and that the two enzymes might have an uneven distribution between the various fragments of the endoplasmic reticulum.

When corrections are made for the mutual contamination of the different cellular subfractions, about 70% of the total palmityl-CoA synthetase activity is localized in the microsomes, and about 30% in the mitochondria. The activity found in the other cellular subfractions is most likely due to mitochondrial or microsomal contamination as indicated by the distribution of the marker enzymes in Table II.

Binding of the palmityl-CoA synthetase to cellular membranes

Isolated mitochondria and microsomes were separately sonicated in a Branson sonifier for four times 15 sec and with an output of 6 A and thereafter centrifuged at 198 000 × *g* for 30 min. The pellets were resuspended in 0.25 M sucrose to a concentration equal to that in the original particle suspensions. Table III shows the results of

TABLE III

BINDING OF THE PALMITYL-CoA SYNTHETASE TO CELLULAR MEMBRANES

The distribution of palmityl-CoA synthetase and some marker enzymes in isolated mitochondria and microsomes after sonication and separation of membranes and the soluble fraction by centrifugation. Details of the sonication procedure are described under RESULTS.

Enzyme	*Mitochondrial fraction % of activity in*		*Microsomal fraction % of activity in*	
	Membranes	*Soluble fraction*	*Membranes fraction*	*Soluble fraction*
D-β-Hydroxybutyrate dehydrogenase	91	9	—	—
Glutamate dehydrogenase	11	89	—	—
Carnitine palmityltransferase	70	30	72*	28*
Palmityl-CoA synthetase	75	25	91	9
Glucose-6-phosphatase	—	—	74	26

* The activity of this enzyme in the microsomal fraction is due to mitochondrial contamination.

enzyme assays in the supernatants and pellets from both mitochondria and microsomes. Some inactivation of the palmityl-CoA synthetase was always found after sonication. Recently we have shown that sonication selectively inactivates the palmityl-CoA synthetase with no obvious effect on β-hydroxybutyrate dehydrogenase, glutamate dehydrogenase, carnitine palmityltransferase or glucose-6-phosphatase[17]. Provided that the inactivation did not exclusively take place in the soluble fraction, the mitochondrial palmityl-CoA synthetase was solubilized to a degree similar to that of carnitine palmityltransferase. In microsomes the synthetase seemed to be more

tightly bound, as lysis after sonication was considerably less than for glucose-6-phosphatase and for the contaminating carnitine palmityltransferase. These findings show that the mitochondrial as well as microsomal palmityl-CoA synthetase are relatively firmly bound to the cellular membranes, although they may be different enzymes.

DISCUSSION

The reported method for the assay of the palmityl-CoA synthetase is a relatively simple and very reproducible procedure. It requires a partially purified carnitine palmityltransferase which must be free from acyl-CoA synthetases and acyl-CoA hydrolase. The formation of palmityl-L-[*Me*-^{3}H]carnitine from palmitate and labelled carnitine requires both CoA and ATP. The palmityl-L-[*Me*-^{3}H]carnitine formed must therefore be a result of an enzymic catalysis of an ATP-dependent enzyme. The conditions for optimal activity in this report are similar to those found in other investigations[1,5].

The reaction R·COOH + CoA + ATP $\rightleftharpoons$ R·CO·CoA + AMP + PP_i involves the formation of several intermediates. BERG[18] suggested an intermediate acyl–AMP complex which may involve Mg^{2+} in a chelating complex as proposed by INGRAHAM AND GREEN[19]. BAR-TANA AND SHAPIRO[20] suggested that there may be two forms of the palmityl-CoA synthetase, one active CoA-containing form, and one inactive form that needs both CoA and ATP for its activation. The present assay method will not be affected by any decrease in free CoA in the incubation medium since palmityl-CoA formed is immediately converted to palmityl-carnitine and CoA by the presence of carnitine palmityltransferase in excess. The amount of labelled carnitine is so large that no dilution will occur with endogenous carnitine in the tissue fractions.

The localization of palmityl-CoA synthetase in rat liver has hitherto not been studied in relation to marker enzymes. The synthetase has been demonstrated both in mitochondria and microsomes of rat liver[1–3,5]. AILHAUD, SAMUEL AND DESNUELLE[21] found the enzyme almost exclusively in the microsomal fraction of intestinal mucosa. CREASEY[4] found the highest specific activity in the nuclear fraction of rat liver, whereas the mitochondria were practically free from acyl-CoA synthetase activity. These results are not supported by our findings. CREASEY[4] used [1-^{14}C]stearate as substrate in his experiments. We found that sufficient endogenous free fatty acids were present in the homogenate and subfractions in many experiments. It is therefore likely that CREASEY had his radioactive substrate diluted to a varying extent. The relatively low total activity found by him is in agreement with this explanation.

YATES, SHEPHERD AND GARLAND[3] found in their experiments about the same specific activity in mitochondria and microsomes, approx. 7 mμmoles/mg protein per min in both fractions. In our experiments the specific activity was about 9 mμmoles/mg protein per min in mitochondria and about 22 mμmoles/mg protein per min in microsomes. The total palmityl-CoA synthetase activity in our experiments was 1.44 μmoles/g tissue per min. Assuming that the composition of the subcellular fractions in the experiments of YATES, SHEPHERD AND GARLAND[3] were identical with ours, a total activity of approx. 0.65 μmole/g tissue per min can be calculated. This difference in total activity may partly be due to that YATES, SHEPHERD AND GARLAND carried out their incubations at 25°.

The main part of the palmityl-CoA synthetase activity is localized in the micro-

somes, but our results confirm that rat liver has a bimodal localization of the enzyme. Furthermore, the present findings show that the palmityl-CoA synthetase is bound to the membranes both in mitochondria and in the microsomes.

The stimulating effect of the particle-free supernatant on the palmityl-CoA synthetase has not been observed previously. The nature of this effect remains to be elucidated. Recently we have shown that the mitochondrial palmityl-CoA synthetase is most likely bound to the outer membrane of these particles[17]. This finding fits well with the observation that the presence of the supernatant stimulated the synthetase both in isolated microsomes and mitochondria. A soluble cytoplasmic substance will presumably be in close contact with the endoplasmic reticulum as well as with the outer membrane of the mitochondria.

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