# ADAPTIVE SYNTHESIS OF RAT LIVER FATTY ACID SYNTHETASE: EVIDENCE FOR IN VITRO FORMATION OF ACTIVE ENZYME FROM INACTIVE PROTEIN PRECURSORS AND 4'-PHOSPHOPANTETHEINE

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Evidence is presented in support of the hypothesis that an important step in the adaptive synthesis of fatty acid synthetase is the conversion of inactive enzyme precursors to active enzyme via the incorporation of the 4'-phosphopantetheine prosthetic group. Fatty acid synthetase activity was generated in vitro when CoA or E. coli acyl carrier protein was incubated with enzymatically inactive extracts from livers of rats fed a fat-free diet for 0-5 hr following starvation, and a factor present in liver extracts from rats refed for more than 6 hr. When ($^{14}$C)-CoA, labelled in the pantetheine moiety, was used in the above system, radioactivity was incorporated into a protein bound form, from which it could be released by mild alkaline hydrolysis.

The "adaptive synthesis" of fatty acid synthetase in rats that are fed a fat free diet following starvation involves the formation of new enzyme protein (1, 2), and it is known that increased liver levels of fatty acid synthetase result from an increased rate of enzyme synthesis with no concomitant change in the rate of degradation (3, 4). Although the appearance of fatty acid synthetase activity in liver is delayed 3-5 hr following refeeding (2, 5), we have recently demonstrated that synthesis of fatty acid synthetase protein commences immediately on refeeding, and that incorporation of pantothenate into fatty acid synthetase parallels the development of fatty acid synthetase activity (6). These findings are consistent with the hypothesis that a holo-apo-enzyme relationship exists between fatty acid synthetase and its biosynthetic precursors. This paper presents further evidence in support of this hypothesis.

## MATERIALS AND METHODS

Materials CoA, acyl CoA esters and ATP were purchased from P-L Biochemicals, Inc. (1-$^{14}$C)-acetyl CoA and D-(1-$^{14}$C)-pantothenic acid were purchased from Amersham/Searle and New England Nuclear Corp. respectively. ($^{14}$C)-CoA, labelled in the pantetheine moiety was prepared as described by Chesterton et al (7).

Animals and diets. Male, Sprague-Dawley rats of 150-200 g each were purchased from North American Laboratory Supply, Winnipeg. Before use, rats were starved for 48 hr, then fed a fat-free diet (Nutritional Biochemicals) as indicated in the text. Animals were killed by cervical dislocation, and their livers were immediately removed on to ice.

Assay of fatty acid synthetase. Fatty acid synthetase activity was measured as described by Butterworth et al (8).

Preparation of Liver Extracts: Crude particle-free supernatants were prepared from livers of rats fed a fat-free diet for varying times following starvation, by the method of Hsu et al (9). The fraction precipitating between 20% and 40% of saturation with ammonium sulfate was then obtained and dialysed overnight against 0.15 M potassium phosphate buffer pH 7.0, containing 1 mM dithiothreitol. The resulting material was termed AS-0, AS-3, AS-12 etc. according to the number of hours the rats were allowed access to the fat-free diet following starvation.

In vitro generation of fatty acid synthetase activity from inactive precursors

The basic procedure was as follows. Enzymatically inactive liver extracts from rats fed a fat-free diet for short periods following starvation eg AS-3 were used as a source of putative enzyme precursors. Extracts from rats refed

TABLE I

GENERATION OF FATTY ACID SYNTHETASE ACTIVITY

| Conditions | ($1-^{14}C$)-acetyl CoA incorporated into long chain fatty acids(pmoles) |
|---|---|
| Complete | 92 |
| Complete minus CoA | 32.6 |
| Complete minus ATP | 87 |
| Complete minus ATP and CoA | 1.9 |
| Complete minus CoA + E. coli ACP (100 µg) | 90.8 |

The complete system contained: AS-3(3 mg); AS-12 from which fatty acid synthetase activity was removed by immunoprecipitation(1.5 mg); CoA (1µM); ATP (0.5 mM); potassium phosphate buffer pH 7.0, 0.15 M. Total volume was 0.3 ml. After 20 min incubation at 38°, the mixture was passed through a Sephadex G-25 column, and the protein eluate was collected in a volume of 1.2 ml. A 0.5 ml sample was assayed for fatty acid synthetase activity.

for longer periods eg. AS-12, were treated with anti-fatty acid synthetase serum to remove endogenous fatty acid synthetase activity, and were then incubated at 37° for 20 min with the preparation of putative enzyme presursors, in the presence of CoA and ATP. Following incubation, mixtures were passed through a 1 x 5 cm column of Sephadex G-25, and the protein eluate was assayed for fatty acid synthetase activity. Further details of this procedure are given in the text as appropriate. The same basic procedure was used to measure incorporation of $^{14}C$ from $(^{14}C)$-CoA labelled in the pantetheine moiety into protein bound form, except that after gel chromatography protein was precipitated with trichloroacetic acid, washed and counted in a liquid scintillation counter.

## RESULTS AND DISCUSSION

We have previously reported that synthesis of fatty acid synthetase protein commenced immediately after feeding starved rats, whereas the appearance of enzyme activity in the liver and the *in vivo* incorporation of $(^{14}C)$-pantothenate into fatty acid synthetase started 3-5 hr later, and paralleled one another (6). We suggested that it should be possible to demonstrate in liver the presence of an enzyme capable of transferring a phosphopantetheine group to putative apo-

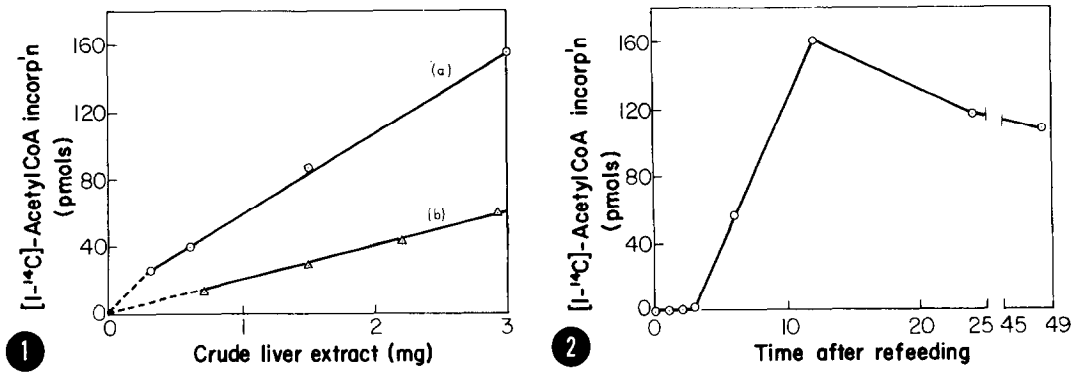


Fig. 1. Dependence of Generation of Fatty Acid Synthetase Activity on Presence of Liver Extracts from (a) 12 hr refed rats, and (b) 3 hr refed rats.

(a) Crude liver extract from 3 hr refed rats (3 mg) was mixed with extract from 12 hr refed rats as indicated.
(b) Crude liver extract from 12 hr refed rats (1.5 mg) was mixed with extract from 3 hr refed rats as indicated. For both (a) and (b) components and conditions of assay were as in Table I.

Fig. 2. Dependence of Fatty Acid Synthetase Activity on Length of Refeeding following starvation. AS-3 (1.5 mg) was mixed with 0.5 mg of 20-40% ammonium sulfate fraction from livers of rats refed for times indicated. Components and conditions of assay were as given in Table I.

fatty acid synthetase, and that the activity of this enzyme should vary during adaptive synthesis of fatty acid synthetase in concert with the observed incorporation of pantothenate. The data presented below supports these suggestions.

Table I shows that fatty acid synthetase activity is generated when AS-3 (as a source of putative apo-enzyme) and AS-12 are incubated together in the presence of CoA. CoA can be replaced by E. coli acyl carrier protein. We have also shown that liver extracts from rats fed for times shorter than 3 hr following refeeding can replace AS-3 as a source of putative apo-enzyme. Such extracts are less active than AS-3 as might be expected if apo-enzyme is being synthesized during the early stages of adaptive synthesis. Extracts from rats refed for longer than 3 hr cannot be used as a source of apo-enzyme because they contain endogenous fatty acid synthetase activity which would interfere with the assay. We therefore used AS-3 as the source of putative apo-enzyme in our experiments. Figure 1 demonstrates that the amount of fatty acid synthetase activity generated is dependent on the quantity of liver extract from both 3 and 12 hr refed rats present during incubation. This can be explained by assuming that the liver extract from 12 hr refed animals contains an enzyme capable of transferring a phosphopantetheine group from CoA (or acyl carrier protein) to a putative apo-enzyme present in liver extracts from 3 hr refed rats. This conclusion is sup-

TABLE II

INCORPORATION OF $^{14}C$ FROM [$^{14}C$]-CoA INTO PROTEIN BOUND FORM

| Conditions | Radioactivity in protein ppt. (d.p.m.) |
|---|---|
| Complete | 390 |
| Complete minus AS-12 | 49 |
| Complete minus AS-3 | 12 |
| Radioactivity released from protein by acid (1M HCl, 100°, 10 min) | 85 |
| Radioactivity released from protein by alkali (pH 12, 70°, 1 hr) | 243 |

The complete system contained: AS-3 (7.5 mg); AS-12 (1.5 mg) from which fatty acid synthetase was removed by immunoprecipitation; ATP (0.55 mM); [$^{14}C$]-CoA (795 dpm, 1.3 nmole); and potassium phosphate buffer pH 7, 0.1 M. Total volume was 0.45 ml. The method of assay was as described previously.

ported by the data in Table II, where it is shown that radioactivity from ($^{14}$C)-CoA, labelled in the pantotheine moiety, is incorporated into protein bound form only when both AS-3 and AS-12 are present. Furthermore a high proportion of radioactivity is released from the protein by treatment with alkali, but not with acid - a finding which is consistent with our knowledge of the nature of the bond between protein and prosthetic group in fatty acid synthetase.

Figure 2 shows that the ability of liver extracts to catalyze the CoA-dependent generation of fatty acid synthetase activity from putative apo-enzyme (AS-3) is dependent on the length of time rats were refed after starvation. This ability does not appear in liver until 3 hr after the onset of refeeding, peaks at about 12 hr, and declines slightly thereafter. This is in excellent agreement with the time course of *in vivo* incorporation of ($^{14}$C)-pantothenate into fatty acid synthetase, and of the development of liver fatty acid synthetase activity during adaptive synthesis (6).

Table I shows that the generation of fatty acid synthetase activity is affected by the presence of ATP. While ATP is not an absolute requirement, it does seem to affect the response of the assay system to CoA. We have been unable to clarify the reason for this phenomenon, but the possibility that it involves protein phosphorylation is attractive in view of the known involvement of hormones and cyclic AMP in controlling the synthesis of fatty acid synthetase (10, 11, 12).

Volpe and Vagelos (13) were unable to demonstrate the presence in rat liver of an enzyme analogous to *E. coli* acyl carrier protein hydrolase, or of putative apo-enzyme in livers of rats fed a fat-free diet for times as short as 4 hr following starvation. However, other reports (6, 14) have supported the possible existence of apo-fatty acid synthetase. The data presented in this paper further substantiates this, and provides evidence that rat liver contains an enzyme capable of converting apo-fatty acid synthetase into the holoenzyme.

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