Acetyl Coenzyme A Carboxylase

THE ROLES OF SYNTHESIS AND DEGRADATION IN REGULATION OF ENZYME LEVELS IN RAT LIVER*

(Received for publication, July 22, 1969)

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SUMMARY

The roles of synthesis and degradation in the regulation of acetyl coenzyme A carboxylase levels in rat liver following fasting and fat-free feeding have been studied. Antibodies prepared to homogeneous chicken liver acetyl-CoA carboxylase were shown to cross-react with rat liver acetyl-CoA carboxylase. Enzyme from both species was totally inactive as well as precipitated by this antibody preparation. Furthermore, quantitative precipitin curves and equivalence point determinations indicated that enzymes from the two species were precipitated in equal quantity by antibody and that the two enzymes had identical turnover numbers. It was also shown that disaggregated, aggregated, and palmityl-CoA-treated enzyme were all equally precipitated by antibody.

Immunological analysis of crude homogenates of rat liver from animals treated with different diets, which had specific activities for acetyl-CoA carboxylase varying over 25-fold indicated that there was a constant amount of immunologically precipitable enzyme per unit of enzyme activity.

The changes in acetyl-CoA carboxylase activity measured after dietary alteration result from changes in the enzyme content of liver rather than from activation or inhibition of preformed enzyme.

The relative rates of acetyl-CoA carboxylase synthesis were determined by quantitative precipitation of the enzyme by antibody after pulse labeling with "H-leucine. There was a 5- to 10-fold increase in the rate of enzyme synthesis after fat-free feeding of previously fasted rats. The rate of degradation of acetyl-CoA carboxylase in rats fed a fat-free diet was found to be first order with a t½ of approximately 48 hours, whereas in fasted rats a t½ of 18 hours was obtained. In rats fed a 12% fat diet the rate of acetyl-CoA carboxylase degradation was similar to that found in animals fed a fat-free diet, whereas the rate of synthesis was diminished, thus suggesting that independent factors regulate the rates of acetyl-CoA carboxylase synthesis and degradation in rat liver.

Fatty acid synthesis in rat liver is inhibited by fasting; when fasted animals are subsequently fed a diet low in fat there is a prompt rise in fatty acid synthesis activity within 24 to 48 hours to levels above those seen in normally fed rats (1, 2). Acetyl coenzyme A carboxylase, the first enzyme in the fatty acid synthesis pathway, mediates these effects on fatty acid synthesis. This enzyme is rate-limiting for fatty acid synthesis, and its activity, as measured in vitro, rises and falls during feeding and fasting (3). Bortz and Lynen (4) have proposed that long chain acyl-CoA compounds, which have been shown to accumulate in the livers of animals deprived of food, mediate the effects on acetyl-CoA carboxylase by means of feedback inhibition. Palmityl-CoA has been shown to inhibit acetyl-CoA carboxylase at low concentrations, and the inhibition is competitive with citrate (5). Thus, citrate converts the enzyme from an inactive protomic form to an aggregated active form (6), and palmityl-CoA stimulates the disaggregation of enzyme to the inactive protomer (5). In this scheme palmityl-CoA could be viewed as an end product inhibitor which increases and decreases in concentration during fasting and with low fat feeding, respectively. In other experiments, Allman, Hubbard, and Gibson (2) noted that when fasted rats were given actinomycin D, there was no rise in fatty acid synthesis activity upon refeeding, suggesting that protein synthesis might be required to produce rises in acetyl-CoA carboxylase activity. In studies of well differentiated rat hepatomas, we have shown that neither acetyl-CoA carboxylase nor fatty acid synthetase of these tumors is subject to the changes in enzyme activity normally observed in host liver following dietary alteration (7). Acetyl-CoA carboxylase was purified from hepatomas and livers to explore the mechanism of this loss of regulation. These studies indicated that acetyl-CoA carboxylases from liver and hepatomas were identical enzymes with similar responsiveness to activation by citrate and to inhibition by palmityl-CoA. One possible explanation for these results was the changes in acetyl-CoA carboxylase activity seen following dietary alterations were due to changing amounts of enzyme rather than to alterations in the
state of activation or inhibition of preformed enzyme. Thus, in liver the amount of enzyme present would rise and fall with low fat feeding and fasting, respectively, whereas in tumors the amount of enzyme present remained constant under these conditions.

In the present studies we have measured the levels of acetyl-CoA carboxylase in rat liver with the use of antibodies to homogeneous chicken liver acetyl-CoA carboxylase. This antibody was found to cross-react with rat liver acetyl-CoA carboxylase both by inactivating and by precipitating the enzyme.

We have demonstrated that extracts with widely varying specific activity of acetyl-CoA carboxylase contain a constant amount of immunologically precipitable enzyme per unit of activity. Therefore, the changes in acetyl-CoA carboxylase activity measured after dietary alteration result from changes in the amount of enzyme present in the extracts. In further studies with the use of $^3$H-leucine, we have defined the rates of activity. Therefore, the changes in acetyl-CoA carboxylase activity measured after dietary alteration result from changes in the amount of enzyme present in the extracts.

Experimental Procedure

Methods

Animals and Diets—Male Sprague-Dawley rats were used in all experiments. Animals were fed either a fat-free diet or a high fat (45% vegetable oil) diet, both of which were purchased from Nutritional Biochemicals, or Purina rat chow. Fresh-frozen chicken livers from laying hens were obtained from Grand Island Biologicals, Grand Island, New York.

Preparation and Assay of Enzymes—Acetyl-CoA carboxylase was isolated from livers of laying hens as described by Gregolin, Ryder, and Lane (8). The sucrose gradient sedimentation pattern of the homogeneous enzyme is shown in Fig. 1. Rats were killed and crude liver homogenates were prepared as described previously (7) with the use of 1 part liver to 1.5 parts buffer. Acetyl-CoA carboxylase activity was measured after passage of an aliquot of crude liver supernatant through a column of Sephadex G-25 by the direct assay described previously (7, 9). When chicken liver acetyl-CoA carboxylase was assayed, the preliminary incubation was omitted. One unit of enzyme activity is defined as 1 μmole of malonyl-CoA formed per min at 37°. Tryptic acid activity was measured as described previously (7), and protein was measured by the method of Lowry et al. (10).

Immunological Procedures—Complete Freund's adjuvant was prepared with Difco Mycobacterium butyricum (11). Homogeneous chicken liver acetyl-CoA carboxylase which had been stored as an ammonium sulfate pellet was dissolved in 0.05 M potassium phosphate, pH 7.5, containing 0.01 mM EDTA and 0.1 mM 2-mercaptoethanol, and was dialyzed for 2 hours against the same buffer. After centrifugation at 4° for 4 hours at 25,000 rpm with a Spinco SW-25 rotor, fractions were collected and assayed for acetyl-CoA carboxylase activity (●—●) and protein content (O—O).

The antibody was then forcibly ejected from a 10-cc glass syringe repeatedly until a thick emulsion was obtained. This mixture was then injected into the footpads of rabbits, each rabbit receiving 0.5 to 1.0 mg of acetyl-CoA carboxylase. Rabbits were bled weekly, and in most experiments peak antibody levels were achieved after 4 to 5 weeks. Once peak antibody levels were achieved, rabbits with high titers were exanguinated and their sera were pooled and frozen at −20°. Serum from unimmunized rabbits was also obtained in the same manner. Ouchterlony double diffusion patterns and quantitative precipitin tests were performed as outlined by Ouchterlony (12) and by Kabat and Mayer (13).

Preparation of Liver Extracts for Immunological Analysis—In preliminary experiments it was found that the 105,000 × g rat liver supernatant solution was unsuitable for quantitative precipitin reactions with antibody because of nonspecific precipitation which occurs in crude rat liver extracts stored at 4°. Thus, it was necessary to develop a rapid purification procedure to avoid nonspecific precipitation. This purification was accomplished by the following procedure. The crude rat liver 105,000 × g supernatant solution (approximately 50 mg of protein per ml) in 0.1 M postassium phosphate, pH 7.5, containing 0.25 M sucrose, 0.01 M 2-mercaptoethanol, 0.07 M potassium bicarbonate, and 0.1 mM EDTA, was poured directly over a column of DEAE-cellulose previously equilibrated with 0.05 M potassium phosphate, pH 7.5, 0.15 M potassium chloride containing 0.01 M 2-mercaptoethanol, and 0.1 mM EDTA. The column load was 1 ml of crude extract per 1.8 ml of bed volume. After the sample was applied, the column was washed with 1 column volume of the same buffer.

This eluted most of the liver protein, including all of the colored and turbid material. The fraction containing acetyl-CoA carboxylase was next eluted in a single column volume of the same buffer containing 0.5 M potassium chloride. Following elution this fraction was concentrated to its original volume or less by Diaflo ultrafiltration with a UM-1 filter. This entire procedure could be completed in less than 2 hours with 80 to 120% yield of enzyme activity and 5- to 10-fold purification. As shown in Table II, there was no significant variation in yield of enzyme with the use of extracts of widely varying specific activity. When this preparation was used for immunological
then divided into 8 aliquots, and sufficient rabbit antiserum was added to determine the recovery of enzyme. This concentrate was eluted with 0.1 M potassium phosphate, pH 7.5, and the protein was eluted with 0.1 M potassium phosphate, pH 7.5, containing 0.01 M 2-mercaptoethanol and 0.1 mM EDTA. The acetyl-CoA carboxylase activity of this fraction was determined. Another aliquot of the 105,000 × g supernatant solution pooled from three to four rats was passed over a Sephadex G-25 column (0.9 × 15 cm), and the protein precipitate formed in tubes containing control serum. Precipitates were collected by centrifugation and, after washing three times in ice-cold 0.9% sodium chloride, were dissolved in formic acid and counted in a liquid scintillation counter. The level of nonspecific coprecipitation of radioactivity in antigen-antibody precipitates was determined by adding unlabeled acetyl-CoA carboxylase and antibody to radioactive supernatant solutions from which the radioactive acetyl-CoA carboxylase had been removed by prior antibody precipitation. Any radioactivity precipitated in this second reaction was presumed to be nonspecific (14). Alternatively, the level of nonspecific precipitation of radioactivity was determined as described by Jost, Khairallah, and Pitot (15), with the use of mixtures of labeled and unlabeled acetyl-CoA carboxylase. The level of nonspecific precipitation obtained by either method was always less than 10% of the total radioactivity precipitated by antibody.

In experiments in which the concentration of acetyl-CoA carboxylase in the antibody precipitation reactions was varied by diluting solutions of enzyme with normal NaCl solution, it was shown that the precipitation of enzyme as measured either by total protein or radioactivity precipitated by antibody was independent of enzyme concentration to levels of enzyme of less than 100 milliunits per ml. In all of the experiments in which H-leucine incorporation was measured, the concentration of radioactive enzyme solution was less than 150 milliunits per ml, unlabeled acetyl-CoA carboxylase was added to ensure that large enough precipitates were formed to minimize losses. Thus, acetyl-CoA carboxylase could be quantitatively precipitated from extracts of different initial specific activity.

**RESULTS**

Initial attempts to obtain sufficient quantities of rat liver acetyl-CoA carboxylase to use as antigen were unsuccessful due to the instability of this enzyme at the later stages of purification. Therefore the more stable chicken liver acetyl-CoA carboxylase was purified to homogeneity and used to immunize rabbits. The specific activity of the chicken liver enzyme obtained was 6 units per mg, a value two-thirds that obtained by Gregolin et al. (8). This difference can be explained by the different assay conditions used, since the intrinsic specific activity which we obtain based on biotin content is also one-third lower than that obtained by Gregolin et al. (8). As shown in Fig. 1, there is good correspondence between protein content and activity on sucrose gradients, and in other experiments it was shown that all of the protein could be converted to a form sedimenting at 12 S in Tris buffer, further indicating that the preparation was homogeneous.

The chicken liver acetyl-CoA carboxylase was used as antigen in the hope that the antibody produced would cross-react with rat acetyl-CoA carboxylase.

**Characterization of Antibody to Acetyl CoA Carboxylase.**—After immunization as described above, rabbits were bled at weekly intervals to determine the presence of antibody. Antibody was assayed by its ability to inhibit acetyl-CoA carboxylase activity in the standard assays. As shown in Fig. 2, acetyl-CoA carboxylase activity was not affected by normal rabbit serum, whereas within 2 weeks after immunization inhibitory activity appeared in the serum of rabbits given injections. The relative amount of antibody contained in serum was estimated by extrapolating the initial slope of inhibition produced by increasing amounts of rabbit serum as shown in Fig. 2. Peak levels of...

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**Fig. 2. Inhibition of chicken liver acetyl-CoA carboxylase by immune rabbit serum.** Reaction mixtures contained final concentrations of 0.05 M Tris-HCl, pH 7.5, 0.02 M MgCl₂, 0.02 M potassium citrate, 0.01 M 2-mercaptoethanol, 1 mg per ml of bovine serum albumin, 2.5 milliunits of enzyme, and the indicated amounts of immune (●—●) or control (▲—▲) rabbit serum in 0.2 ml. After incubation at 37°C for 20 min, acetyl-CoA carboxylase activity was measured by addition of ATP, acetyl-CoA, and KH³¹CO₃, as described previously (7).
antibody were obtained after 4 to 5 weeks as determined in this manner. It is clear that the antibody inactivates acetyl-CoA carboxylase without the necessity for precipitation since these inhibition studies were performed without centrifugation of the preliminary incubation mixture of enzyme and antibody. When reaction mixtures were centrifuged at 50,000 × g for 10 min after prior incubation and the supernatant solution was subsequently assayed, there was a more marked inhibition of enzyme activity at low levels of antibody. This result suggests that in the presence of excess enzyme, with relatively few antibody molecules bound to each mole of enzyme, the antibody-enzyme complex retains some catalytic activity. However, when excess antibody was present, there was no activity present in the suspended antibody enzyme complexes, indicating that additional binding of antibody leads to loss of catalytic activity. When rabbit antiserum was added to rat liver, acetyl-CoA carboxylase inhibition similar to that shown in Fig. 2 was seen. Thus, the antibody produced inactivated enzyme from both rat and chicken. Fig. 3 shows the Ouchterlony double diffusion analysis of rabbit antiserum with homogeneous chicken liver enzyme and the 105,000 × g supernatant from rats fed a fat-free diet (having relatively high acetyl-CoA carboxylase levels). There was a single precipitation band formed with both rat and chicken enzyme with a line of complete identity indicating that the antibody produced against chicken enzyme reacts well with rat acetyl-CoA carboxylase. With some batches of antibody there was slight spurring present, indicating some antigenic differences between the two enzymes. This experiment also suggests that a single protein is precipitated from crude rat liver by this antibody preparation.

The results of a quantitative precipitin reaction with the use of homogeneous chicken liver enzyme and partially purified rat liver enzyme are shown in Fig. 4. There was an equivalent precipitation of both enzymes, again indicating the high degree of cross-reactivity of the two enzymes with the antiserum. Furthermore, when the enzyme activity of the supernatant solution after removal of the antibody-enzyme complex was measured, identical equivalence points for enzyme from the two sources were found. Enzyme (200 milliunits) from either chicken or rat was precipitated by 0.05 ml of rabbit antiserum, as shown in Fig. 4. This indicates that acetyl-CoA carboxylase from rat and chicken liver has essentially identical intrinsic specific activities since antibody precipitates equal amounts of both enzymes as measured by either mass or activity. Assuming a specific activity for the homogeneous enzyme of 6 units per mg of protein, it can be calculated that at equivalence there are approximately 15 moles of antibody bound per mole of proteom-
eter (molecular weight, 400,000 (16)). This suggests that there must be multiple antigenic sites on the enzyme and that there is a relatively large area available for antibody binding.

In order to use antibody to determine the amount of acetyl-CoA carboxylase present in rat liver, it was necessary to indicate that antibody could precipitate all forms of the enzyme. Should antibody precipitate only the active form of the enzyme, it would be of no use in determining the total amount of enzyme, both active and inactive, in crude extracts. Acetyl-CoA carboxylase is known to occur both as an inactive 400,000 molecular weight protomeric form as well as an aggregated active 4 to 8 × 10^6 molecular weight form (16). The inactive protomer can be converted to the active aggregated form in the presence of citrate and other tricarboxylic acids (6).

As noted above, palmitoyl-CoA inhibits acetyl-CoA carboxylase by competing with citrate and converting the enzyme to an inactive protomeric form. Rat liver enzyme was prepared in each of these forms, and the quantitative precipitin reactions of each were compared (Fig. 5). This experiment indicates that the antibody can precipitate both aggregated and disaggregated enzyme as well as palmitoyl-CoA-treated enzyme.

**Effect of Antibody on Fatty Acid Synthetase Activity**—Since it has been proposed that the fatty acid synthetase multienzyme complex may be associated with acetyl-CoA carboxylase in vivo (17), and, further, because the liver content of fatty acid synthetase has been shown to vary with diet in a manner similar to that seen with acetyl-CoA carboxylase, we next tested the effect of antibody to acetyl-CoA carboxylase on fatty acid synthetase activity in crude 105,000 × g rat liver supernatant solutions. No inhibition or apparent precipitation of fatty acid synthetase activity occurred while acetyl-CoA carboxylase activity was completely inhibited (Table I). Although this result does not exclude association in vivo of the fatty acid synthetase complex with acetyl-CoA carboxylase, it does indicate that the two activities do not coprecipitate with this antibody preparation.

**Acetyl-CoA Carboxylase Content of Rats Fed Various Diets**—Rats weighing 125 g were subjected to four different dietary treatments in order to obtain liver homogenates with different specific activities for acetyl-CoA carboxylase. The specific activity of enzyme in these homogenates was determined as described under "Methods." Rats fed a fat-free diet for 48 hours had enzyme with a specific activity of 13.8 milliunits per mg. The corresponding specific activities in crude 105,000 × g supernatants from rats fed a high fat diet, Purina rat chow, and fasted for 48 hours were 0.57 milliunit per mg, 6.0 milliunits per mg, and 2.0 milliunits per mg, respectively. Increasing amounts of each of these preparations were assayed in the presence of 0.5 µl of rabbit antiserum, and the acetyl-CoA carboxylase activity in the supernatant solution was measured after centrifugation of the preliminary incubation mixtures. No activity was found in supernatant solutions from any of the preparations tested until 0.8 to 0.9 milliunit of enzyme was added, at which point activity was found in all four preparations upon further addition of enzyme (Fig. 6). On the basis of enzyme activity, therefore, all four preparations have the same equivalence point (the point at which activity first appears in the supernatant solution) despite the 25-fold variation in specific activity. Thus, although the amount of protein actually added to the reaction mixtures varied over 25-fold at the equivalence point, the amount of enzyme activity contained in each preparation accurately reflected the amount of immunologically reactive enzyme present. This experiment indicates that the differences in specific activity of acetyl-CoA carboxylase seen upon dietary manipulation are actually due to different amounts of enzyme in the livers of these animals. Thus, the changes in liver acetyl-CoA carboxylase activity in animals fed different diets can be entirely explained on the basis of changing amounts of enzyme, and inhibition of pre-formed enzyme by increased levels of palmitoyl-CoA does not play a role in this phenomenon as measured in vitro.

**Leucine Incorporation into Acetyl-CoA Carboxylase**—The rates of acetyl-CoA carboxylase synthesis and degradation were examined under conditions of fasting and fat-free feeding in order to determine the mechanism by which the altered enzyme levels.

### Table I

<table>
<thead>
<tr>
<th>Antiserum added (µl)</th>
<th>Acetyl-CoA carboxylase activity</th>
<th>Fatty acid synthetase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8.2</td>
<td>8.0</td>
</tr>
<tr>
<td>0.4</td>
<td>2.2</td>
<td>8.8</td>
</tr>
<tr>
<td>1.2</td>
<td>0.3</td>
<td>8.8</td>
</tr>
<tr>
<td>4.8</td>
<td>0</td>
<td>8.7</td>
</tr>
</tbody>
</table>

### Effect of antiserum on fatty acid synthetase

Reaction mixtures containing 1.0 µg of protein from a 105,000 × g rat liver supernatant solution were initially incubated as described in Fig. 2 with the indicated amounts of antibody for 20 min at 37°. After subsequent centrifugation at 50,000 × g for 10 min, the supernatant solution was assayed for acetyl-CoA carboxylase activity. Alternatively, the supernatant solution after prior incubation with antibody was assayed for fatty acid synthetase activity.
seen in these conditions were obtained. These experiments were carried out by means of techniques similar to those used by Schimke (18) in studying inducible enzymes of amino acid catabolism. For these experiments two rats initially weighing 140 g were given injections of 500 μCi of 3H-leucine; 40 min later the livers were removed and homogenized, and acetyl-CoA carboxylase was determined as described under "Methods." The incorporation of 3H-leucine into both total liver protein and acetyl-CoA carboxylase activity and leucine incorporation into acetyl-CoA carboxylase were determined as described under "Methods."

![Graph](Fig. 6) Immunological analysis of acetyl-CoA carboxylase in rat liver extracts. The 105,000 X g liver supernatant solutions pooled from three rats fed different diets were filtered over Sephadex G-25 as described under "Methods." Aliquots of each of these preparations were assayed by adding 0.5 μl of rabbit anti-serum to the initial supernatant solutions as described in the legend of Fig. 2. After prior incubation these mixtures were centrifuged at 50,090 X g for 10 min and the acetyl-CoA carboxylase activity in the resulting supernatant solution was measured. □-□, high-fat (45% fat) fed for 1 week; ■-■, fasted 48 hours, ○-○, Purina rat chow fed, ●-●, fat-free fed 72 hours.

Table II

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Mean wt</th>
<th>Acetyl-CoA carboxylase activity</th>
<th>3H-Leucine incorporation into acetyl-CoA carboxylase</th>
<th>Total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>105,000 X g supernatant</td>
<td>DEAE-cellulose eluate</td>
<td>Total</td>
</tr>
<tr>
<td>Protein</td>
<td>mg</td>
<td>units</td>
<td>units</td>
<td>cpm</td>
</tr>
<tr>
<td>Fat free 72 hrs → fast 48 hrs</td>
<td>145</td>
<td>11.0</td>
<td>17.5</td>
<td>17.2</td>
</tr>
</tbody>
</table>

Table III

Rats were fed as indicated in groups of three. The group of rats fasted 48 hours were given injections of 500 μCi of 3H-leucine. Experiments 1 and 2 were carried out together with a single batch of leucine whereas Experiment 3 was performed subsequently with a different lot of leucine. See "Methods" for experimental details.

![Table](Table III) Synthesis of acetyl-CoA carboxylase

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Mean wt</th>
<th>Enzyme activity</th>
<th>Acetyl-CoA carboxylase total</th>
<th>3H-Leucine incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>units/mg</td>
<td>units</td>
<td>cpm</td>
</tr>
<tr>
<td>1. Fasted 60 hours</td>
<td>170</td>
<td>0.95</td>
<td>860</td>
<td>3920</td>
</tr>
<tr>
<td>2. Fat-free fed 48 hours</td>
<td>176</td>
<td>14.0</td>
<td>8740</td>
<td>2960</td>
</tr>
<tr>
<td>3. Fat-free fed 48 hours</td>
<td>178</td>
<td>10.3</td>
<td>3820</td>
<td>2920</td>
</tr>
</tbody>
</table>
rate constant for synthesis, as noted previously by Schimke (18). Fat-free fed animals even greater than our estimate, although it greater with more rapidly degraded proteins, thus tending to guanido-labeled arginine, which is not reutilized, is 5 days (20, however, it seems unlikely that this provides a major error since the true half-life due to some reutilization of leucine; estimate the half-lives, and the degree of overestimation is 21). As noted by Koch (22), reutilization of isotope leads to varied from 3 to 4 days and the half-life estimate by the use of this isotope is approximately 18 hours. These values may overestimate the true half-life due to some reutilization of leucine; however, it seems unlikely that this provides a major error since the half-life estimate by the use of guanido-labeled arginine, which is not reutilized, is 5 days (20, 21). As noted by Koch (22), reutilization of isotope leads to overestimated half-lives, and the degree of overestimation is greater with more rapidly degraded proteins, thus tending to make the differences in rate of degradation between fasted and fat-free fed animals even greater than our estimate, although it seems unlikely that this is of major significance since our estimates predict accurately the changes in enzyme activity actually observed. Under steady state conditions it is possible to calculate the rate constant for synthesis, as noted previously by Schimke (18). In the above experiment the rats fed a fat-free diet approximate the differences in rate of enzyme synthesis under these conditions. Furthermore, the differences in synthetic rate between the two groups of rats with the use of this technique are in general agreement with the relative differences in leucine incorporation shown in Tables II and III. Thus, it appears that in shifting from fasting to fat-free feeding there was approximately a 6-fold increase in the rate of enzyme synthesis, as well as approximately a 2.5-fold fall in the rate of enzyme degradation.

When rats are fed Purina rat chow, acetyl-CoA carboxylase levels intermediate between fasting and fat-free fed levels are seen. When the turnover of enzyme was measured in rats fed this diet, the results shown in Fig. 8 were obtained. Since the rats used were presumably in a steady state, the results are plotted as counts per min incorporated into acetyl-CoA carboxyl-
ase per g of soluble liver protein. Thus, the $t_1$ for degradation of acetyl-CoA carboxylase in rats fed this diet is 50 hours, a value similar to that found in rats fed a fat-free diet. This result suggests that the fat content of the diet does not influence the degradation rate of acetyl-CoA carboxylase although the synthetic rate is affected.

The rate of acetyl-CoA carboxylase synthesis calculated from Fig. 8 was 0.4 pg per hour per rat, a value intermediate between levels found in fasted and fat-free fed rats.

**DISCUSSION**

In a previous study of acetyl-CoA carboxylase activity in liver and hepatomas we demonstrated (7) that, whereas marked changes in enzyme activity occur in liver after fat-free feeding of fasted animals, no change in activity occurred in the hepatomas of these same animals. Comparison of acetyl-CoA carboxylase purified from liver and hepatomas suggested that the two enzymes were identical and that the altered responsiveness in tumors could not be explained on the basis of a tumor enzyme with alteration in either activation by citrate or inhibition by palmitoyl-CoA or malonyl-CoA. This suggested that the differences in acetyl-CoA carboxylase activity seen in livers from rats fed various diets could be due to actual changes in enzyme content. Thus, in liver the rate of enzyme synthesis or degradation changes upon dietary manipulation whereas in tumors no changes occur.

The present study has verified the hypothesis indicating that the changes in acetyl-CoA carboxylase activity as measured in vivo which occur with fat-free feeding and fasting can be entirely explained by changes in the enzyme content in liver rather than by activation or inhibition of pre-formed enzyme. This conclusion is subject to the reservation that liver does not contain a form of acetyl-CoA carboxylase which is both inactive and immunologically unreactive. Although our experiments indicate that acetyl-CoA carboxylase may be quantitatively precipitated from extracts containing either aggregated or disaggregated enzyme, they do not exclude the possibility that antibody preferentially binds to one form of the enzyme. Thus, it is possible that antibody binding to one form of the enzyme shifts the equilibrium between protomer and polymer until all of the enzyme is precipitated in the preferred form. Studies of direct binding of $^{131}I$-labeled antibody to different forms of the enzyme yielded large aggregates even when a great excess of enzyme was used. Thus, it will be necessary to prepare Fab antibody fragments to perform satisfactory binding experiments.

Numerous other factors may influence the activity of acetyl-CoA carboxylase in vivo (23). These include availability of Krebs cycle acids (24), inhibition by long chain acyl-CoA compounds (4, 25), long chain acylcarnitine levels (26), and other as yet unknown factors. Korchak and Masoro (27) have shown that fatty acid synthesis in liver slices from rats fasted for 24 hours is depressed more than can be accounted for by the acetyl-CoA carboxylase activity as measured in extracts of these slices. This discrepancy may be explained by one or more of the above factors or by other factors which operate in the intact cell, such as the rate of utilization of fatty acid for lipid synthesis. Our results on the rates of synthesis and degradation of acetyl-CoA carboxylase suggest that the actual level of enzyme present in liver is probably important in regulating the level of fatty acid synthesis on a day-to-day basis, whereas more short term regulation of this pathway cannot be accomplished by this mechanism. Thus, the 18-hour half-life of acetyl-CoA carboxylase in fasted animals precludes changes in enzyme content accounting for rapid changes in the ability to synthesize fatty acids.

Although the present study has indicated that fat-free feeding induces both an increased rate of enzyme synthesis as well as a stabilization or decrease in enzyme turnover, it has not elucidated the mechanism by which these changes occur. The compound which induces the acetyl-CoA carboxylase after fat-free feeding is unknown. Allman et al. (2) have shown that linoleate levels parallel the changes in fatty acid synthesis induced by diet. After fat-free feeding, linoleate levels in liver fall rapidly; dietary supplementation with small amounts of linoleate can prevent the full induction of fatty acid synthesis whereas feeding other lipids has no effect. Whether linoleate acts directly to inhibit enzyme synthesis or whether its effect is secondary to some other effector is unknown.

Experiments with rats fed Purina rat chow indicate that the effects on acetyl-CoA carboxylase synthesis and degradation can be dissociated. Thus, in comparing these rats to fat-free fed animals, no significant difference in enzyme turnover is noted although the synthetic rates vary. This result indicates that separate factors may regulate the synthesis and degradation of acetyl-CoA carboxylase.

The degradation rate ($t_1$) for liver acetyl-CoA carboxylase in these studies varied from 18 to 50 hours in rats fed different diets. These degradation rates are slower than degradation rates of enzymes such as tryptophan pyrrolase ($t_1$, 2 hours) or tyrosine-glutamic transaminase ($t_1$, 2 hours), whereas other enzymes such as arginase ($t_1$, 96 hours) are degraded more slowly than acetyl-CoA carboxylase (18). It should be emphasized that rats weighing 125 to 175 g were used for all of the experiments on acetyl-CoA carboxylase. Preliminary experiments suggest that synthetic and degradation rates may vary according to the size and age of the animal. Thus, synthetic rates for acetyl-CoA carboxylase are somewhat higher in younger rats. Schmeke has obtained a mean $t_1$ for degradation of total liver protein with the use of guanido-labeled arginine of 3.3 days in rats weighing 80 to 100 g, whereas in rats weighing 125 g a value of 4 to 5 days was obtained (20, 21).

In all studies reported thus far, the changes in acetyl-CoA carboxylase and fatty acid synthetase activities which occur after either dietary manipulation or diabetes are coordinate. That is true also of the hepatomas which have been studied. Although the enzyme levels in hepatomas are fixed and insensitive to changes in diet, the enzyme levels in each hepatoma line are different from any other and are constant and characteristic for each tumor. The acetyl-CoA carboxylase and fatty acid synthetase activities in each tumor are always relatively alike. When the acetyl-CoA carboxylase level is 80% of its fully induced level, suggesting that even in hepatomas the fatty acid-synthesizing enzymes remain coordinate. This consistent coordinate behavior of the enzymes of fatty acid synthesis suggests that the enzymes of this pathway may constitute an operon (28) or its mammalian equivalent.

It would be interesting to compare the rates of synthesis and degradation of fatty acid synthetase with those of acetyl-CoA carboxylase in order to verify this hypothesis. Burton, Collins, and Porter, (29) in a preliminary report, have noted that the rise in fatty acid synthetase activity following refeeding fasted rats
is associated with increased leucine incorporation into fatty acid synthetase, indicating that increased enzyme synthesis does occur. More detailed analysis in parallel with studies of acetyl-CoA carboxylase is clearly indicated. In current experiments we are comparing the rates of acetyl-CoA carboxylase synthesis and degradation in hepatomas. Preliminary results indicate that the level of enzyme activity in hepatomas parallels the content of immunologically reactive acetyl-CoA carboxylase in these tumors. Furthermore, whereas a marked increase in acetyl-CoA carboxylase synthesis is seen after fat-free feeding in liver from tumor-bearing animals, no change in rate of synthesis occurred in the tumors of these same animals.

Acknowledgments—We wish to thank Drs. C. K. Osterland, C. Parker, and H. Eisen for their helpful suggestions.

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J. Biol. Chem. 1969, 244:6254-6262.

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