The Regulation of Fatty Acid Biosynthesis in Rat Hepatomas*

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SUMMARY

The dietary regulation of fatty acid synthesis de novo in two transplantable hepatomas, 7777 and 9618A, has been studied by comparison of the acetyl coenzyme A carboxylase and fatty acid synthetase activities of tumor and host liver, both in animals fasted 48 hours and in those subsequently refed a fat-free diet for 48 hours. Neither acetyl-CoA carboxylase nor fatty acid synthetase of these hepatomas was subject to the changes in enzyme concentration normally observed in host liver following dietary alteration. Thus, in livers of refed animals acetyl-CoA carboxylase activity was 12- to 29-fold greater than in the livers of fasted animals. Neither tumor studied showed any change in enzyme levels upon refueling 48-hour fasted animals; 1.4 mmol of malonyl-CoA were formed per mg per min for hepatoma 7777 and 3.3 mmol of malonyl-CoA were formed per mg per min for hepatoma 9618A. These activities of enzyme are 5- to 10-fold greater than fasting levels in host liver.

Fatty acid synthetase levels in host livers increased 1- to 20-fold after refueling. Again, neither tumor showed significant changes in enzyme activity upon refueling fasted animals. Both acetyl-CoA carboxylase and fatty acid synthetase from hepatoma were inhibited by palmitoyl-CoA despite their lack of response to dietary alteration.

Acetyl-CoA carboxylase was purified 104-fold from rat liver and 67-fold from hepatoma 7777 to define further this lack of regulation. Studies of the purified enzymes disclosed that the tumor enzyme was essentially identical with that derived from liver in terms of heat inactivation, affinities for acetyl-CoA and ATP, activation and aggregation by citrate, product inhibition by malonyl-CoA, pH optima, and intrinsic specific activity as determined from biotin content.

These experiments suggest that the acetyl-CoA carboxylase from the tumors is the same enzyme as that from host liver, and that the increased activity in the tumors is due to an increased amount of enzyme rather than a structurally altered enzyme.

The well differentiated rat hepatomas have been the subject of extensive investigation because of the opportunity which these tumors present to compare the biological properties of a tumor to that of the hepatic cell of origin within the same host (1, 2). These tumors have been called "minimal deviation" hepatomas because of morphological and biochemical similarity to normal liver, yet no two are alike (3, 4). Previous studies have attempted to define the essential alteration which leads to malignancy. It is obvious that malignant cells have altered control mechanisms which lead to uncontrolled cell division but the molecular deviation which leads to this condition remains obscure.

Although great biochemical variation occurs in the hepatomas studied previously, one striking finding has been a loss of regulation of cholesterol biosynthesis in these tumors. Siperstein has shown that inhibition of hepatoma cholesterol biosynthesis upon feeding rats cholesterol does not occur while inhibition does occur in the host. This finding has been consistent in 14 rat hepatomas, one mouse tumor, and two human hepatomas (5, 6).

Further derangement in the control of lipid metabolism is suggested by studies of Sabino et al. (7) and of Elwood and Morris (8) in which the incorporation of acetate into fatty acids was measured in rat hepatomas. Thus, in normal rats the incorporation of acetate into fatty acids was markedly inhibited by fasting and stimulated after refueling. These authors have shown that the rates of acetate incorporation into tumor fatty acids are not affected by dietary alterations in each of five well differentiated hepatomas.

We have attempted to define the mechanism of this alteration of control of fatty acid synthesis in these tumors by study of the enzymes of fatty acid synthesis. These studies have shown that...
the levels of both acetyl-CoA carboxylase and of fatty acid synthetase in two hepatomas do not change following dietary alteration. Further studies with purified acetyl-CoA carboxylase from rat tumors show that the tumor enzyme is the same enzyme as that present in normal liver.

**EXPERIMENTAL PROCEDURE**

**Methods**

**Animals and Diets**—Hepatomas 7777 (Generations 30, 32 and 34) and 9618A (Generation 3) from male Buffalo strain rats were used in these studies. Tumor cells were inoculated intramuscularly into both hind legs and the rats were shipped by air to St. Louis shortly after tumor implantation. Animals were fed Purina chow until the tumors reached a size of 0.5 to 1.5 cm in diameter and then were transferred to individual cages for the various dietary experiments. Fat-free diet was purchased from Nutritional Biochemicals. In preliminary experiments it was shown that acetyl-CoA carboxylase and fatty acid synthetase activity in liver rose progressively after refeeding fasted animals, reaching a peak by 48 hours and remaining at this level thereafter. Thus, 48 hours of refeeding were used in the dietary experiments.

**Preparation and Assay of Enzymes**—The rats were stunned by a blow on the head and, following cervical dislocation, the tumors and livers were quickly removed. Tissues were minced and then homogenized with three strokes in a Potter-Elvehjem homogenizer in buffer (12) containing 0.1 M potassium phosphate (pH 7.5), 0.07 M potassium bicarbonate, 0.1 mM EDTA, and 0.01 M 2-mercaptoethanol at 0°. The homogenate was adjusted to 0.25 M sucrose and centrifuged at 49,000 × g for 10 min. The supernatant solution was recentrifuged at 60,000 × g for 60 min and the resulting supernatant solution was used to determine fatty acid synthetase activity. An aliquot of the crude supernatant was passed through a column of G-25 Sephadex and the protein fraction was used to determine acetyl-CoA carboxylase activity. Protein was measured by the method of Lowry et al. (9).

**Fatty Acid Synthetase Assay**—Fatty acid synthetase was measured as incubations were for 15 min at 37°.

**Acetyl-CoA Carboxylase Assay**—The activity of this enzyme was assayed in two ways. The first, with the Sephadex-treated crude supernatant, measures malonyl-CoA formation from acetyl-CoA directly. Reaction mixtures contained 20 μmoles of Tris-HCl (pH 7.5), 6 μmoles of magnesium chloride, 7 μmoles of potassium citrate, 0.4 μmole of 2-mercaptoethanol, 0.3 mg of bovine serum albumin, and enzyme in a volume of 0.26 ml. After preincubation at 37° for 30 min, 0.7 μmole of ATP, 0.05 μmole of acetyl-CoA, and 6.0 μmoles of 14C potassium bicarbonate (0.6 μc per μmole) were added, yielding a total volume of 0.33 ml. After incubation at 37° for 5 min, 0.05 ml of 10% perchloic acid was added. After centrifugation, 0.1 ml of this 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 purified yeast fatty acid synthetase, as described by Matsushashi, Matsushashi, and Lynen (11).

**Purification of Acetyl-CoA Carboxylase—Acetyl-CoA carboxylase** was purified from both livers and tumors by a procedure modified from that used by Matsushashi, Matsushashi, and Lynen (11).

Fifty male Sprague-Dawley rats weighing approximately 200 g were fasted for 48 hours and then refed a fat-free diet for 48 hours prior to removal of the livers. The livers were removed and homogenized and the crude supernatant solution was obtained as described above. The protein concentration of this solution was adjusted to 25 mg per ml with the same buffer. Solid ammonium sulfate was then added to this solution at 4° with stirring to a final concentration of 8 g/100 ml. After stirring for 15 min, the solution was centrifuged at 49,000 × g for 15 min and the precipitate was discarded. Ammonium sulfate, 8.2 g/100 ml, was again added and, after stirring and centrifugation, the precipitate was dissolved in a minimum volume of 0.02 M potassium phosphate, pH 7.5. This buffer and all subsequent buffers used in the purification also contained 0.01 M 2-mercaptoethanol and 0.1 mM EDTA. This solution was diluted 5-fold in 0.1 mM EDTA-0.01 M 2-mercaptoethanol, and calcium phosphate gel (0.75 g of gel per g of protein) was added with stirring. After centrifugation at 2,000 × g for 6 min, the supernatant was discarded and the gel was eluted with 100 ml of 0.02 M potassium phosphate, pH 7.5. This elution was repeated twice and the eluates were discarded. The gel was next eluted five times with 0.10 M potassium phosphate, pH 7.5. These eluates were pooled and solid ammonium sulfate was added to a final concentration of 16.5 g/100 ml. After centrifugation the precipitate was dissolved in 0.04 M potassium phosphate, pH 7.5, and was dialyzed against the same buffer for 3 hours. This solution was then diluted slightly to a conductivity of 4 mho at 4° and poured over a column of DEAE-cellulose (Whatman DE 23) which had been previously equilibrated with 0.04 M potassium phosphate, pH 7.5. (The column load was 8 g protein per liter of bed volume with columns with 10 to 20 times height to diameter.) The column was first washed with 1 column volume of 0.04 M potassium phosphate, pH 7.5, and then eluted with 10 column volumes of phosphate buffer in a linear gradient from 0.04 M to 0.2 M. Fractions were collected and those containing the maximum acetyl-CoA carboxylase activity were pooled and solid ammonium sulfate was added to a final concentration of 16.5 g/100 ml. The precipitate was collected by centrifugation and dissolved in a minimum volume of 0.05 M potassium phosphate, pH 7.0. The results of the purification of the liver enzyme are summarized in Table I. The same procedure was used to purify acetyl-CoA carboxylase from the hepatoma 7777. In this case 50 male Buffalo rats bearing tumors with a diameter of 0.5 to 1.5 cm were used without previous dietary alterations. The tumors were carefully dissected free from adherent muscle prior to homogenization. The results of this purification are shown in Table II.

**Materials**

Malonyl-CoA (12) and acetyl-CoA (13) were synthesized as were the 14C-acetyl and 14C-malonyl derivatives. Yeast fatty acid synthetase was purified by the method of Lynen through the calcium phosphate gel step (14). Calcium phosphate gel was prepared by the method of Keilin and Hartree (15). Palmitoyle-CoA was synthesized by the method of Goldman and Vagelos (16). Other substrates and cofactors were obtained from commercial sources. Lactobacillus plantarum 8014 obtained from the American Type Culture Collection was used to assay biotin.
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First ammonium sulfate

Crude supernatant

Calcium phosphate gel

Second ammonium sulfate

DEAE-cellulose column plus third ammonium sulfate

zyme pooled from two to four rats. Control rats were Buffalo rats of the same age as tumor-bearing animals.

The dietary regulation of fatty acid synthesis de novo was investigated in two transplantable hepatomas, 9618A which is a highly differentiated and very slowly growing hepatoma (7 months average generation time) and 7777 which is less differentiated and rapidly growing (1.5 months average generation time).

**Acetate-μmol of 14CO2 fixed into malonyl-CoA per min at 37°C.

**Crude supernatant

First ammonium sulfate

Calcium phosphate gel

Second ammonium sulfate

DEAE-cellulose column plus third ammonium sulfate

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**One unit is 1 μmol of 14CO2 fixed into malonyl-CoA per min at 37°C.

### TABLE II

**Acetate-CoA carboxylase purification from hepatoma 7777**

<table>
<thead>
<tr>
<th>Step</th>
<th>Total activity units</th>
<th>Specific activity (μmol/mg protein)</th>
<th>Fold purified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude supernatant</td>
<td>77</td>
<td>0.00136</td>
<td>5.4</td>
</tr>
<tr>
<td>First ammonium sulfate</td>
<td>60</td>
<td>0.0073</td>
<td>18</td>
</tr>
<tr>
<td>Calcium phosphate gel</td>
<td>31.6</td>
<td>0.0245</td>
<td></td>
</tr>
<tr>
<td>DEAE-cellulose column plus second and third ammonium sulfate</td>
<td>8.9</td>
<td>0.091</td>
<td>67</td>
</tr>
</tbody>
</table>

### TABLE III

**Effect of diet on acetate-CoA carboxylase activity in hepatomas**

Acetate-CoA carboxylase activity was measured by the CO2 fixation assay described under "Experimental Procedure." Animals were fasted for 48 hours with water and normal 0.9% NaCl ad libitum, and, where indicated, refed a fat-free diet for 48 hours. Each value represents the activity of the enzyme pooled from two to four rats. Control rats were Buffalo rats of the same age as tumor-bearing animals.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Dietary manipulation</th>
<th>Ratio (2:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Fasted 48 hrs</td>
<td>2. Fasted-fat-free diet 48 hrs</td>
</tr>
<tr>
<td></td>
<td>mmoles malonyl-CoA formed/mg/min</td>
<td></td>
</tr>
<tr>
<td>Control rat liver</td>
<td>0.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Control rat liver</td>
<td>0.3</td>
<td>6.0</td>
</tr>
<tr>
<td>7777 tumor</td>
<td>1.67</td>
<td>1.23</td>
</tr>
<tr>
<td>7777 host liver</td>
<td>0.23</td>
<td>6.7</td>
</tr>
<tr>
<td>9618A tumor</td>
<td>3.2</td>
<td>3.5</td>
</tr>
<tr>
<td>9618A host liver</td>
<td>0.37</td>
<td>4.3</td>
</tr>
</tbody>
</table>

### TABLE IV

**Effect of diet on fatty acid synthetase activity in hepatomas**

Fatty acid synthetase activity was measured as described under "Experimental Procedure." The other conditions were as described in Table III.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Dietary manipulation</th>
<th>Ratio (2:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Fasted 48 hrs</td>
<td>2. Fasted-fat-free diet 48 hrs</td>
</tr>
<tr>
<td></td>
<td>mmoles 14C-malonyl-CoA incorporated into fatty acids/mg/min</td>
<td></td>
</tr>
<tr>
<td>Control rat liver</td>
<td>1.3</td>
<td>10.1</td>
</tr>
<tr>
<td>Control rat liver</td>
<td>1.5</td>
<td>18.1</td>
</tr>
<tr>
<td>7777 tumor</td>
<td>0.82</td>
<td>1.12</td>
</tr>
<tr>
<td>7777 host liver</td>
<td>0.83</td>
<td>16.9</td>
</tr>
<tr>
<td>9618A tumor</td>
<td>3.7</td>
<td>4.9</td>
</tr>
<tr>
<td>9618A host liver</td>
<td>0.56</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Biotin was hydrolyzed from the enzyme in 3.6 N H2SO4 for 1 hour at 120°C with internal biotin standards used to correct for losses during hydrolysis.

### RESULTS

The dietary regulation of fatty acid synthesis de novo was investigated in two transplantable hepatomas, 9618A which is a highly differentiated and very slowly growing hepatoma (7 months average generation time) and 7777 which is less differentiated and rapidly growing (1.5 months average generation time).
from these studies that the control of both fatty acid synthetase and acetyl-CoA carboxylase is altered in these tumors. Since acetyl-CoA carboxylase is rate-limiting for fatty acid synthesis in vitro, and since this enzyme appears to be a regulatory enzyme (19), it was purified to study the mechanism of the altered control in these tumors.

Purification of Acetyl-CoA Carboxylase—Because the enzyme is unstable, the purification of this enzyme from both liver (Table I) and tumor (Table II) proved difficult. It was most stable when stored in 0.02 to 0.05 M phosphate buffer, pH 7.5, frozen in liquid nitrogen. Under these conditions, about 50% of enzyme activity was lost in 1 month with frequent freezing and thawing while enzyme not thawed remained stable. At both 4° and 20°, 80 to 100% of its activity was lost in 1 week.

The liver enzyme obtained from rats refed the fat-free diet was purified 164-fold. Because of the tendency of this enzyme to aggregate, it was difficult to assess its purity. Assuming that its biotin content is similar to the acetyl-CoA carboxylase from homogeneous chicken enzyme (20). The enzyme from hepatoma 7777 was purified 164-fold. Because of the tendency of this enzyme to aggregate, it was difficult to assess its purity. Assuming that its biotin content is similar to the acetyl-CoA carboxylase from homogeneous chicken enzyme (20). The enzyme from hepatoma 7777 was purified only 67-fold, largely because of the poor recovery of enzyme obtained in the final stages of the purification.

Palmityl-CoA Inhibition—The mechanism of regulation of long chain fatty acid synthesis is unknown, but it has been suggested that palmityl-CoA is a feedback inhibitor of acetyl-CoA carboxylase activity (21). Recently the physiological significance of such inhibition has been questioned (22, 23). The effect of palmityl-CoA on acetyl-CoA carboxylase activity with tumor and liver enzyme is shown in Table V. Both enzymes are equally inhibited by palmityl-CoA, indicating that the lack of response of the tumor enzyme to starvation cannot be explained by the inability of this enzyme to be inhibited by high tissue levels of palmityl-CoA. In a similar experiment the effect of palmityl-CoA on the crude fatty acid synthetase from host liver and tumor was compared as shown in Table VI. Again, the hepatoma enzyme is equally inhibited by palmityl-CoA.

Heat Inactivation—Enzymes purified through the first ammonium sulfate fractionation were used for these experiments. As shown in Fig. 1, when the tumor and host liver enzymes were heated in the presence of citrate at 47° essentially identical rates of inactivation occurred with a t 1 of 4.1 min, suggesting that the two enzymes are identical. When the enzymes were heated in the absence of citrate, there was a biphasic inactivation curve with some differences between the liver and tumor enzymes. It is known that citrate activates acetyl-CoA carboxylase by causing an aggregation from an inactive protomeric form to an active polymeric form (19). The fact that the initial inactivation is more rapid in the absence of citrate and later parallels the rate in the presence of citrate suggests that the initial rapid inactivation in the absence of citrate represents denaturation of disaggregated enzyme, and the latter points (after 5 min) represent the small fraction of enzyme which is aggregated in the absence of citrate. The difference between the tumor and liver enzymes observed here may be due to isolation of the enzyme from the tumor in a more highly activated state as supported by the experiment recorded in Table VII in which host liver enzyme was completely dependent on citrate while tumor enzyme retained 24% of its activity in the absence of citrate. The requirements for the noncitrate-dependent portion of the activity in the tumor enzyme are seen to be the same as those for acetyl-CoA carboxylase. Furthermore, all of the counts per min fixed in the absence of citrate could be isolated by DEAE-cellulose chromatography as malonyl-CoA (10). Alternative interpretations for these data are that the tumor contains two acetyl-CoA carboxylase enzymes, one which requires citrate and a second which does not, or that the tumor contains a single enzyme which has been isolated in a partially activated form. The latter alternative is supported by an experiment in which the tumor enzyme was purified through the DEAE-cellulose column step and the activity of fractions was compared with and without citrate (Fig. 2). Citrate-dependent and citrate-independent activities cochromatograph on this column, suggesting that the tumor contains a single enzyme which has been isolated in a partially activated state. This hypothesis is further substantiated by the fact that, upon aging of the dilute enzyme, that portion of the activity not dependent upon citrate diminished. Furthermore, enzyme isolated from rats bearing hepatoma 7777 in some experiments, as well as from animals with 96188, failed to show this noncitrate-dependent component of activity. The reason that enzyme is sometimes isolated from hepatoma 7777 in a partially activated form remains obscure. The avian acetyl-CoA carboxylase is isolated in an active aggregated form under
FIG. 1. Heat inactivation of acetyl-CoA carboxylase from hepatoma 7777 and rat liver. Incubation mixtures contained 5 μmoles of potassium phosphate (pH 7.5), 0.1 mg of albumin, 0.3 μmole of manganese chloride, 0.5 mg of enzyme, and 1 μmole of citrate where indicated in a total volume of 0.1 ml. Tubes containing citrate were preincubated for 30 min prior to heating. The reaction mixtures were heated at 47°C and aliquots were removed for assay at various time points. ▲, 7777 enzyme; ◇, liver enzyme.

FIG. 2. DEAE-cellulose chromatography of acetyl-CoA carboxylase from hepatoma 7777. Conditions for chromatography are described under "Experimental Procedure." Aliquots of each fraction were assayed for acetyl-CoA carboxylase activity with (●) and without (◇) citrate.

FIG. 3. Sucrose density gradient sedimentation (25) of acetyl-CoA carboxylase from hepatoma 7777. Sucrose gradients (5 to 20%) were prepared in a Spinco gradient maker. Samples containing 0.30 mg of enzyme, 0.3 μmole of manganese chloride, and 1 μmole of citrate where indicated were layered on the gradients after preincubation of citrate-containing samples. Samples were sedimented at 50,000 rpm in an SW50 Spinco rotor for 200 min, and 22 fractions were collected from each tube. The tubes are numbered from the bottom of the tube. Values were estimated from an internal standard of catalase. ●, with citrate; ◇, minus citrate. Aliquots of fractions were assayed for acetyl-CoA carboxylase activity as described under "Experimental Procedure."

Table VII

Effect of citrate on acetyl-CoA carboxylase from hepatoma 7777 and host liver

<table>
<thead>
<tr>
<th></th>
<th>Plus citrate</th>
<th>Minus citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPM/μM, 5%</td>
<td>CPM/μM, 0%</td>
</tr>
<tr>
<td>Host liver</td>
<td>2657</td>
<td>30</td>
</tr>
<tr>
<td>Hepatoma 7777</td>
<td>1872</td>
<td>450</td>
</tr>
<tr>
<td>Hepatoma 7777-ATP</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Hepatoma 7777-MgCl₂</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Hepatoma 7777-acetyl-CoA</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

conditions in which the mammalian enzyme is inactive and disaggregated. From other studies it is clear that changes in pH, ionic strength, and salt concentration can all affect the state of aggregation of this enzyme (24).

Sucrose Gradient Sedimentation—The sucrose gradient sedimentation pattern of the hepatoma 7777 enzyme is shown in
comparisons of the acetyl-CoA carboxylase enzymes from hepatoma and rat liver, the kinetic properties of the purified enzyme from hepatoma 7777 and partially purified enzyme from hepatoma 9618A were compared to the highly purified rat liver enzyme and 9618A host liver partially purified enzyme. The results of these kinetic studies were plotted by the method of Lineweaver and Burk (26) as shown in Figs. 4, 5, and 6. Table VIII summarizes the Michaelis constants \( (K_m) \) derived from these and other experiments. The \( K_m \) values for Mg-ATP and Mn-ATP differed from each other significantly, but there was no significant difference in the values obtained for the liver and hepatoma enzymes. Similarly, the \( K_m \) values for another substrate, acetyl-CoA, vary no more than 2-fold within each experimental group, and all of the enzymes studied were activated by citrate at equal levels. Product inhibition by malonyl-CoA was also studied since it has been suggested recently that this inhibition may be of physiological significance in regulating fatty acid synthesis (27). In these experiments the purified liver enzyme was compared to hepatoma 7777 enzyme. In both cases malonyl-CoA inhibited the carboxylase reaction and the inhibition was competitive with acetyl-CoA as has been shown previously (27). The \( K_i \) for malonyl-CoA determined by the method of Dixon (28) was \( 4.5 \times 10^{-5} \) M for the hepatoma 7777 enzyme and \( 3.5 \times 10^{-5} \) M for the purified liver carboxylase.

**Table VIII**

Michaelis constants of acetyl-CoA carboxylase

<table>
<thead>
<tr>
<th>Substrate</th>
<th>9618A host liver</th>
<th>9618A hepatoma</th>
<th>Purified rat liver</th>
<th>7777 hepatoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn-ATP</td>
<td>( 3.0 \times 10^{-4} )</td>
<td>( 4.3 \times 10^{-4} )</td>
<td>( 8 \times 10^{-6} )</td>
<td>( 1.4 \times 10^{-4} )</td>
</tr>
<tr>
<td>Mg-ATP</td>
<td>( 1 \times 10^{-4} )</td>
<td>( 1 \times 10^{-4} )</td>
<td>( 1.6 \times 10^{-4} )</td>
<td>( 1.6 \times 10^{-4} )</td>
</tr>
<tr>
<td>Acetyl-CoA</td>
<td>( 8.3 \times 10^{-3} )</td>
<td>( 8.3 \times 10^{-3} )</td>
<td>( 8 \times 10^{-3} )</td>
<td>( 1 \times 10^{-3} )</td>
</tr>
<tr>
<td>Potassium citrate</td>
<td>( 8.3 \times 10^{-3} )</td>
<td>( 8.3 \times 10^{-3} )</td>
<td>( 8 \times 10^{-3} )</td>
<td>( 1 \times 10^{-3} )</td>
</tr>
</tbody>
</table>
These kinetic studies all support the conclusion that the enzyme derived from the hepatomas is the same as that derived from liver.

Further support for this hypothesis is obtained from determination of the biotin content of these enzymes. The 164-fold purified rat liver enzyme contained 0.12 mg of biotin per g of protein. Thus the intrinsic activity of this enzyme is 2080 moles of malonyl-CoA formation per mole of biotin per min at 37°C. The 67-fold purified enzyme from hepatoma 7777 contained 0.0099 mg of biotin per g of protein, yielding an intrinsic specific activity of 2220 moles of malonyl-CoA per mole of biotin per min at 37°C. Thus, the tumor and liver enzymes have almost identical intrinsic specific activity. These values obtained for rat liver are quite similar to the value of 3100 moles of CoA per mole of biotin per min at 37°C obtained by Gregolin et al. for the acetyl-CoA carboxylase from chicken liver (20).

In further experiments it was shown that the activity of the hepatoma 7777 enzyme paralleled that from liver with varying pH with the optimum at pH 7.5.

**Discussion**

Previous studies of the regulation of cholesterol biosynthesis in 14 different transplantable rat hepatomas have indicated a loss of the normal inhibition of this pathway by dietary cholesterol. While the mechanism of this inhibition is complex and not fully understood at the molecular level, Siperstein and Fagan have suggested that the defect lies in a structural alteration of the enzyme β-hydroxy β-methylglutaryl-CoA reductase resulting in loss of end product feedback inhibition (5, 6). Studies by Sabine et al. (7) and Elwood and Morris (8) suggest that this consistent abnormality in the regulation of cholesterol biosynthesis may also extend to another pathway of lipid biosynthesis, namely, fatty acid biosynthesis. They reported that acetate incorporation into the tumors did not vary with feeding and fasting in five different tumors tested. In the present study the lack of dietary regulation is shown in two additional transplantable hepatomas.

There are several possible explanations for the loss of the normal dietary control of this pathway. It is possible that the tumor utilizes a different pathway for fatty acid synthesis, or that one or more of the tumor enzymes is structurally modified so that it responds differently to the normal controlling influences. These hypotheses seem unlikely in view of data obtained in this study. The requirements of the tumor enzymes were shown to be the same as those of the enzymes from normal liver. Furthermore, acetyl-CoA carboxylase and fatty acid synthetase of the tumors were both inhibited by palmitoyl-CoA to the same extent as the liver enzymes. The acetyl-CoA carboxylase from the tumors was studied extensively. In these studies, the tumor enzymes were essentially identical with the liver enzymes by the following criteria: heat inactivation, affinity for acetyl-CoA and ATP, activation and aggregation by citrate, product inhibition by malonyl-CoA, pH optima, and intrinsic specific activity. Thus, it appears that the hepatoma enzyme is identical with that of liver, suggesting that the altered control does not represent an example of deleted feedback inhibition. In other studies, Cho and Pitot (2) have shown that tryptophan pyrrolase and threonine dehydrase show altered environmental control of enzyme levels in minimal deviation hepatomas. Structural and antigenic properties of these enzymes suggest their identity with normal liver enzymes.

From our experiments it is not possible to define further the defective control of fatty acid synthesis. By analogy to bacterial systems (29), it is possible that the alteration is at the level of repressor control of enzyme levels. Thus, in the tumor, repressor may not be formed or, alternatively, it is incapable of combining with an operator gene for the fatty acid synthesis pathway. The coordinate change in the enzymes of fatty acid synthesis is consistent with this hypothesis. Experiments of Allman, Hubbard, and Gibson (30) suggest that the normal increase of the enzymes of fatty acid synthesis following refeeding of 48-hour fasted animals requires new enzyme synthesis. Other possible explanations include greater stability of the enzymes in the tumor as well as lack of production of some unknown feedback inhibitor.

Host factors such as blood supply have not been excluded as important in the observed changes; however, previous studies by Siperstein with the cholesterol system and by Pitot studying tryptophan pyrrolase induction by tryptophan indicate that these factors cannot explain the altered controls in these pathways (2).

One of the tumors studied (9618A) appears to be one of the least deviated hepatomas developed to date. This tumor is one of the few hepatomas which has normal glycogen levels. Furthermore, it is one of only two out of 38 hepatomas studied which have a diploid number of chromosomes and a completely normal karyotype (31). From our studies it is clear that the regulation of fatty acid biosynthesis is abnormal in this tumor. Our finding extends the number of hepatomas shown to lack normal dietary control of the fatty acid synthesis to seven. The seeming consistency of alteration in regulation of both cholesterol and fatty acid biosynthesis is interesting but the relation of these findings to the carcinogenesis remains obscure.

If the levels of acetyl-CoA carboxylase in vivo reflect the rate of fatty acid synthesis in vivo, it is notable that in the relatively small tumors studied in these experiments as much as 4 mmoles of malonyl-CoA could be incorporated into fatty acid per rat per day. In fasted animals this represents a 10- to 30-fold increase over the hepatic synthesis of fatty acid in the host liver. These differences may be magnified greatly as the tumors grow and could well contribute to the weight loss ultimately observed in these animals. Since lipid presumably does not accumulate in these tumors, the fatty acid in excess of that required for tumor growth must be oxidized. This potential "short circuit" energy could thus contribute to inanition in these animals.

**Acknowledgment**—The authors wish to thank Dr. D. R. Moranzo for the histological diagnosis of these hepatomas.

**References**

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