Regulation of Volatile Fatty Acid Uptake by Mitochondrial Acyl CoA Synthetases of Bovine Liver\textsuperscript{1,2}

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ABSTRACT
Mitochondria of bovine liver contain acyl CoA synthetases necessary for the uptake of propionate, butyrate, and valerate whereas acetate is bound only weakly. Purification of these enzymes separated a distinct propionyl CoA synthetase highly specific for propionate and acrylate and a butyrate-activating fraction with broad substrate specificity for short and medium chain fatty acids. Evidence from kinetic studies and sucrose density centrifugation suggested that this latter fraction was composed of two enzymes, a butyryl CoA synthetase and a valeryl CoA synthetase. The apparent molecular weights of the propionyl, butyryl, and valeryl CoA synthetases were 72,000, 67,000, and 65,000. The Michaelis-Menten constants of propionyl CoA synthetase for propionate, adenosine 5'-triphosphate, and coenzyme A were 1.3 \times 10^{-3} \text{M}, 1.3 \times 10^{-3} \text{M}, and 6.3 \times 10^{-4} \text{M}. Enzyme activity is regulated by the concentration of propionate in portal blood. Relative to propionyl, butyryl, or valeryl CoA synthetases little acetyl CoA synthetase could be demonstrated.

In ruminants hepatic metabolism is such that use of acetate as an energy source is minimum. This ensures that an alternative energy source to glucose, as acetate units, will reach the extrahepatic tissues. Separation of a distinct propionyl CoA synthetase regulated by the concentration of propionate in portal blood is significant because a primary role of ruminant liver is to synthesize glucose from ruminally derived propionate.

INTRODUCTION
In ruminant animals microbial fermentation in the rumen leads to production of large quantities of volatile fatty acids, principally acetate, propionate, and butyrate. Little dietary hexose is available for absorption from the gastrointestinal tract, thus making the animal critically dependent on gluconeogenesis for provision of glucose in both fed and fasted states (32). As a consequence of this lack of dietary hexose, the ruminant animal has evolved a unique system of metabolism which allows it to spare glucose for essential body functions and to use ruminally derived volatile fatty acids as alternative substrates for both generation and storage of energy. In addition, up to 50\% of the total glucose requirement of the animal can be met by synthesis from propionate, a process in liver (15, 20).

Cellular membranes are freely permeable to volatile fatty acids. For uptake and subsequent tissue metabolism they first must be trapped within a particular cellular compartment by conversion into the impermeable coenzyme A derivative (29). This “activation reaction” is catalyzed by a series of enzymes termed acyl CoA synthetases and has been the subject of several reviews (11, 21).

Studies with tissue homogenates have demonstrated that different tissues activate different volatile fatty acids (4). For example, liver tissue activates propionate, butyrate, and valerate but shows marginal activity with acetate; heart tissue can activate acetate and propionate; and kidney tissue can activate all these substrates. However, work on characterizing acyl CoA synthetases from these tissues has not been extensive. Until recently,
based upon purification studies on mitochondrial forms of these enzymes from different animal species, it generally was accepted that there were three acyl CoA synthetases. Acetyl CoA synthetase (EC.6.2.1.1) has been purified from mitochondria of beef heart by Campagnari and Webster (2), and from goat mammary mitochondria by Cook et al. (6), and from bovine mammary mitochondria by Qureshi and Cook (28) where it was active on acetate, propionate, and acrylate. A butyryl CoA synthetase (EC.6.2.1.2), active on C₄ to C₁₂ saturated straight chain fatty acids, has been purified by Mahler et al. (23) from mitochondria of beef liver and by Groot (10) from mitochondria of guinea pig. Similar preparations have been obtained from pig kidney and rabbit liver (16), from pig liver particles (14), and rat liver (19), suggesting the enzyme is common to liver and perhaps kidney of many species. A long chain acyl CoA synthetase (EC.6.2.1.3) has been purified partly from rat liver microsomes (1) and active on saturated and unsaturated fatty acids C₁₂ to C₁₈.

This classification has proved to be too simplistic. As early as 1964, from studies with tissue homogenates, Cook and colleagues proposed a distinct propionyl CoA synthetase. This enzyme has been purified from mitochondria of sheep liver by Latimer (18) and from mitochondria of guinea pig liver by Groot (10). The enzyme exhibits a high specificity for propionate (Kₘ = 43 mM) and little affinity for other short chain volatile fatty acids. Webster et al. (31) purified a butyrate-activating enzyme from mitochondria of bovine heart. This enzyme is different from butyryl CoA synthetase purified by Mahler et al. (23) from beef liver. Groot (10), using mitochondria of guinea pig liver, and Killenberg et al. (17), using mitochondria of beef liver, isolated a salicylate enzyme exhibiting maximal activation toward hexanoate and benzoate. This enzyme also activates butyrate. Activation of short chain volatile fatty acids in guinea pig mitochondria would be a consequence of activation by three enzymes, one directed towards propionate activation, propionyl CoA synthetase, whereas butyrate activation would be from two enzymes, "Mahler" enzyme and salicylate enzyme.

Different patterns of volatile fatty acid activation by various bovine tissues could not be accounted for adequately by the acyl CoA synthetases described in the scientific literature. The purpose of the work in this and companion papers was to purify enzymes activating fatty acids from tissues of lactating Holstein cows and to measure kinetics further to study how enzyme activity and volatile fatty acid uptake are controlled. Liver, kidney, and heart tissue were chosen because these tissues show different patterns of activation of volatile fatty acids and because physiological functions of these organs differ.

MATERIALS AND METHODS

Enzyme Assay

Enzyme activity was determined as in (26) except that each fraction was assayed for its ability to activate acetate, propionate, butyrate, and valerate. One unit of enzyme is the amount which catalyzes disappearance of 1 nmol of coenzyme A per min.

Protein Determination

Protein was determined by the method of Lowry et al. (22). During column chromatography protein content of the effluent was measured in various fractions by the method of Warburg and Christian (30).

Isolation of Mitochondria

Liver tissue was obtained from lactating Holstein cows slaughtered at a local abattoir. No data were available on previous milking history of these animals. After removal from the animal the liver was transported on ice to the laboratory. All subsequent steps were at 4°C. Connective tissue membrane was removed, and the tissue was sliced and then ground by a meat grinder. The ground tissue then was homogenized in a Waring blender with 1 part tissue to 2 parts of 0.13 M KCl containing 2.5 mM 2-mercaptoethanol and adjusted to pH 8.0 with 1N ammonium hydroxide. Homogenization was for 10 s at medium speed followed by 10 s at low speed. The homogenate was transferred to 1-liter bottles and centrifuged at 100 x g in an MSE centrifuge for 15 min. After centrifugation the 100 x g supernatant was filtered through eight layers of cheesecloth and recentrifuged at 20,000 x g in a sorvall RC-2B centrifuge for 30 min. The 20,000 x g pellet containing the mitochondria was re-

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suspended in .13 M KCl containing 2.5 mM 2-mercaptoethanol and 10% glycerol (pH 8.0). For each gram of mitochondrial pellet 3 ml of buffer was used, and resuspension was by teflon homogenizer. Enzymes activating fatty acid were liberated from mitochondria by a sonifier cell disrupter (Heat Systems Ultrasonic, Inc., L. I., NY). This process was by standard horn immersed in 300-ml quantities of mitochondrial suspension. Sonication time was 2 min at a setting of 3. The sonicated extract then was centrifuged at 30,000 × g for 30 min. The supernatant was designated as mitochondrial extract.

Ammonium Sulfate Fractionation

To each liter of mitochondrial extract 243 g of solid ammonium sulfate was added slowly with stirring. The pH then was adjusted to 8.0 with 1 N ammonium hydroxide and the solution allowed to stir gently for 1 h. The solution was centrifuged for 30 min at 30,000 × g. To the supernatant a further 285 g of solid ammonium sulfate was added per liter of solution, allowed to stir gently for 1 h, and then centrifuged as before. The pellet so obtained (80% precipitate) was resuspended in .05 M Tris-HCl containing 2.5 mM 2-mercaptoethanol and 10% glycerol and stored frozen at −20°C until required for chromatography.

DEAE–23 Cellulose Chromatography

DEAE–23 cellulose was precycled, degassed, and equilibrated as described in the Whatman information leaflet on advance ion exchange celluloses. Column dimensions were 2.5 cm × 47 cm. Column equilibration was overnight with .005 M Tris-HCl containing 2.5 mM 2-mercaptoethanol and 10% glycerol (pH 7.5). Ammonium sulfate precipitate was dialyzed against the equilibration buffer for 40 min and then diluted to 1.5 to 2.0 mg/ml. Approximately 800 mg of protein was used. The sample was washed onto the column with 350 ml of equilibration buffer followed by 700 ml .01 M Tris-HCl containing 2.5 mM 2-mercaptoethanol and 10% glycerol. The column was eluted with 500 ml of a linear KCl gradient of 0 to .6 M in 2.5 mM 2-mercaptoethanol, .01 M Tris-HCl, and 10% glycerol (pH 7.5) at a flow of 60 ml/h. Fractions containing enzyme with the highest specific activity were combined and concentrated by ultrafiltration with a Dia-flow cell. The concentrated protein was stored at −20°C.

Calcium Phosphate Gel Chromatography

Calcium phosphate gel was prepared ac-

<p>| TABLE 1. Purification of propionyl CoA synthetase from bovine liver mitochondria. |
|------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total units C₃</th>
<th>Specific activity (units/mg protein)</th>
<th>Yield</th>
<th>Fold purification C₃</th>
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<tr>
<td>Mitochondrial suspension</td>
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<td>47,264</td>
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<td>29</td>
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<tr>
<td>Mitochondrial extract c</td>
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<td>35,185</td>
<td>10</td>
<td>56</td>
<td>37</td>
</tr>
<tr>
<td>Ammonium sulfate precipitate</td>
<td>241</td>
<td>29,631</td>
<td>12</td>
<td>123</td>
<td>62</td>
</tr>
<tr>
<td>DEAE–23 cellulose</td>
<td>50</td>
<td>18,920</td>
<td>30</td>
<td>378</td>
<td>97</td>
</tr>
<tr>
<td>Calcium phosphate gel</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First peak</td>
<td>11</td>
<td>1,000</td>
<td>15</td>
<td>100</td>
<td>386</td>
</tr>
<tr>
<td>Second peak</td>
<td>1.6</td>
<td>5,809</td>
<td>195</td>
<td>3,631</td>
<td>208</td>
</tr>
</tbody>
</table>

a A unit is defined as the amount of enzyme which catalyzes the disappearance of 1 nmol of coenzyme A per min.

b C₂ = acetate; C₃ = propionate; C₄ = butyrate; C₅ = valerate.

c Prepared by sonication of mitochondria. Amount of liver tissue used = 100 g; wet weight of mitochondria = 14 g.

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cording to the method of Miller et al. (25).

Column dimensions were 3 cm x 15 cm. The column was equilibrated overnight in .001 M potassium phosphate buffer (pH 7.0) containing 10% glycerol and 2.5 mM 2-mercaptoethanol. The extract from a DEAE-23 cellulose column was dialyzed for 40 min against equilibration buffer. The dialyzed sample was diluted to approximately .5 to 1.0 mg/ml and 50 to 150 mg of protein applied to the column. The enzyme activity was eluted with a stepwise gradient of increasing concentrations of potassium phosphate buffer (pH 7.0) from .001 M to .5 M. All buffers contained 10% glycerol and 2.5 mM 2-mercaptoethanol. Flow was approximately 120 ml/h. Fractions with the highest specific activity were pooled and concentrated by ultrafiltration by the Dia-flow cell. The enzyme protein was stored frozen at -20°C in .05 M Tris-HCl containing 10% glycerol and 2.5 mM 2-mercaptoethanol.

**Electrophoresis**

Enzyme purity was assessed by the technique of native polyacrylamide disc electrophoresis as outlined by Davis (7). Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was used to determine subunit molecular weight of purified enzyme protein (Welton and Felgner, personal communication). Gels were prepared according to the procedure of Fairbanks et al. (8).

**Sucrose Density Centrifugation**

Molecular weights of the various enzyme preparations were determined by the procedure of Martin and Ames (24).

**Enzyme Kinetic Data**

Michaelis-Menten constants (K<sub>m</sub>) and maximal velocities (V<sub>max</sub>) were determined from Lineweaver-Burk and Eadie-Hofstee plots by linear regression. Theoretical curves were obtained from K<sub>m</sub> and V<sub>max</sub> derived from the Eadie-Hofstee plot.

**RESULTS**

Although the enzymes did not bind to DEAE-23 cellulose, substantial purification could be achieved with this support (Table 1). Activity was associated with a single peak; propionate, butyrate, and valerate, but not acetate, were activated. After concentration of tubes that contained fatty acid activating ability, the sample was applied to a calcium phosphate gel column and enzyme activity monitored with propionate, butyrate, and valerate as substrates. The chromatogram (Figure 1) shows that the activity could be separated into two components, one which activated primarily butyrate and valerate but with some propionate activating ability and one which activated only propionate. Based on evidence to be presented later, the former fraction will be the butyrate-activating fraction.

![Figure 1](image-url)
and the latter propionyl CoA synthetase.

Purification of the two enzymes from liver mitochondria is in Table 1. Mitochondrial extract can activate propionate, butyrate, and valerate but has only marginal ability to activate acetate (Table 1). With DEAE-23 cellulose and calcium phosphate gel chromatography a 114-times purification of propionyl CoA synthetase was achieved. Both enzymes after concentration were stable for months if stored in the presence of 10\% glycerol at -20\°C.

Electrophoresis of propionyl CoA synthetase showed one major band and several minor bands. The pattern was the same on gels of differing acrylamide content. However, it is not known whether enzyme activity was associated with the major band or with one of the minor bands. Electrophoresis of the butyrate-activating fraction showed at least five major bands, indicating heterogeneity of the preparation.

Molecular weights were determined for both enzymes by the technique of sucrose density centrifugation (Figures 2, 3). The apparent molecular weight of propionyl CoA synthetase was 73,400. Sucrose density centrifugation of the butyrate-activating fraction (Figure 3) indicated two enzymes activating fatty acid, a butyryl CoA synthetase of apparent molecular weight 67,000 and a valeryl CoA synthetase of apparent molecular weight 65,000.

Sodium dodecyl sulfate polyacrylamide
electrophoresis of propionyl CoA synthetase gave six minor bands and one major band. Molecular weights of the proteins composing the minor bands were 68,000, 60,000, 57,000, 50,000, 45,000, 40,000, and the molecular weight of the protein composing the major band was 35,000. Because minor impurities were in the preparation, no definitive statements can be made on the subunit structure of this enzyme although it is tempting to speculate that the protein is a dimer of apparent molecular weight 70,000 composed of two subunits of molecular weight 35,000.

The effect of propionate concentration of propionyl CoA synthetase activity is in Figure 4. Insets are Lineweaver-Burk and Eadie-Hofstee plots of the same data. A $K_m$ of $1.28 \times 10^{-3}$ M was obtained from the Lineweaver-Burk plot ($r = .996$). A $K_m$ of $1.30 \times 10^{-3}$ M was obtained from the Eadie-Hofstee plot ($r = -.975$). Line plots were straight in both cases, indicating only one enzyme with activating ability toward propionate. Figure 5 shows the effect of ATP concentration on activity of propionyl CoA synthetase. A $K_m$ of $9.51 \times 10^{-4}$ M and $13.33 \times 10^{-4}$ M for ATP were from the Lineweaver-Burk and Eadie-Hofstee plots of the data ($r = .926; r = -.868$). Figure 6 shows the effect of coenzyme A concentration of propionyl CoA synthetase activity. The Lineweaver-Burk plot gave a $K_m$ of $6.0 \times 10^{-4}$ M ($r = .997$). The Eadie-Hofstee plot of the same data gave a $K_m$ of $6.3 \times 10^{-4}$ M ($r = .964$). Enzyme activity with various other substrates is in Table 2. The enzyme exhibits maximal activity with propionate followed by acrylate. Some activity is obtained with crotonate and salicylate as substrates.

Effect of propionate and butyrate concentration on activity of the butyrate-activating enzyme is in Figures 7 and 8. In this case Eadie-Hofstee plots of the data did not give straight lines indicating at least two enzymes activating fatty acids. In the presence of two
enzymes $K_m$'s cannot be meaningful. This fraction had a low affinity for propionate. Twice the enzyme concentration was required to measure propionate activation than when butyrate activation was measured (Figures 7 and 8). Effects of various substrates on the activity of butyrate-activating enzyme are in Table 3. The enzyme had a broad affinity for short and medium chain fatty acids. Activity was maximal when benzoate was a substrate. Butyrate, valerate, hexanoate, and acrylate were all about equally active as substrates.

**DISCUSSION**

Studies of purification of enzymes activating fatty acids from mitochondrial tissue of liver demonstrate a distinct propionyl CoA synthetase with a high specificity for propionate but not for the other short chain fatty acids. Although purification of this enzyme was substantial (Table 1), the preparation was not homogeneous as evaluated by the technique of polyacrylamide gel electrophoresis. However, Eadie-Hofstee plot of the kinetic data yielded straight line plots indicating that the enzyme preparation was not contaminated by other enzymes activating propionate (Figures 4, 5, and 6).

We believe that this enzyme activating propionate is similar to the propionyl CoA synthetase isolated from guinea pig liver mitochondria by Groot (10) and from sheep liver by Latimer (18). The substrate specificities are similar, with maximal activation on propionate followed by acrylate (10). Furthermore, $K_m$'s for propionate, ATP, and coenzyme A are of similar magnitudes (Figures 4, 5, and 6) to those obtained by those workers.

As a result of activation by propionyl CoA synthetase, propionate is trapped within the mitochondrion as the coenzyme A derivative. It seems reasonable to suppose that this enzyme, as the first committed step in the sequence of reactions leading to the synthesis of glucose, plays a major role in control of the
process. The $K_m$ of the partially purified enzyme for propionate is $1.3 \times 10^{-3}$ M (Figure 4). The concentration of propionate in portal blood can range from .3 mM to 2 mM depending on both the type of feed and time after feeding (3, 5). Since propionate can diffuse readily through cellular membranes, propionate concentration in cytoplasm would be within the same range as concentration in portal blood. Thus, the concentration of propionate reaching the mitochondria may be within the range of the $K_m$ of the enzyme, i.e., $3 \times 10^{-3}$ M to $2 \times 10^{-3}$ M. As a result the metabolic pathways of liver mitochondrial propionate metabolism must be regulated by availability (concentration in portal blood) of propionate. A reduction in propionate concentration in portal blood will decrease activity of the enzyme because it is not saturated with substrate. This will decrease flux of propionate through the pathways of propionate metabolism such as gluconeogenesis. Small fluctuations in concentration of propionate in portal blood will cause relatively large changes in the amount of propionate available for glucose synthesis. Diets which yield greater productions of ruminal propionate

### TABLE 2. Substrate specificity of propionyl CoA synthetase purified from bovine liver mitochondria.

<table>
<thead>
<tr>
<th>Substrates tested</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>0</td>
</tr>
<tr>
<td>Propionate</td>
<td>100</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0</td>
</tr>
<tr>
<td>Valerate</td>
<td>0</td>
</tr>
<tr>
<td>Hexanoate</td>
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<td>Heptanoate</td>
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</tr>
<tr>
<td>Octanoate</td>
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</tr>
<tr>
<td>Acrylate</td>
<td>85</td>
</tr>
<tr>
<td>Maleate</td>
<td>0</td>
</tr>
<tr>
<td>Crotonate</td>
<td>15</td>
</tr>
<tr>
<td>Fumarate</td>
<td>0</td>
</tr>
<tr>
<td>Benzoate</td>
<td>0</td>
</tr>
<tr>
<td>Salicylate</td>
<td>8</td>
</tr>
</tbody>
</table>

*Fatty acids (racemic mixtures) were used at a concentration of 5 µmol.

Figure 6. Effect of coenzyme A concentration on the activity of propionyl CoA synthetase partly purified from liver mitochondria. The inset on the left is the Lineweaver-Burk plot, and the inset on the right is the Eadie-Hofstee plot of the same data. . . . visual fit of data, —— theoretical line.
should increase propionate availability and conversion to glucose. This would be equivalent to a fine control on the system, because once propionate has been absorbed into portal blood, rapid conversion of propionate to propionyl CoA and thus to glucose would occur.

Activity of propionyl CoA synthetase is low in the fetus and newborn calf and increases as rumen fermentation become established (unpublished). This increase in activity is reduced when animals are maintained on a liquid diet suggesting that propionyl CoA synthetase is substrate inducible. Also, propionyl CoA synthetase activity in liver mitochondria increases as lactation progresses, and this may be from increased grain feeding during lactation, which increases propionate production in the rumen. Consequently, feeding ingredients favorable to an active rumen propionic acid fermentation may not only control activity of propionyl CoA synthetase per se but also may induce synthesis of enzyme protein in liver tissue and increase the potential for glucose synthesis from propionate.

For a number of reasons butyrate and valerate activation of liver mitochondrial suspensions cannot be accounted for by a single enzyme, although it eluted as a single component on calcium phosphate gel (Figure 1). First, Eadie-Hofstee plots of kinetic data (Figures 7 and 8) gave curvilinear plots, indicating more than one molecular species activating volatile fatty acid. Second, sucrose density centrifugation (Figure 3) of the butyrate-activating fraction indicated more than one molecular species activating volatile fatty acids. Finally, Groot et al. (11), working with the enzymes activating fatty acids of guinea pig mitochondria, could separate not only a propionyl CoA synthetase but also an enzyme activating medium chain fatty acids (classical/butyryl CoA synthetase first described by Mahler et al. (23)) and a salicylate activating
enzyme. Enzyme activating medium chain fatty acids had maximal activity on hexanoate followed by octanoate and butyrate. The enzyme activating salicylate had maximal activity on benzoate and hexanoate with less activity on octanoate and butyrate. The butyrate-activating fraction isolated from bovine liver mitochondria activated both benzoate, characteristic of the salicylate enzyme, and octanoate, characteristic of the medium chain acyl CoA synthetase. It probably is composed of two enzymes which can activate butyrate. It is believed that these two enzymes could be separated by phosphocellulose chromatography (10). On the basis of the sucrose density centrifugation experiment (Figure 3) it is likely that one enzyme might activate primarily butyrate (butyryl CoA synthetase) and the other primarily valerate (valeryl CoA synthetase).

Groot et al. (11) suggested that in the guinea pig these two enzymes may be involved in initiation of oxidation of medium chain fatty acids. They also may serve a role in excretion of aromatic carboxylic acids by activation followed by conjugation with glycine (9, 17). In the ruminant another role might be to remove all of the butyrate, which has escaped metabolism to β-hydroxybutyrate by the rumen epithelium, from portal blood. Such a role would be compatible with the observation that little butyrate appears in peripheral blood and must be taken up by liver. Acetate is not a major source of energy for ruminant liver. It is proposed that butyrate replaces this function of acetate in ruminant liver.

Because liver propionyl CoA synthetase has a low $K_m$ ($1.3 \times 10^{-3} M$) and high affinity for propionate (Figure 4) and the liver fraction activating butyrate has a low affinity for propionate (Figure 7), these enzymes may function in a manner analogous to the glucokinase-hexokinase system for glucose uptake in nonruminants. At low or normal substrate concentrations propionyl CoA synthetase can activate all propionate. When larger amounts of

![Figure 8](image_url)

Figure 8. Effect of butyrate concentration on the activity of the butyrate fraction partly purified from liver mitochondria. The inset on the left is the Lineweaver-Burk plot, and the inset on the right is the Eadie-Hofstee plot of the same data. —— theoretical line or curve, . . . visual fit of data.
TABLE 3. Substrate specificity of butyryl CoA synthetase purified from bovine liver mitochondria.a

<table>
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<tr>
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</tr>
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<tbody>
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<td>Acetate</td>
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<tr>
<td>Salicylate</td>
<td>38</td>
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</table>

aFatty acids (racemic mixtures) were used at a concentration of 5 μmol.

propiionate are presented to the liver, the butyrate-activating enzyme becomes important in propionate activation. This ensures that all propionate in portal blood is taken up by the liver for glucose synthesis.

Liver mitochondria contained negligible amounts of acetyl CoA synthetase, the enzyme necessary for the uptake of acetate. Acetate activation in the cytosolic compartment of liver tissue is also low (unpublished). In contrast, nonruminant animals are characterized by significant ability to activate acetate in both mitochondrial and cytosolic fractions of liver tissue (11). Thus in ruminants, ruminally derived acetate passes from portal blood to peripheral blood with little intermediary metabolism in liver. The physiological basis for this phenomenon is probably twofold. First, it ensures that an energy furnishing substrate, acetate, is made available to peripheral tissues such as kidney and heart which may require it when alternative substrates such as glucose are in short supply. Second, it ensures no synthesis of lipid in the cytosolic fraction of ruminant liver via acetate activation to acetyl CoA and subsequent synthesis into long chain fatty acids. Because glucose is precluded also as a carbon source for fatty acid synthesis in ruminant liver (13), this allows the primary role of ruminant liver to be synthesis of glucose and not fat, i.e., gluconeogenesis can occur at all times. In ruminants lipogenesis occurs primarily in adipose tissue (12, 13). Gluconeogenesis and lipogenesis are processes which compete for ATP and carbon skeletons and, thus, cannot occur at maximal rates at the same time in the same organ. In the nonruminant it is not as important that acetate be excluded from mitochondrial metabolism in liver tissue, since glucose is available as an energy-furnishing substrate for peripheral tissues. Moreover, gluconeogenesis does not occur continuously. Fatty acid synthesis and gluconeogenesis can occur in monogastric liver because they are separated in time.

We conclude that in the ruminant, liver metabolism is adapted to low blood glucose concentrations, not only by absence of citrate cleavage enzyme (12) but also by absence of mitochondrial and cytosolic forms of acetyl CoA synthetase. These modifications and the presence of distinct propionyl, butyryl, and valeryl CoA synthetase ensure that propionate, butyrate, and valerate are taken up by ruminant liver and that acetate is metabolized by extrahepatic tissues. The role of the acyl CoA synthetases in hepatic metabolism is in Figure 9.

REFERENCES


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