Intracellular Localization of the 3-Hydroxy-3-methylglutaryl Coenzyme A Cycle Enzymes in Liver

SEPARATE CYTOPLASMIC AND MITOCHONDRIAL 3-HYDROXY-3-METHYLGLUTARYL COENZYME A GENERATING SYSTEMS FOR CHOLESTEROGENESIS AND KETOGENESIS*

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SUMMARY

Acetoacetyl-CoA thiolase and 3-hydroxy-3-methylglutaryl coenzyme synthase which comprise the 3-hydroxy-3-methylglutaryl-CoA-generating system(s) for hepatic cholesterogenesis and ketogenesis exhibit dual mitochondrial and cytoplasmic localization. Twenty to forty per cent of the thiolase and synthase of avian and rat liver are localized in the cytoplasmic compartment, the remainder residing in the mitochondria. In contrast, 3-hydroxy-3-methylglutaryl-CoA lyase, an enzyme unique to the "3-hydroxy-3-methylglutaryl-CoA cycle" of ketogenesis, appears to be localized in the mitochondrion. The small proportion, 4 to 8%, of this enzyme found in the cytoplasmic fraction appears to arise via leakage from the mitochondria during cell fractionation in that its properties, pH and stability, are identical to those of the mitochondrial lyase. These results are consistent with the view that ketogenesis which involves all three enzymes, acetoacetyl-CoA thiolase, 3-hydroxy-3-methylglutaryl-CoA synthase and 3-hydroxy-3-methylglutaryl-CoA lyase, occurs exclusively in the mitochondrion, whereas cholesterogenesis, a pathway which involves only the 3-hydroxy-3-methylglutaryl CoA synthesizing enzymes, is restricted to the cytoplasm.

Further fractionation of isolated mitochondria from chicken and rat liver showed that all three of the 3-hydroxy-3-methylglutaryl-CoA cycle enzymes are soluble and are localized within the matrix compartment of the mitochondrion. Likewise, cytoplasmic acetoacetyl-CoA thiolase and 3-hydroxy-3-methylglutaryl-CoA synthase are soluble cytosolic enzymes, no thiolase or synthase activity being detectable in the microsomal fraction.

Chicken liver mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase activity consists of a single enzymic species with a pH of 7.2, whereas the cytoplasmic activity is composed of at least two species with pH values of 4.8 and 6.7. Thus it is evident that the mitochondrial and cytoplasmic species are molecularly distinct as has been shown to be the case for the mitochondrial and cytoplasmic acetoacetyl-CoA thiolases from avian liver (CLINKENBEARD, K. D., SUGIYAMA, T., MOSS, J., REED, W. D., AND LANE, M. D. (1973) J. Biol. Chem. 248, 2275).

Substantial mitochondrial 3-hydroxy-3-methylglutaryl-CoA lyase activity is present in all tissues surveyed, while only liver and kidney possess significant mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase activity. Therefore, it is proposed that tissues other than liver and kidney are unable to generate acetoacetate because they lack the mitochondrial synthase.

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), is synthesized from acetyl-CoA by a two-step reaction sequence (Scheme 1, Reactions i and 2) catalyzed by acetoacetyl-CoA-(AcAc-CoA)thiolase and HMG-CoA synthase (1-4). By 1958 Rudney and Lynen and their collaborators had determined that HMG-CoA is an intermediate in two major pathways in animal liver, the synthesis of cholesterol and acetoacetate (1, 2). Based on these facts, Gould and Popjak proposed (5) that HMG-CoA is synthesized in common for both cholesterogenesis and ketogenesis, and that a branch point occurs between these two pathways at the first reaction unique to each pathway, i.e. the reduction of HMG-CoA to mevalonate and the cleavage of HMG-CoA to acetoacetate as illustrated in Scheme 1 (Reactions 3 and 4, respectively). This concept of a branched pathway for cholesterogenesis and ketogenesis has been generally accepted (6-9).

The work of Bucher et al. (10) on the subcellular distribution

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Unlabeled and [1-14C]acetyl-CoA were prepared from the anhydride by the method of Simon and Shemin (21); acetoacetyl-CoA was similarly prepared; however, diketene replaced the anhydride. Unlabeled and (R,S)-[3-14C]HMG-CoA were synthesized as described by Goldfarb and Pitot (22). The [1-14C]acetyl-CoA and (R,S)-[3-14C]HMG-CoA were purified by DEAESepharose chromatography (23). Lactate dehydrogenase (EC 1.1.1.27), malate dehydrogenase (EC 1.1.1.37), adenylate kinase (EC 2.7.4.3), monooamine oxidase (EC 1.4.3.4), succinate dehydrogenase (EC 1.3.99.1), citrate synthase (EC 4.1.3.7), and acetoacetyl-CoA thiolase (EC 2.3.1.9) were assayed as described elsewhere (13, 24-28). Assays were conducted at 30°C and reaction mixtures contained 0.2% (w/v) Triton X-100 where indicated; Triton X-100 had no inhibitory effect on the enzymes assayed. Protein was determined by the methods of Lowry et al. (99) or Warburg and Christian (30) or by the biuret method employing deoxycholate (31) as indicated.

Subcellular Fractionation of Liver—Animals were killed by decapitation and livers were removed and with iced cold homogenizing buffer containing 0.25 M sucrose, 0.1 mM EDTA, and 2.0 mM Hepes, pH 7.2. All operations were conducted at 4°C. Two grams of cubed liver suspended in 10 ml of homogenizing buffer were disrupted by three passes of a motor-driven Teflon pestle in a loose fitting glass homogenizer. The homogenate was then gently resuspended in 10 ml of homogenizing buffer using a test tube filled with ice as a pestle, after which the suspension was resedimented at 11,300 X g for 15 min. This pellet which contained mitochondria and nuclei was resuspended as above and used directly (Table I, Fraction I). Subcellular fractionation by this procedure gave reasonably good resolution of cytoplasm and mitochondria as judged by the localization of lactate dehydrogenase, citrate synthase, marker enzymes for the cytoplasmic and mitochondrial fractions, respectively (Table I). Since the usual low speed centrifugation (600 X g) was omitted to avoid the loss of mitochondria with the nuclei, essentially quantitative recovery of mitochondrial enzyme activities was achieved. This is important, particularly with avian liver because of the tendency of these mitochondria to clump. A final concentration of 0.2% (w/v) of Triton X-100 was employed in all enzyme assays in Table I, and in other experiments as indicated in table and figure legends, to release membrane-occluded enzyme activities.

The cytoplasmic fraction (Table I, Fraction II) was fractionated further by centrifugation at 100,000 X g for 1 h. The supernatant from this centrifugation, i.e., the cytosolic fraction (Table IV, Fraction I), was then further fractionated into mitochondrial and cytoplasmic fractions, respectively (Table IV). Since the usual high speed centrifugation (100,000 X g) was omitted to avoid the loss of mitochondria with the nuclei, essentially quantitative recovery of mitochondrial enzyme activities was achieved. This is important, particularly with avian liver because of the tendency of these mitochondria to clump. A final concentration of 0.2% (w/v) of Triton X-100 was employed in all enzyme assays in Table I, and in other experiments as indicated in table and figure legends, to release membrane-occluded enzyme activities.

Intramitochondial fractionation, livers were homogenized as described above except that the homogenizing buffer also contained 0.1% (w/v) bovine serum albumin. The homogenate was centrifuged at 160 X g for 10 min to remove nuclei and the supernatant from this centrifugation was resedimented at 6700 X g to collect the mitochondria. This mitochondrial pellet was gently resuspended in 3 ml of the homogenizing buffer (containing 0.1% bovine serum albumin) and resedimented at 9700 X g for 15 min. The washed mitochondria were resuspended in 2 ml of homogenizing buffer (containing 0.1% bovine serum albumin) and further fractionated as indicated in Tables IV and V.

Isoelectric Focusing—Isoelectric points of HMG-CoA synthase and HMG-CoA lyase preparations were determined using an LKB 8101 electrofocusing column. The dense electrode solution consisted of 0.4 ml of monochloethanolamine, 12 g of sucrase, and 14 ml of water, while the light electrode solution contained 0.1 ml of phosphoric acid and 10 ml of water. For the column gradient, the dense solution contained 28 g of sucrase, 50 ml of 2-mercaptoethanol, and 75% of the appropriate Ampholine, in some cases the sample, and water to bring the final volume to 42 ml; the light

It was observed that a substantial fraction of the mitochondrial marker enzymes sedimented at 600 X g, whereas insignificant amounts of the cytoplasmic marker enzyme, lactate dehydrogenase, was found in the pellet under these conditions.

EXPERIMENTAL PROCEDURE

Materials and Miscellaneous Methods—Coenzyme A, NAD, NADH, ADP, and ATP were purchased from P-L Biochemicals, diketene from Sigma, 3-hydroxy-3-methylglutaric acid from Schwarz/Mann, and Ampholine 8141 from LKB. [1-14C]Acetate and [3-14C]HMG were obtained from New England Nuclear, while hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) were purchased from Boehringer-Mannheim. 3-Methylglutaconic acid was a gift from Dr. F. Huchler (Bowman-Bray School of Medicine).
Intracellular distribution of acetoacetyl-CoA thiolase, HMG-CoA synthase, and HMG-CoA lyase activity in chicken liver

Livers from 20 8-week-old white Leghorn cockerels fed ad libitum were fractionated into mitochondria (Fraction I, also containing nuclei and some unbroken cells) and cytoplasm (Fraction II) after which enzyme assays were conducted in the presence of 0.2% Triton (w/v) as described under “Experimental Procedure.” HMG-CoA synthase and HMG-CoA lyase were assayed spectrophotometrically; 100 mm glycyglycine buffer, pH 8.8, was employed for HMG-CoA synthase assays. Protein was determined by the biuret method (31).

“Homogenate” refers to enzymatic activity in the homogenate prior to the separation of the subcellular fractions, and this activity is taken as 100% for the calculation of percentages shown in parentheses. Enzyme activity is expressed as units (1 μmol of substrate converted/min)/g wet weight of liver ± the standard deviation of the mean.

### Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Acetocetyl-CoA thiolase</th>
<th>HMG-CoA synthase</th>
<th>HMG-CoA lyase</th>
<th>Citrate dehydrogenase</th>
<th>Lactate dehydrogenase</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>93 ± 14 (100%)</td>
<td>1.9 ± 0.4 (100%)</td>
<td>7.3 ± 1.4 (100%)</td>
<td>6.3 ± 1.0 (100%)</td>
<td>240 ± 40 (100%)</td>
<td>236 ± 26</td>
</tr>
<tr>
<td>I. Mitochondria</td>
<td>47 ± 12 (51%)</td>
<td>1.6 ± 0.1 (84%)</td>
<td>6.6 ± 1.0 (90%)</td>
<td>5.5 ± 0.9 (87%)</td>
<td>26 ± 5 (19%)</td>
<td>115 ± 13</td>
</tr>
<tr>
<td>(plus nuclei)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II. Cytoplasm</td>
<td>41 ± 8 (44%)</td>
<td>0.4 ± 0.1 (21%)</td>
<td>0.3 ± 0.2 (4%)</td>
<td>0.1 ± 0.1 (2%)</td>
<td>210 ± 30 (88%)</td>
<td>115 ± 9</td>
</tr>
<tr>
<td>Total recovery in Fractions I + II</td>
<td>88 (95%)</td>
<td>2.0 (105%)</td>
<td>6.9 (94%)</td>
<td>5.6 (89%)</td>
<td>235 (98%)</td>
<td>233</td>
</tr>
</tbody>
</table>

Mitochondria

Isolated mitochondria from chicken liver were prepared as described under “Experimental Procedure” except that the washed mitochondria were resuspended in 50 mm potassium phosphate buffer, pH 7.0, instead of homogenizing buffer. This mitochondrial suspension (20 mg of protein/ml) was sonicated for three 20-s periods at 4°C with a Bronwill Biosonik sonicator described under “Experimental Procedure” except that the washed mitochondria were resuspended in 50 mM potassium chloride, pH 8.0, 20 nmol of EDTA, 40 nmol of [l- or 2-]14C]acetyl-CoA (2 X 10^6 cpm/pmol), 10 nmol of acetoacetyl-CoA, and 0.1 to 1.0 milliunit of HMG-CoA synthase in a total volume of 0.2 ml. The reaction is initiated by addition of [14C]acetyl-CoA to the otherwise complete reaction mixture after a preliminary incubation for 2 min at 30°C. At Z, 4, 6, and 8 min after addition of acetyl-CoA, 0.04-ml aliquots are removed and pipetted into glass vials (15 X 45 mm) containing 0.1 ml of 6 N HCl, and the acidified aliquot is taken to dryness at 65°C in a forced-draft oven. Water and the liquid scintillator are added and nonvolatile 14C activity (as [14C]HMG-CoA plus 3-[14C]methylglutaconyl-CoA) is determined with a scintillation spectrometer. Under the conditions described, a linear increase in nonvolatile 14C activity with time is observed until at least 70% of the added acetoacetyl-CoA is consumed and the rate of incorporation is proportional to the amount of synthase added as shown in Fig. 1A. When highly purified preparations of HMG-CoA synthase are employed, the sole radioactive product of the assay following alkaline deactivation of thioesters was found to be 3-hydroxy-3-methylglutaric acid as judged by paper chromatography (Whatman 3MM paper; n-amylformate-formic acid solvent ascending (32), Rf = 0.63, and 1-butanol-acetic acid-water ascending (33), Rf = 0.72). With crude preparations of HMG-CoA synthase, two radioactive products are observed, 3-hydroxy-3-methylglutarate and 3-methylglutaconate, at a near equilibrium ratio of 4:1 (34). The sum of the radioactivity in these two products was equal to the nonvolatile radioactivity observed in the HMG-CoA synthase assay. The 3-methylglutaconyl-CoA observed presumably arose by the action of HMG-CoA dehydrogenase (EC 4.2.1.18) present in the crude enzyme preparations. It should be noted that the radiochemical assay cannot be employed for enzyme preparations containing active HMG-CoA lyase. Lyase activity of crude avian liver extracts can be quantitatively activated by a procedure described under “HMG-CoA Lyase Assay.”

For crude enzyme preparations containing HMG-CoA lyase, a spectrophotometric assay modified from that of Ferguson and Rudney (35) was generally used. The complete assay mixture solution contained 50 μl of 2-mercaptoethanol, 25% of the appropriate Ampholine, and water to a final volume of 50 ml. After adding the dense electrode solution to the electrofocusing column, a linear density gradient, prepared from the dense and light solutions with a gradient mixer, was applied to the column. Enzyme samples were either mixed with an equal volume of the dense solution and added to the column at the midpoint of the gradient or, when the volume of the sample was >2 ml, mixed with the dense solution and applied with the gradient. The light electrode solution was applied to the top of the column and focusing conducted at 4°C with the voltages for the periods indicated, after which the column contents were collected fractionally and the pH of each fraction was determined immediately at 4°C. HMG-CoA Synthase Assays—HMG-CoA synthase activity can be accurately measured in enzyme extracts free of HMG-CoA lyase by following the incorporation of [1- or 2-14C]acetyl-CoA into HMG-CoA or its enzymic dehydration product, 3-methylglutaconyl-CoA. The complete reaction mixture contains 20 μmol of Tris (Cl-) pH 8.0, 20 nmol of EDTA, 40 nmol of [l- or 2-]14C]acetyl-CoA (2 X 10^6 cpm/μmol), 10 nmol of acetoacetyl-CoA, and from enzyme preparations containing active HMG-CoA synthase, a total activity (as [14C]HMG-CoA plus 3-[14C]methylglutaconyl-CoA) is determined with a scintillation spectrometer. Under the conditions described, a linear increase in nonvolatile 14C activity with time is observed until at least 70% of the added acetoacetyl-CoA is consumed and the rate of incorporation is proportional to the amount of synthase added as shown in Fig. 1A. When highly purified preparations of HMG-CoA synthase are employed, the sole radioactive product of the assay following alkaline deactivation of thioesters was found to be 3-hydroxy-3-methylglutaric acid as judged by paper chromatography (Whatman 3MM paper; n-amylformate-formic acid solvent ascending (32), Rf = 0.63, and 1-butanol-acetic acid-water ascending (33), Rf = 0.72). With crude preparations of HMG-CoA synthase, two radioactive products are observed, 3-hydroxy-3-methylglutarate and 3-methylglutaconate, at a near equilibrium ratio of 4:1 (34). The sum of the radioactivity in these two products was equal to the nonvolatile radioactivity observed in the HMG-CoA synthase assay. The 3-methylglutaconyl-CoA observed presumably arose by the action of HMG-CoA dehydrogenase (EC 4.2.1.18) present in the crude enzyme preparations. It should be noted that the radiochemical assay cannot be employed for enzyme preparations containing active HMG-CoA lyase. Lyase activity of crude avian liver extracts can be quantitatively activated by a procedure described under “HMG-CoA Lyase Assay.”

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Intramitochondrial distribution of acetoacetyl-CoA thiolase, HMG-CoA synthase, and HMG-CoA lyase of liver

Isolated mitochondria were prepared as described under "Experimental Procedure" and subsequently fractionated into outer membrane plus intracristal space (Fraction I) and inner membrane-matrix vesicles (Fraction II) by the method of Schnaitman and Greenwald (28). HMG-CoA synthase and HMG-CoA lyase were assayed as described in Table II, while acetoacetyl-CoA (Aaac-CoA) thiolase, succinate dehydrogenase (Succ. dehydr.), citrate synthase, monoamine oxidase, and adenylate kinase were assayed as described under "Experimental Procedure." Triton X-100 (0.2%, w/v) was used in all enzyme assays. Mitochondria had acceptor control ratios as in Table II prior to digitonin treatment. "Mitochondria" refers to isolated digitonin-treated mitochondria. Distribution is expressed in percentages.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>A. Chicken Mitochondria</th>
<th>B. Rat Mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Intracristal space + outer membrane</td>
<td>100 (350)</td>
<td>100 (135)</td>
</tr>
<tr>
<td>II. Matrix + inner membrane</td>
<td>100 (25)</td>
<td>100 (31)</td>
</tr>
<tr>
<td>Total recovery in Fractions I + II</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

Table III

<table>
<thead>
<tr>
<th>Proteina</th>
<th>Aaac-CoA thiolase</th>
<th>HMG-CoA synthase</th>
<th>HMG-CoA lyase</th>
<th>Succ. dehydr.</th>
<th>Citrate synthase</th>
<th>Monoamine oxidase</th>
<th>Adenylate kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteina</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>A. Chicken Mitochondria</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>B. Rat Mitochondria</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Aaac-CoA thiolase has an $K_m = 16.1 \times 10^{-5}$ M$^{-1}$ in this buffer. Mitochondria HMG-CoA synthase is inhibited by MgCl$_2$ (19) and is assayed in the absence of MgCl$_2$ in Tris (Cl$^-$), pH 8.2, where acetoacetyl-CoA has an $K_m = 3.6 \times 10^{-6}$ M$^{-1}$. In experiments in which both cytoplasmic and mitochondrial HMG-CoA synthase were assayed, glycylglycine (Na$^+$), pH 8.8, was used without MgCl$_2$ where acetoacetyl-CoA has an $K_m = 7.8 \times 10^{-6}$ M$^{-1}$.

HMG-CoA synthase activity is equal to one-half the rate of acetoacetyl-CoA consumption for preparations, e.g., crude mitochondrial or cytoplasmic extracts, in which acetoacetyl-CoA thiolase activity > HMG-CoA synthase activity. This correction is made to account for the CoA-dependent consumption of acetoacetyl-CoA catalyzed by thiolase, the extent of which is governed by the amount of CoA generated by HMG-CoA synthase. As illustrated in Fig. I, A and B, the activities determined by the radiochemical and spectrophotometric assays are in good agreement.

**Fig. 1.** Dependence of HMG-CoA synthetic rate on enzyme concentration. The rate of HMG-CoA synthase was determined by the (A) radiochemical and (B) spectrophotometric (using 10$^6$ $\text{ arbitrary units}$) assay of acetoacetyl-CoA thiolase with a specific activity of 0.1 unit/mg of protein (20) and free of acetoacetyl-CoA thiolase was employed. Protein was determined by the Warburg and Christian method (30).

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**HMG-CoA Lyase Assays**—A new radiochemical assay for HMG-CoA lyase was developed which has the advantage of increased sensitivity over the spectrophotometric assay (36). The lyase-catalyzed conversion of [3-$^14$C]HMG-CoA (not volatile when taken to dryness in 6 N HCl and 10 min at 95°), to its volatile product, [3-$^14$C]acetoacetate, is measured. The reaction is initiated by the addition of 0.5 to 3.5 milliunits of HMG-CoA lyase to a reaction mixture containing 20 $\mu$mol of Tris (Cl$^-$), pH 8.2, and 40 $\mu$mol of (R,S)-[3-$^14$C]-HMG-CoA (specific activity, 5 to 20 $\times 10^6$ cpm/$\mu$mol) in a total volume of 0.2 ml. After 2, 4, 6, and 8 min of incubation at 30°, the final aliquots are transferred to glass vials containing 0.1 ml of 6 N HCl and 10 mm volatile 3-14C activity determined as described for the radiochemical HMG-CoA synthase assay.

As shown in Fig. 2, A and B, the disappearance of nonvolatile 3-14C activity, i.e., [3-$^14$C]HMG-CoA, is linear with time and the rate of the reaction is proportional to enzyme concentration within the limits specified above. Moreover, the end point of the reaction is reached when approximately 50% of the (R,S)-[3-$^14$C]HMG-CoA has been cleaved which is consistent with the fact that the lyase acts only on (S)-HMG-CoA (37). That this assay in fact measures
chondrion. Only 4% of the cellular lyase activity is found in the cytoplasm, and this small percentage is probably the result of the mitochondrial enzyme. HMG-CoA lyase, appears to be exclusively localized within the mitochondrial fraction of liver, the ketogenic enzyme, HMG-CoA synthase, and HMG-CoA dehydrogenase (Table I). A similar intracellular enzyme distribution was obtained with rat liver (results not shown) where 30 and 20% of the total cellular acetoxacyl-CoA thiolase and HMG-CoA synthase activities, respectively, were present in the cytoplasmic fraction, the remainder being mitochondrial.

Intracellular Distribution of Acetoacetyl-CoA Thiolase, 3-Hydroxy-3-methylglutaryl-CoA Synthase, and 3-Hydroxy-3-methylglutaryl-CoA Lyase—It has been assumed and earlier studies in agreement. The presence of 5 mM dithiothreitol in the assay increases HMG-CoA lyase activity by approximately 1.5-fold. HMG-CoA lyase is a calcium-containing metalloenzyme (39), which is consistent with our observation that the activity of the chicken liver enzyme is rapidly lost at 4°C in the presence of agents (12 mM EGTA or EDTA, 5 mM ATP, or 20 mM phosphate) which form stable complexes with calcium; moreover, stability is enhanced by 5 mM Ca²⁺ or Mg²⁺. Dialysis of chicken liver HMG-CoA lyase against a 1000-fold excess of 20 mM potassium phosphate buffer, pH 7.0, at 4°C for 24 hours results in complete loss of lyase activity. This property is of special utility for measurement of HMG-CoA synthase activity by the biochemical technique in preparations containing HMG-CoA lyase. In some experiments, as indicated in the text, HMG-CoA lyase was measured by the spectrophotometric assay (36).

RESULTS

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lyase are localized within one of the two soluble compartments of the mitochondrion, i.e. the intracistral space or the matrix.

In order to determine which soluble compartment within the mitochondrion contains the HMG-CoA cycle enzymes, chicken and rat liver mitochondrial preparations were fractionated by the method of Schnaitman and Greenwalt (26) to separate the mitoplast (inner membrane-matrix vesicle) from the outer membrane and intracistral space components. The ability of this procedure to resolve these submitochondrial fractions from both chicken and rat liver is verified by the distribution of appropriate marker enzymes (Table III). Essentially all of the succinate dehydrogenase and citrate synthase, inner membrane, and matrix enzymes (26, 44), respectively, were found to be associated with the mitoplast. Monoamine oxidase, an accepted outer membrane marker (26, 44), also yielded the expected intramitochondrial distribution pattern although in chicken liver mitochondria (Table III) is not understood, this phenomenon was observed consistently.

As illustrated in Table III, the three HMG-CoA cycle enzymes, acetoacetyl-CoA thiolase, HMG-CoA synthase, and HMG-CoA lyase, were found primarily in the fraction containing the matrix and inner membrane components. Since these enzymes had already been shown to be soluble and not membrane-bound (Table II), it is evident that the ketogenic pathway is compartmentalized within the mitochondrial matrix. This is in basic agreement with a recent report of Chapman et al. (45) who found thiolase and HMG-CoA lyase to be localized within the matrix compartment. However, these investigators studied neither the distribution of these enzymes between mitochondria and cytoplasm nor the localization of HMG-CoA synthase.

Distinct Forms of HMG-CoA Synthase in Mitochondrial and Cytosolic Compartments of Liver—Recently it was established (13, 14) on the basis of several criteria, including isoelectric focusing, that different forms of acetoacetyl-CoA thiolase are present in the mitochondrion and cytoplasm of liver. This and the fact that HMG-CoA synthase activity is present (Table I) in both of these cell compartments implies dual intra- and extramitochondrial localization of HMG-CoA synthase from acetyl-CoA thiolase (Table IV, Fraction 1) of both chicken and rat liver; no activity of either enzyme could be detected in the washed mitochondrial matrix fraction (Table IV, Fraction 1) of both chicken and rat liver; no activity of either enzyme could be detected in the washed microsomal fraction. Thus, it appears that the intracytoplasmic localization of the HMG-CoA generating system which presumably provides precursor for cholesterol synthesis, is cytosolic.

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HMG-CoA Lyase—Not all animal tissues can synthesize acetoacetate from acetyl-CoA; in fact, it appears that this pathway is restricted to animal liver (47). Moreover, liver appears to be the only tissue unable to metabolize ketones, i.e., acetoacetate and \( \beta \)-hydroxybutyrate, at significant rates (48). Mitochondrial acetoacetyl-CoA thiolase is involved in both the synthesis of ketones by liver as well as the utilization of ketones by extrahepatic tissues. Consistent with these physiological roles of mitochondrial acetoacetyl-CoA thiolase, this enzyme is present in all animal tissues surveyed (14). The inability of extrahepatic tissues to synthesize ketones has been attributed to the absence of mitochondrial HMG-CoA lyase or HMG-CoA synthase, or both (47). Mitochondrial HMG-CoA synthase appears to function only in ketogenesis while HMG-CoA lyase, in addition to hepatic ketogenesis, participates in leucine catabolism (36, 37). As shown in Table V, liver and kidney contain significant activities of mitochondrial HMG-CoA synthase, whereas other tissues, such as heart, ileum, brain and skeletal muscle, do not contain detectable levels of this enzyme, i.e. <0.01 unit/g wet weight of tissue. To confirm this finding, mitochondrial HMG-CoA synthase activity was estimated using antisera prepared against purified chicken liver mitochondrial HMG-CoA synthase (19). By the Ouchterlony double diffusion technique, precipitin lines were observed for tissues such as liver and kidney which contain >0.02 unit of HMG-CoA synthase activity/g of tissue, while no precipitin line could be detected for heart, brain, ileum, or skeletal muscle.

**TABLE IV**

| Tissue Distribution of Mitochondrial HMG-CoA Synthase and HMG-CoA Lyase in Liver |
|----------------------------------|---------------------|---------------------|---------------------|
|                                  | Acetoacetyl-CoA thiolase | HMG-CoA synthase | Lactate dehydrogenase | Protein |
| A. Chicken                       | %                     | %                   | %                   | %       |
| Cytoplasm                        | 100                   | 100                 | 100                 | 100     |
| Fraction                         | (%)                   | (%)                 | (%)                 | (%)     |
| I. Cytosol                       | 88                    | 87                  | 84                  | 62      |
| II. Microsomes                   | 0                     | 0                   | 3                   | 36      |
| Total recovery in Fractions I + II | 88                   | 87                  | 87                  | 98      |
| B. Rat                           | %                     | %                   | %                   | %       |
| Cytoplasm                        | 100                   | 100                 | 100                 | 100     |
| Fraction                         | (%)                   | (%)                 | (%)                 | (%)     |
| I. Cytosol                       | 90                    | 80                  | 84                  | 61      |
| II. Microsomes                   | 0                     | 0                   | 8                   | 24      |
| Total recovery in Fractions I + II | 99                   | 80                  | 92                  | 85      |

* Milligrams of protein in the cytoplasmic fraction/g wet weight of liver.

**Fig. 5.** Isoelectric focusing of mitochondrial and cytosolic HMG-CoA synthase activities of chicken liver. Isoelectric focusing was conducted as described under "Experimental Procedure." A, mitochondrial matrix was prepared as described in Table II and focusing of 0.33 unit of mitochondrial HMG-CoA synthase activity (90 mg of protein) was carried out in 1% Ampholine 3141 for 70 hours at 300 volts; HMG-CoA synthase was determined spectrophotometrically in 100 mM Tris (Cl-) buffer, pH 8.2, as described under "Experimental Procedure." B, cytosol was prepared as described under "Experimental Procedure" and focusing of 0.36 unit of cytosolic HMG-CoA synthase activity (102 mg of protein) conducted in 1% Ampholine 1809-101 for 69 hours at 300 volts; HMG-CoA synthase activity was measured by the radiochemical assay with [1-\( ^{14} \)C]acetyl-CoA (specific activity, 2.56 \( \times 10^4 \) cpm/\( \mu \)mol); fraction size was 1 ml in A and 2 ml in B. The recovery of HMG-CoA synthase activity following isoelectric focusing was 37% for A and 26% for B.
subcellular compartments, cholesterol synthesis in the cytoplasm of liver contains all of the enzymes required for the conversion of which commit acetyl-CoA to cholesterol or acetoacetate synthesis. Rather, these two biosynthetic processes occur in different subcellular compartments, cholesterol synthesis in the cytoplasm and acetoacetate formation in the mitochondria. The cytoplasm of liver contains all of the enzymes required for the conversion of HMG-CoA to cholesterol, while mitochondrial acetyl-CoA synthesis is not in equilibrium with the mitochondrial acetyl-CoA pool from which acetoacetate is synthesized.

**Table V**

Distribution of mitochondrial HMG-CoA synthase and HMG-CoA lyase in various tissues of the chicken

<table>
<thead>
<tr>
<th>Tissue</th>
<th>HMG-CoA synthase</th>
<th>HMG-CoA lyase</th>
<th>Ketogenic capacity (from Ref. 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1.6 ± 0.4</td>
<td>8.1 ± 2.2</td>
<td>+</td>
</tr>
<tr>
<td>Kidney</td>
<td>&lt;0.01 ± 0.03</td>
<td>0.1 ± 0.4</td>
<td>+</td>
</tr>
<tr>
<td>Heart</td>
<td>&lt;0.01</td>
<td>1.8 ± 0.6</td>
<td>-</td>
</tr>
<tr>
<td>Brain</td>
<td>&lt;0.01</td>
<td>1.0 (1.5-1.7)</td>
<td>-</td>
</tr>
<tr>
<td>Ileum</td>
<td>&lt;0.01</td>
<td>0.5 ± 0.3</td>
<td>-</td>
</tr>
<tr>
<td>Muscle</td>
<td>&lt;0.01</td>
<td>0.6 (0.5-0.7)</td>
<td>-</td>
</tr>
</tbody>
</table>

* One unit equals 1.0 μmol of substrate converted/min.

In contrast, the same tissues all contained significant levels of HMG-CoA lyase activity (Table V). These results are in contradiction to similar studies conducted with the rat where HMG-CoA lyase activity was found to be absent in brain and skeletal muscle (47). Rat brain also was tested for HMG-CoA lyase activity as described in Table V with the finding that the mitochondrial activity was 0.7 unit/g wet weight of brain (average value for four rats). This level of activity also was confirmed by measuring the [3-14C]acetoacetate formed (38) instead of the [3-14C]HMG-CoA used.

Since kidney is the only extrahepatic tissue known to be capable of ketogenesis (47), albeit at a slow rate, it is evident that ketogenic capacity is directly correlated to the presence of mitochondrial HMG-CoA synthase activity. Ketogenic capacity is not correlated to the tissue distribution of either mitochondrial acetoacetyl-CoA thiolase or HMG-CoA lyase. This is reasonable since both mitochondrial acetoacetyl-CoA thiolase and HMG-CoA lyase serve extrahepatic functions other than ketogenesis, e.g. β oxidation of fatty acids and ketone body utilization (14) as well as leucine catabolism (36, 37).

**DISCUSSION**

Cholesterogenesis and ketogenesis are diverse functions of liver which require independent regulation, yet the first two steps of these pathways are chemically identical (Scheme 1). Upon cursory examination it might seem logical that the flux of acetyl units into these diverging pathways necessarily would be determined at the first committed step(s) beyond the HMG-CoA branch point, i.e. at the HMG-CoA reductase reaction of cholesterogenesis and at the HMG-CoA lyase reaction of acetoacetate formation (Scheme 1). However, the present study indicates that the reductase and lyase reactions are not the steps which commit acetyl-CoA to cholesterol or acetoacetate synthesis. Rather, these two biosynthetic processes occur in different subcellular compartments, cholesterol synthesis in the cytoplasm and acetoacetate formation in the mitochondria. The cytoplasm of liver contains all of the enzymes required for the conversion of HMG-CoA to cholesterol (49), while only mitochondria appear to possess the lyase needed to convert HMG-CoA to acetoacetate and acetyl-CoA (Table I, Figs. 3 and 4). Consistent with the independence of these diverse pathways is the finding that the cytoplasmic, as well as the mitochondrial, compartment of liver has the capacity to synthesize HMG-CoA, i.e. both contain acetoacetyl-CoA thiolase and HMG CoA synthase (Table I, Ref. 13). Hence, the segment common to both cholesterogenesis and ketogenesis has dual localization. Segregation of the cytoplasmic and mitochondrial HMG-CoA pools is insured by the impermeability of the inner mitochondrial membrane to CoA derivatives (11); consequently, HMG-CoA generated in the cytoplasm apparently serves as precursor for cholesterol, while mitochondrially generated HMG-CoA serves as precursor for acetoacetate. This point was confirmed recently by the finding that the cytoplasmic acetyl-CoA pool as precursor for cholesterol synthesis has been shown (13) to possess molecular properties distinct from its mitochondrial, i.e. ketogenic, counterpart. Compelling evidence has also been obtained which shows the mitochondrial (19) and cytoplasmic (15, 20) HMG-CoA synthases to be different proteins. Both synthases have been purified to homogeneity from chicken liver and can be distinguished immunologically as well as by other means. Also important is the fact that the cytoplasmic acetoacetyl-CoA thiolase and HMG-CoA synthase of liver appear subject to negative feedback control by dietary cholesterol, while the corresponding mitochondrial enzymes are not (15, 20).

Cholesterol synthesis occurs in most, if not all animal tissues, although in most extrahepatic tissues the process is relatively slow (50). The tissue distribution of cytoplasmic acetoacetyl-CoA thiolase, the first enzyme in cholesterogenesis, is correlated with the tissue distribution of cholesterol synthetic capacity and has been found in all tissues surveyed (14). Selected tissues of the chicken were surveyed for cytoplasmic HMG-CoA synthase. Liver was found to have the highest activity, 0.3 unit/g wet weight of tissue and extrahepatic tissues, such as kidney (0.1 unit/g) and brain (0.02 unit/g), contained significant activities. The presence of cytoplasmic HMG-CoA synthase in liver, kidney, and brain, as well as in heart and ileum, was confirmed by immunodiffusion with antiserum against homogeneous cytoplasmic HMG-CoA synthase from chicken liver. Hence, the broad tissue distribution of cytoplasmic thiolase and HMG-CoA synthase is compatible with that of cholesterogenesis.

In contrast to the ubiquity of cholesterogenesis, not all animal tissues can synthesize acetoacetate from acetyl-CoA. This pathway, i.e. the HMG-CoA cycle, appears restricted to liver and perhaps kidney (12). Moreover, liver is the only tissue known to be unable to utilize acetoacetate at a significant rate (48); thus, its export to extrahepatic tissues is facilitated. Mitochondrial acetoacetyl-CoA thiolase is involved in both the synthesis of ketones by liver, as well as the utilization of ketones by extrahepatic tissues. Consistent with these physiological roles is the fact that this enzyme has been found in all animal tissues surveyed (14). The inability of extrahepatic tissues to synthesize ketones has been attributed to the absence of mitochondrial IIM-CoA lyase or IIM-CoA synthase, or both (47). In this...
connection, mitochondrial HMG-CoA synthase appears to function primarily in ketogenesis, while HMG-CoA lyase participates in both hepatic ketogenesis and in the leucine catabolic pathway (37, 51). As illustrated in Table V, liver and kidney contain significant mitochondrial HMG-CoA synthase activity, whereas in other tissues, such as heart, ileum, brain, and skeletal muscle, the enzyme appears to be absent, i.e. <0.01 unit/g wet weight of tissue. In contrast, the same tissues contained significant levels of mitochondrial HMG-CoA lyase activity (Table V). These results are in disagreement with those conducted with the rat where HMG-CoA lyase activity was not detectable in brain and skeletal muscle (47, 52). Rat brain tested for HMG-CoA lyase activity as described in Table V contains 0.7 unit of the mitochondrial enzyme/g wet weight of tissue (average of four rats). This level of activity was confirmed by the direct measurement of [3-14C]acetacacetate formed from [3-14C]-HMG-CoA. The presence of HMG-CoA lyase in all extrahepatic tissues surveyed is consistent with the occurrence of leucine catabolism primarily in extrahepatic tissues (53). In view of the tissue distribution of mitochondrial thiolase, synthase, and lyase discussed above, we suggest that the ability of a tissue to synthesize acetacacetate is determined by its mitochondrial content of HMG-CoA synthase.

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REFERENCES

40. Wieland, O. (1968) in Advan. Metab. Disord. 3, 7
42. Allred, J. B. (1973) Biochim. Biophys. Acta 297, 22
Intracellular localization of the 3-hydroxy-3-methylglutaryl coenzyme A cycle enzymes in liver. Separate cytoplasmic and mitochondrial 3-hydroxy-3-methylglutaryl coenzyme A generating systems for cholesterogenesis and ketogenesis.

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