Feedback Regulation of Cholesterol Biosynthesis:

STUDIES WITH CHOLESTYRAMINE

By Lawrence W. White

ABSTRACT

Regulation of hepatic cholesterol biosynthesis is thought to occur at the step involving formation of mevalonate from 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA); this reaction is catalyzed by HMG-CoA reductase. Oral administration of cholestyramine, an agent that interferes with cholesterol reabsorption and is associated with a compensatory increase in hepatic cholesterol synthesis, was used to evaluate feedback regulation of cholesterol biosynthesis. Liver slices and cell-free fractions were prepared from control rats and rats fed 3% cholestyramine for 3–7 days. Cholestyramine feeding was associated with a four- to eightfold increase in incorporation of [14C]acetate into cholesterol and mevalonate. However, there was no effect on incorporation of [14C]acetate into CO₂, fatty acids, or acetoacetate or on incorporation of [14C]mevalonate into cholesterol, indicating stimulation at an early regulatory site prior to mevalonate formation. In fractionated liver homogenates, incorporation of [14C]acetate, [14C]acetyl-CoA, and [14C]HMG-CoA into mevalonate increased markedly following cholestyramine administration. In the absence of microsomes, incorporation of [14C]acetate and [14C]acetyl-CoA into HMG was also increased. Changes in incorporation of [14C]acetate into HMG, mevalonate, and cholesterol corresponded to changes in product synthesis. I concluded that (1) interruption of the enterohepatic circulation of cholesterol causes a release of feedback inhibition of cholesterol synthesis, (2) mevalonate synthesis catalyzed by HMG-CoA condensing enzyme and HMG-CoA reductase, and (3) HMG-CoA condensing enzyme, by limiting the availability of HMG-CoA, may have an important regulatory function in cholesterol biosynthesis.

KEY WORDS compensatory mechanisms mevalonate 3-hydroxy-3-methylglutarate hypocholesterolemic agents acetoacetate synthesis rat liver
adequately assess the possibility of regulation at points prior to HMG-CoA formation (6). Evaluation of all reactions prior to MVA synthesis has suggested that feedback inhibition by dietary cholesterol involves both the reaction catalyzed by HMG-CoA reductase and the preceding reaction catalyzed by HMG-CoA condensing enzyme (3-hydroxy-3-methylglutaryl-CoA acetoacetyl-CoA lyase, EC 4.1.3.5) (7). However, inhibition of HMG-CoA condensing enzyme was relatively small (approximately 50%) compared with inhibition of HMG-CoA reductase (greater than 90%) (7); furthermore, other workers, using different techniques, have concluded that cholesterol feeding has no effect on HMG-CoA condensing enzyme (3, 8).

Oral administration of cholestyramine, an anion exchange resin, results in an interruption of the normal enterohepatic circulation of bile salts, an increase in the fecal excretion of bile acids and neutral steroids (9, 10), and a reduction in the plasma cholesterol concentration in animals and man (9, 11). However, prolonged interference with cholesterol resorption may be counteracted by increased endogenous cholesterol synthesis with no net change in tissue concentration (12, 13). It is now clear that cholestyramine administration is associated with a marked enhancement in cholesterol synthesis, as shown by an accelerated rate of decay of plasma cholesterol specific activity after administration of radioactive cholesterol (10, 14, 15) and by a marked increase in [14C]acetate incorporation into cholesterol following cholestyramine feeding (16). Because cholestyramine decreases intestinal absorption of cholesterol, resulting in a compensatory increase in hepatic synthesis, administration of this agent was used to further explore feedback regulation of cholesterol biosynthesis.

Methods

Male Sprague-Dawley rats weighing 100–300 g were fed ground Purina rat chow (control) or ground chow with added cholestyramine\(^1\) at a level of 3% of the diet. After 3–7 days of treatment, rats were killed by decapitation, their livers were rapidly removed and chilled, and slices or homogenate fractions were prepared. At the time of death, blood was collected in beakers containing heparin, plasma was separated by centrifugation, and plasma cholesterol was determined by the method of Zlatkis et al. (17). The liver samples were added to 20 volumes of 10% KOH in 50% ethanol and refluxed for 4 hours. After cooling, the mixture was extracted twice with 2 volumes of hexane. The hexane was evaporated to dryness, and the cholesterol in the residue was measured by the colorimetric method of Zlatkis et al. (17).

Slices.—Approximately 500 mg of liver slices, prepared with a McIlwain tissue chopper, were incubated in 5 ml of Krebs-Ringer's bicarbonate buffer, pH 7.2-7.4, containing 20 mM glucose and labeled substrate consisting of either 4 mM \([14C]\)acetate (5 μc/flask) or 1 mM \([2-14C]\)MVA (0.1 μc/flask). In some flasks, the segment of the pathway before MVA synthesis was evaluated by adding 20 μM MVA as a trap. Medium and contents were bubbled with 95% O2-5% CO2 for 5 minutes and incubated with shaking for 90 minutes at 37°C.

Following incubation, slices were blotted and saponified, and cholesterol was extracted as described by Daly et al. (18). Digitonide formation, washing, colorimetric analysis, and radioactive assay were performed as outlined by Kabara (19). Incorporation of \(^{14}C\) into MVA, fatty acids, and CO2 was measured by methods previously detailed (20). Content, incorporation of \(^{14}C\), and specific activity of acetoacetate were determined as previously described (6).

Homogenates.—To localize the biochemical sites of action, cell-free preparations were used. Individual reactions between acetate and MVA were studied by using \([14C]\)acetate, \([14C]\)acetyl-CoA, and \([14C]\)HMG-CoA as substrates and by separating the hepatic microsomal fraction, which contains HMG-CoA reductase, from the 105,000-g supernatant (soluble) fraction, which contains all enzymes involved in generation of HMG-CoA. These methods have been described in detail previously (6, 7). To assay the earlier reactions, \([14C]\)acetate or \([14C]\)acetyl-CoA was incubated with the soluble fraction derived from 180 mg of liver from control or cholestyramine-fed rats, and incorporation into HMG was determined. To assay microsomal HMG-CoA reductase, \([14C]\)acetate, \([14C]\)acetyl-CoA, or \([14C]\)HMG-CoA was incubated in the presence of an MVA trap with microsomes derived from 600 mg of liver from control or cholestyramine-fed rats. When \([14C]\)acetate or \([14C]\)acetyl-CoA was used, a pooled soluble fraction from control rats

\(^1\)Cholestyramine resin was generously supplied by Dr. C. L. Rendub of Mead Johnson Research Center, Evansville, Indiana.
TABLE 1
Effect of Cholestyramine on Substrate Incorporation into Cholesterol by Rat Liver Slices

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control (mumoles/mg cholesterol)</th>
<th>Cholestyramine (mumoles/mg cholesterol)</th>
<th>Ratio, treated to control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1-14C]Acetate</td>
<td>42.5 ± 6.8 (4)</td>
<td>385 ± 43.9 (5)</td>
<td>8.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>[1-14C]Acetate</td>
<td>73.7 ± 42.1 (4)</td>
<td>329 ± 62.0 (4)</td>
<td>4.45</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>[2-14C]MVA</td>
<td>290 ± 30.3 (5)</td>
<td>327 ± 18.9 (5)</td>
<td>1.12</td>
<td>&lt;0.4</td>
</tr>
</tbody>
</table>

All rats were fed chow with or without cholestyramine for 3 days. Values are means ± se; number of rats tested is given in parentheses. Statistical evaluations of t and probability were performed by standard methods (22).

Materials. — [14C]HMG anhydride and [14C] HMG-CoA were prepared as described by Louw et al. (21). Cholestyramine resin is the insoluble chloride salt of an anion exchange resin, a large polymer containing quaternary ammonium groups attached to a styrene-2% divinylbenzene skeleton. Other materials were prepared or obtained as previously described (6).

Results
Cholestyramine administration at a level of 3% of the diet had no effect on weight gain. Plasma cholesterol concentration decreased in all treated rats, but this decrease was not significant in all cases. There were no significant changes in liver cholesterol concentration.

To study the overall pathway of cholesterol synthesis, as well as selected segments of the

TABLE 2
Effect of Cholestyramine on Incorporation of [1-14C]Acetate into MVA, CO2, Fatty Acids, and Acetoacetate by Rat Liver Slices

<table>
<thead>
<tr>
<th>Product</th>
<th>Control (mumoles/g liver)</th>
<th>Cholestyramine (mumoles/g liver)</th>
<th>Ratio, treated to control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVA</td>
<td>48.0 ± 11.0 (5)</td>
<td>235 ± 26.5 (5)</td>
<td>4.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MVA</td>
<td>29.2 ± 4.9 (4)</td>
<td>176 ± 35 (4)</td>
<td>6.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CO2</td>
<td>2664 ± 82 (5)</td>
<td>1869 ± 110 (5)</td>
<td>0.91</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>605 ± 135 (5)</td>
<td>926 ± 233 (5)</td>
<td>1.53</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>64.8 ± 5.5 (5)</td>
<td>64.3 ± 9.4 (5)</td>
<td>0.99</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>37.3 ± 9.7 (4)</td>
<td>35.2 ± 9.3 (4)</td>
<td>0.95</td>
<td>&gt;0.5</td>
</tr>
</tbody>
</table>

All rats were fed chow with or without cholestyramine for 3 days. Values are means ± se; number of rats tested is given in parentheses.

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TABLE 3
Effect of Cholestyramine on HMG-CoA Reductase in Rat Liver Homogenate Fractions

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Days of cholestyramine</th>
<th>Incorporation into MVA</th>
<th>Ratio, treated to control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A ([14C]acetate + control soluble fraction)</td>
<td>3</td>
<td>14.7 ± 3.1</td>
<td>264 ± 11.4</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>19.3 ± 1.5</td>
<td>197 ± 18.6</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>42.9 ± 6.6</td>
<td>383 ± 56.6</td>
<td>8.9</td>
</tr>
<tr>
<td>B ([14C]acetyl-CoA + control soluble fraction)</td>
<td>3</td>
<td>0.789 ± 0.144</td>
<td>28.2 ± 2.9</td>
<td>35.7</td>
</tr>
<tr>
<td>C ([14C]HMG-CoA)</td>
<td>3</td>
<td>8.34 ± 1.27</td>
<td>164 ± 11.7</td>
<td>19.7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>10.6 ± 1.99</td>
<td>138 ± 19.6</td>
<td>13.9</td>
</tr>
</tbody>
</table>

Values are means ± SE for four rats. Incubations were performed in air at 37° for 1 hour. Contents of each incubation flask in a volume of 7 ml were as follows. A: Soluble and microsomal fractions, 20 μmoles [1-14C]acetate (5 μc), 0.4 μmoles CoA, 20 μmoles ATP, 10 μmoles EDTA, 12 μmoles MgCl2, 50 μmoles MVA, 20 μmoles glucose-6-phosphate, 10 μmoles NADP, 2 units glucose-6-phosphate dehydrogenase, and 400 μmoles potassium phosphate buffer (pH 7.0). B: Soluble and microsomal fractions, 2 μmoles [14C]acetyl-CoA (2 μc), 10 μmoles EDTA, 7 μmoles dithiothreitol, 50 μmoles MVA, 20 μmoles glucose-6-phosphate, 10 μmoles NADP, 2 units glucose-6-phosphate dehydrogenase, and 100 μmoles potassium phosphate buffer (pH 7.0). C: Microsomal fraction, 1 μmol [14C]HMG-CoA (0.3 μc), 10 μmoles EDTA, 70 μmoles dithiothreitol, 50 μmoles MVA, 20 μmoles glucose-6-phosphate, 10 μmoles NADP, 2 units glucose-6-phosphate dehydrogenase, and 200 μmoles potassium phosphate buffer (pH 7.0).

pathway, rat liver slices were used. The effect of cholestyramine on incorporation of [14C]substrate into cholesterol by slices is shown in Table 1. Incorporation of [14C]acetate into cholesterol increased markedly following cholestyramine administration, whereas incorporation of [2-14C]MVA into cholesterol was not affected, indicating that

TABLE 4
Effect of Cholestyramine on HMG-CoA Condensing Enzyme in Rat Liver Soluble Fraction

<table>
<thead>
<tr>
<th>Condition</th>
<th>Days of cholestyramine</th>
<th>Incorporation into HMG (fmoles substrate)</th>
<th>Ratio, treated to control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A ([14C]acetate + soluble fraction)</td>
<td>3</td>
<td>26.0 ± 6.7</td>
<td>147.8 ± 4.0</td>
<td>5.67</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>45.9 ± 8.4</td>
<td>122 ± 14.1</td>
<td>2.66</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>28.3 ± 6.4</td>
<td>78.3 ± 7.8</td>
<td>2.77</td>
</tr>
<tr>
<td>B ([14C]acetyl-CoA + soluble fraction)</td>
<td>3</td>
<td>35.3 ± 1.1</td>
<td>57.2 ± 1.7</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>35.7 ± 2.4</td>
<td>47.6 ± 2.7</td>
<td>1.34</td>
</tr>
<tr>
<td>C ([14C]acetate + soluble and microsomal fractions)</td>
<td>3</td>
<td>51.3 ± 1.1</td>
<td>12.3 ± 1.2</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>52.5 ± 3.0</td>
<td>31.8 ± 5.1</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>27.9 ± 2.7</td>
<td>16.4 ± 3.5</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>3*</td>
<td>27.9 ± 2.7</td>
<td>51.5 ± 4.6</td>
<td>1.84</td>
</tr>
</tbody>
</table>

Values are means ± SE for four rats. Incubations were performed in air at 37° for 1 hour. Contents of each incubation flask in a volume of 7 ml were as follows. A: Soluble fraction of 105,000-g supernatant fluid, 20 μmoles [14C]acetate (5 μc), 0.4 μmoles CoA, 20 μmoles ATP, 2 μmoles EDTA, and 400 μmoles potassium phosphate buffer (pH 7.0). B: Soluble fraction, 2 μmoles [14C]acetyl-CoA (2 μc), 2 μmoles EDTA, 7 μmoles dithiothreitol, and 100 μmoles potassium phosphate buffer (pH 7.0). C: All flasks contained normal soluble fractions and either normal or cholestyramine microsomes, except where the values are designated by an asterisk. These flasks contained both soluble and microsomal fractions from normal or treated rats. Other contents of incubation flasks are as in Table 3A.
cholestyramine influenced the segment of the biosynthetic pathway prior to MVA formation.

The segment of the pathway before MVA synthesis was evaluated directly by adding unlabeled MVA to the incubation medium at a concentration of 20 mM to trap MVA formed from [14C]acetate (Table 2). Incorporation of [14C]acetate into MVA increased four- to sixfold in slices from cholestyramine-fed rats, but there was no significant effect on acetoacetate formation or on incorporation of [14C]acetate into CO₂, fatty acids, or acetoacetate, suggesting a site of action beyond the branch points for acetoacetate, CO₂, and fatty acid synthesis, i.e., between acetoacetyl-CoA and MVA.

To determine the biochemical sites of action more precisely, fractionated homogenates were used. In the presence of microsomes from cholestyramine-treated rats, there was a marked increase in incorporation of [14C]acetate, [14C]acetyl-CoA, or [14C]HMG-CoA into MVA, indicating greatly increased activity of HMG-CoA reductase (Table 3).

The effect of cholestyramine on incorporation of [14C]acetate or [14C]acetyl-CoA into HMG is shown in Table 4 and Figure 1A. When this effect was measured in the absence of microsomes (Table 4), so that HMG-CoA was an end product, incorporation of both [14C]acetate and [14C]acetyl-CoA into HMG increased following cholestyramine feeding, indicating an effect on HMG-CoA condensing enzyme. When microsomes were also present (Table 4), the increased incorporation into HMG was no longer apparent. With the normal soluble fraction present in all flasks, permitting the same rate of HMG-CoA generation, addition of microsomes from cholestyramine-treated rats resulted in increased MVA formation but decreased accumulation of

![FIGURE 1](https://example.com/figure1.png)

**FIGURE 1**

Incorporation of [14C]acetate into HMG or MVA in the presence or the absence of microsomes. Conditions of incubation are outlined in the text. A: Two experiments performed in the absence of the microsomal fraction are shown. Flasks contained the soluble fraction from control or cholestyramine-fed rats. Flask contents are described in Table 5. B: Two additional experiments are shown in which all flasks contained the soluble fraction from control rats and microsomes from either control or cholestyramine-treated rats. Flask contents are described in Table 4. Values are means ± se for four rats, expressed as µmoles of [14C]acetate incorporated into HMG or MVA.
radioactivity in HMG (Fig. 1B); this effect is presumably the result of increased HMG-CoA removal following enhanced HMG-CoA reductase activity.

The relative roles of soluble and microsomal fractions in determining the effects of cholestyramine were examined more closely by combining microsomal and soluble fractions from control or cholestyramine-treated rats (Fig. 2). When both fractions were from cholestyramine-fed rats, there was a marked increase in incorporation into MVA with a relatively small increase in incorporation into HMG. When microsomes from normal rats were present, addition of soluble fraction from cholestyramine-treated rats resulted in an increase in both HMG and MVA synthesis, indicating that HMG-CoA formation, dependent on the soluble fraction, was limiting subsequent MVA formation. Similarly, when microsomes from cholestyramine-treated rats were present, addition of soluble fraction from cholestyramine-treated rats led to a marked increase in MVA formation. Addition of microsomes from cholestyramine-treated rats resulted in decreased accumulation of radioactivity in HMG and increased MVA formation; this phenomenon occurred in the presence of soluble fraction from normal rats or soluble fraction from cholestyramine-treated rats.

To determine whether incorporation of labeled substrate into product was a reflection of product synthesis, the specific activity of acetoacetate was determined, permitting correction for dilution of intermediate pools. Synthesis of HMG, MVA, and cholesterol was compared with incorporation of labeled substrate (Table 5). The specific activity of acetoacetate formed by liver slices or homogenates was not influenced by cholestyramine treatment; consequently, increased incorporation of [14C]acetate into products correlated with increased HMG, MVA, or cholesterol synthesis.

Discussion

Cholestyramine, one of several pharmacologic agents used to decrease plasma cholesterol concentration in an attempt to prevent atherosclerosis (11, 23), is considered one of the more effective drugs for treatment of type II hyperlipoproteinemia (24). However, the decrease in plasma cholesterol concentration has not been impressive in many cases, and in some patients a hypocholesterolemic effect has not been obtained (10, 14, 25).

Cholestyramine produces a negative cholesterol balance with increased excretion of endogenous neutral and acidic steroids (10, 14). The increment in cholesterol excreted can come from newly synthesized cholesterol, necessitating an increased rate of synthesis, or it can result from a depletion of tissue or plasma cholesterol pools. An increased rate of turnover of body cholesterol has been found in patients following cholestyramine treatment (14). The drug probably fails to induce a decrease in plasma cholesterol concentration in many patients, because the rate of cholesterol synthesis increases enough to compensate.
TABLE 5

Effect of Cholestyramine on Synthesis of HMG, MVA, and Cholesterol

<table>
<thead>
<tr>
<th>Product</th>
<th>Incorporation (mmoles [%]Acetate/g tissue)</th>
<th>Synthesis (mmoles product/g tissue)</th>
<th>Ratio, treated to control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Cholestyramine</td>
<td>Control</td>
</tr>
<tr>
<td>MVA</td>
<td>48.0 ± 11.0</td>
<td>235 ± 26.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>29.2 ± 4.9</td>
<td>176 ± 35</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>90.4 ± 13.8</td>
<td>728 ± 83</td>
<td>8.1</td>
</tr>
<tr>
<td>HMG*</td>
<td>28.3 ± 6.4</td>
<td>78.3 ± 7.8</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. Conditions are described in the text.

*Assay was performed with 105,000-g soluble fraction of liver homogenates instead of the rat liver slices used when MVA or cholesterol synthesis was measured.

for the increased excretion (10). In the present study, the compensatory increase in synthesis was sufficient to prevent changes in liver cholesterol concentration and to minimize changes in plasma cholesterol concentration.

As previously emphasized, earlier conclusions regarding sites of regulation of cholesterol synthesis were based on assumptions that may not be valid (6). Other workers have shown that cholestyramine produces a twelvefold increase in incorporation of [%]Acetate into hepatic cholesterol, with only a two- to threefold increase in [%]MVA incorporation into cholesterol (16). These studies measured incorporation, rather than synthesis; furthermore, they measured the effect on the entire pathway without localizing the site of regulation. If treatment resulted in an altered degree of dilution of acetyl-CoA by unlabeled material, incorporation of [%]MVA, but not [%]MVA, would be affected (6). By determining acetocacetate specific activity, I found that dilution of intermediates by endogenous substrates was not affected by treatment and showed that synthesis of cholesterol or MVA was markedly increased. In addition, the sites of action that account for the increased cholesterol synthesis following cholestyramine administration have been clearly delineated and involve increased activity at two key steps before MVA synthesis: HMG-CoA condensing enzyme and HMG-CoA reductase are both affected. These studies emphasize the importance of studying reactions involved in MVA formation other than the one catalyzed by HMG-CoA reductase.

The amount of HMG-CoA converted to MVA will depend not only on the activity of HMG-CoA reductase but on the quantity of HMG-CoA generated by earlier reactions. For several reasons, the reaction catalyzed by HMG-CoA reductase has been considered an appropriate regulatory step in sterol biosynthesis (2-7). However, insufficient consideration has been given to possible regulation at the preceding step involving condensation of acetyl-CoA and acetocetyl-CoA to form HMG-CoA. This step might also be considered a logical control point on theoretical grounds (6): the reaction is irreversible and is uniquely situated, influencing the concentration of acetyl-CoA available for oxidation or fatty acid synthesis, the concentration of acetocetyl-CoA available for ketone synthesis, and the concentration of HMG-CoA available for sterol synthesis. Furthermore, if HMG-CoA was not a significant precursor of acetocacetate, then the step catalyzed by HMG-CoA condensing enzyme would represent the first reaction beyond the last quantitatively significant branch point, and feedback inhibition at this point would be analogous to that in several bacterial systems (3, 6).

The importance of HMG-CoA condensing enzyme has not been generally recognized. Using short-duration cholesterol feeding (10 hours), Shapiro and Rodwell (8) showed that there was no effect on HMG-CoA condensing enzyme, in contrast to effects previously

*Circulation Research, Vol. XXXI, December 1972
demonstrated with more prolonged cholesterol feeding (7). Siperstein and Fagan (3) were unable to demonstrate changes in incorporation of [14C]acetate into HMG following cholesterol feeding and suggested that synthesis of HMG was unaffected by dietary cholesterol. However, the microsomal fraction containing HMG-CoA reductase was also present in their experiments so that HMG was not an end product. In the presence of microsomes, net accumulation of HMG-CoA depends on both the rate of HMG-CoA formation and the rate of reduction of HMG-CoA to MVA, as previously discussed (6, 7). This fact is well exemplified in the experiments shown in Figure 1, in which cholestyramine treatment resulted in increased incorporation of [14C]acetate into HMG when microsomes were absent and HMG was an end product but decreased incorporation into HMG when microsomes were present and cholestyramine treatment increased the rate of reduction of HMG-CoA to MVA several fold. The use of a single system containing both soluble and microsomal fractions will obscure changes in the rate of HMG-CoA generation. Under these circumstances, increased activity of HMG-CoA condensing enzyme will be associated with increased MVA formation, since any increment in HMG-CoA formed will be converted to MVA. In the absence of microsomes, HMG-CoA condensing enzyme activity is decreased by cholesterol feeding (7) and markedly increased by interruption of the enterohepatic circulation of cholesterol with a release of feedback inhibition of cholesterol synthesis.

Several workers have assayed HMG-CoA condensing enzyme by measuring acetoacetate formation (26-28), based on the assumption that this reaction proceeds entirely via HMG-CoA, as suggested by Lynen et al. (29). However, it is now clear that acetoacetate can also be formed by acetoacetyl-CoA decylation (30, 31). The present study showed that the activity of HMG-CoA condensing enzyme, as measured directly, was markedly increased following cholestyramine, but there was no increase in acetoacetate formation, indicating that assays that rely on acetoacetate formation as a measure of HMG-CoA condensing enzyme are not valid.

Regulation of two key reactions in the pathway prior to MVA formation suggests synchronous influences analogous to those on groups of rate-limiting enzymes governing reactions in pathways of gluconeogenesis and glycolysis (32). In addition, reactions beyond MVA may also be involved in regulation of cholesterol biosynthesis (33). However, in contrast to the studies of Huff et al. (16), no stimulation of the reaction pathway beyond MVA was found under the conditions of the present experiments. Since hypcholesterolemic effects of cholestyramine are attenuated by a compensatory increase in cholesterol biosynthesis, agents that will prevent increased activity of HMG-CoA condensing enzyme, HMG-CoA reductase, or both should, if used concomitantly with cholestyramine, produce an effect on plasma and tissue cholesterol pools more dramatic than that obtained with cholestyramine alone.

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References


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