Inhibition of Hepatic Acetyl Coenzyme A Carboxylase by Hypolipidemic Agents*

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MICHAEL E. MARAGOUDAKIS

From the Research Department, CIBA Pharmaceutical Company, Summit, New Jersey 07901

SUMMARY

The effects of two hypolipidemic agents, 2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxyjpropionic acid and ethyl 2-(p-chlorophenyl)-2-methylpropionate, on the activity of a highly purified preparation of acetyl coenzyme A carboxylase, obtained from chicken livers, were studied.

Both compounds are inhibitors of acetyl-CoA carboxylase, but they have no inhibitory effect on a number of other enzymes tested, including fatty acid synthetase. The inhibition cannot be prevented by addition of exogenous biotin.

Kinetic analysis indicates that the inhibition is competitive for acetyl-CoA and isocitrate and noncompetitive for ATP and bicarbonate for both drugs. Mixed inhibition studies indicate that both drugs act on the same site of the enzyme and that this site is different from the site of inhibition by palmitoyl-CoA. Some insight into the mechanism of the inhibition is provided by the kinetic analysis, heat inactivation studies, sucrose gradient sedimentation, and Arrhenius plots.

The results suggest that the effect of these compounds as lipid-lowering agents may be accounted for by inhibition of acetyl-CoA carboxylase in vivo.

The major effect of 2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxyjpropionic acid (2) and ethyl 2-(p-chlorophenyl)-2-methylpropionate (3) is to reduce the plasma level of cholesterol-rich and triglyceride-rich lipoproteins. This reduction of circulating lipids in CPIB-treated animals and humans has been well established in many laboratories, but the mechanism of this effect is unknown (3-11).

Since the original report by Thorp and Waring (2), numerous reports on CPIB have appeared, and many hypotheses have been advanced concerning the mode of action of this drug. It has been suggested by indirect evidence (12-14) that displacement of thyroxine from its plasma-binding proteins is involved in the mechanism of action of CPIB. Contrary to this hypothesis of indirect action, many reports support the contention that CPIB directly alters the enzymatic process of lipid synthesis from acetate (15, 16). Experiments with liver slices indicated that the site of inhibitory action is between acetate and mevalonate (15).

On the other hand, Teal and Gamble (17) presented evidence which suggested that the enzymatic block by CPIB is between squelene and cholesterol in the reaction sequence.

Ubiquinone has been also implicated as being involved in the mode of action of CPIB (18, 19). More recently, Burch (20) found increased activity in the enzyme acetoacetyl decarboxylase in CPIB-treated animals and suggested that CPIB may be acting by making acetoacetyl coenzyme A unavailable for the biosynthetic process.

The object of this investigation was to determine whether hepatic acetyl-CoA carboxylase and/or fatty acid synthetase are affected by CPIB and TPIA to an extent that would indicate that these enzymes could be the locus of action. Acetyl-CoA carboxylase is, in fact, inhibited by both compounds, and this inhibition has been studied in some detail.

EXPERIMENTAL PROCEDURE

ATP, acetyl-CoA, glutathione, EDTA, pyruvate kinase, lactate dehydrogenase, biotin, and palmityl-CoA were obtained from Sigma. Avidin and "fat-free" diet were products of Nutritional Biochemicals. D-L-Isocitric acid (trisodium salt) was obtained from J. T. Baker Chemical Company (Phillipsburg, New Jersey). NaH14CO3 and 1,3-14C-malonyl-CoA were purchased from New England Nuclear.

CPIB is a product of Imperial Chemical Industries (Wilmslow, England). TPIA is an experimental compound synthesized by Dr. W. Bencze (2), and 14C-TPIA was prepared by Dr. T. Thompson in our laboratories. All chemicals used were of analytical grade and were used without further purification. The structural formulas of these compounds are shown in Fig. 1.

Purification of Acetyl-CoA Carboxylase—Acetyl-CoA carboxylase was extracted from fresh chicken livers, obtained from a local slaughterhouse, or from the livers of laying hens which were fasted for 48 hours and subsequently fed for 48 hours with a low fat diet. This dietary alteration causes an at least 10-fold increase in the acetyl-CoA carboxylase activity. The enzyme was purified by the method of Gregolin, Ryder, and Lane (21), and of Goto et al. (22), with minor modifications.
Inhibitors of Acetyl-CoA Carboxylase

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the carboxylation of acetyl-CoA.

Crude extract. 6,740
First, (NH₄)₂SO₄. 4,520
Ca₃(PO₄)₃ gel. 14,400
DEAE-cellulose. 34
with bovine albumin as standard.

Minus bovine albumin.. 2,817
Minus glutathione. 9,409
Minus acetyl-CoA.. 1,104
Minus MgCl₂.. 19
Minus ATP......................... 43
Minus isocitrate. 1,104

Second, (NH₄)₂SO₄. 470

Table I

Purification of acetyl-CoA carboxylase from chicken liver

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Protein</th>
<th>Total activity</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract.</td>
<td>6,740</td>
<td>412,000</td>
<td>190</td>
<td>0.0004</td>
</tr>
<tr>
<td>First, (NH₄)₂SO₄</td>
<td>4,520</td>
<td>56,700</td>
<td>119</td>
<td>0.0021</td>
</tr>
<tr>
<td>Ca₃(PO₄)₃ gel.</td>
<td>14,400</td>
<td>10,880</td>
<td>262</td>
<td>0.0024</td>
</tr>
<tr>
<td>Second, (NH₄)₂SO₄</td>
<td>470</td>
<td>438</td>
<td>12.1</td>
<td>0.0280</td>
</tr>
<tr>
<td>DEAE-cellulose.</td>
<td>34</td>
<td>71.4</td>
<td>18</td>
<td>0.2500</td>
</tr>
</tbody>
</table>

* A unit of enzyme is defined as that amount which catalyzes
1 µmole of acetyl-CoA per min under the standard assay conditions.

** Protein was determined by the method of Lowry et al. (23)
with bovine albumin as standard.

Table II

Requirements for acetyl-CoA carboxylase activity purified
from chicken liver

<table>
<thead>
<tr>
<th>Components</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>11,247</td>
</tr>
<tr>
<td>Minus ATP</td>
<td>45</td>
</tr>
<tr>
<td>Minus MgCl₂</td>
<td>19</td>
</tr>
<tr>
<td>Minus acetyl-CoA</td>
<td>21</td>
</tr>
<tr>
<td>Minus isocitrate</td>
<td>1,104</td>
</tr>
<tr>
<td>Minus glutathione</td>
<td>9,409</td>
</tr>
<tr>
<td>Minus bovine albumin</td>
<td>2,817</td>
</tr>
<tr>
<td>Minus enzyme</td>
<td>30</td>
</tr>
</tbody>
</table>

* H¹⁴CO₂⁻ fixation assay is as described under "Experimental Procedures." Enzyme is purified acetyl-CoA carboxylase from chicken liver, 65 µg of protein (Lowry) (specific activity, 0.25). Incubation time was 10 min.

Results

Inhibition of Acetyl-CoA carboxylase by TPIA and CPIB—The activity of acetyl-CoA carboxylase was measured by the H¹⁴CO₂⁻ fixation assay.
fixation assay in the presence of increasing concentrations of CPIB (sodium salt). As shown in Table III, the enzymatic activity falls as the concentration of the drug is increased. The same effect was obtained in the presence of TPIA (sodium salt) in the reaction mixture, however, at much lower concentrations of inhibitor (Table IV).

The concentration of drug which is required to inhibit the enzyme by 50% (I_50) under the experimental conditions is I_50 = 1.25 × 10^{-3} M for CPIB and I_50 = 7.5 × 10^{-4} M for TPIA. TPIA is therefore about 17 times more potent than clofibrate as an inhibitor of acetyl-CoA carboxylase. TPIA exerts its effect in vitro at lower doses than does clofibrate. The decrease of the activity is linear when plotted against the log of drug concentration. The inhibition is evident at all stages of purification of the enzyme and is of the same magnitude throughout the narrow pH range for enzyme activity (pH 6.5 to 8.0).

**Effect of TPIA and CPIB on Other Enzymes**—Concentration levels of the above compounds which drastically inhibit acetyl-CoA carboxylase have no inhibitory effect in vitro on laetic dehydrogenase, malic dehydrogenase, isocitric dehydrogenase, enolase, phosphoenolpyruvate mutase, pyruvate kinase, creatine kinase, and fatty acid synthetase.

The effect of TPIA and CPIB on the multienzyme complex which catalyzes the synthesis of fatty acids from malonyl-CoA was of special interest. As shown in Table V, fatty acid synthetase is stimulated to a significant extent at low concentrations of these compounds and only at high concentrations does the activity drop abruptly.

The presence of 1.25 × 10^{-3} M TPIA in the fatty acid synthetase system has a stimulatory effect of about 19%, whereas the same concentration in the acetyl-CoA carboxylase system causes 100% inhibition. Similarly, CPIB at the level of 8.4 × 10^{-3} M causes a 51% stimulation of fatty acid synthetase but inhibits acetyl-CoA carboxylase by 95%.

**Effect of Biotin on Inhibition of Acetyl-CoA Carboxylase by TPIA, CPIB, and Avidin**—Avidin is a specific biotin-binding protein from egg white; it forms stoichiometric complexes with biotin (K_d = 10^{-14} M) (28) and the biotinyl moiety of enzymes (29).

The inhibition of acetyl-CoA carboxylase caused by avidin can be prevented if exogenous biotin is present in the reaction mixture. Under our assay conditions, the addition of 0.2 mg of avidin reduces the enzyme activity by 83%. When 0.2 mg of avidin plus 0.1 mg of d-biotin are present in the reaction mixture, before the addition of the enzyme, the enzyme activity is not affected by the presence of avidin. d-Biotin alone has no effect on the activity of acetyl-CoA carboxylase. On the other hand, the presence of d-biotin along with either TPIA or CPIB at concentrations that cause about 50% inhibition, does not prevent the inhibitory action of the drugs. These data would indicate that the biotinyl moiety of the enzyme is not involved in the inhibition by TPIA or CPIB.

**C-TPIA Binding to Proteins**—Table VI shows the results of equilibrium dialysis experiments in which the binding affinity of three proteins for TPIA was measured at 0°C (27). The C values indicate the highest binding affinity for acetyl-CoA carboxylase. C-TPIA, however, is bound to other proteins by what is probably a hydrophobic type of interaction.
**TABLE VI**

Relative binding activities (C values) for [14C]-TPIA of acetyl-CoA carboxylase, lactic dehydrogenase, and avidin

<table>
<thead>
<tr>
<th>Protein</th>
<th>C values at 0°F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-CoA carboxylase</td>
<td>0.53</td>
</tr>
<tr>
<td>Lactic dehydrogenase</td>
<td>0.30</td>
</tr>
<tr>
<td>Avidin</td>
<td>0.24</td>
</tr>
</tbody>
</table>

*Binding studies were performed by equilibrium dialysis at 0°F for 72 hours. Binding affinity is expressed by C = |I|/[I]P, where I is bound TPIA (cpm/ml), I is unbound TPIA (cpm/ml), and P is protein (mg/ml).*

**Fig. 2.** The effect of acetyl-CoA concentration on enzyme activity and TPIA inhibition. Conditions were the same as described under "Experimental Procedure." Acetyl-CoA carboxylase, about 4 X 10⁻³ unit (purified after the third ammonium sulfate stage (see Table I)), was present in each tube. Initial velocity V is expressed as counts per minute of H¹³CO₃ fixed in 5 min per assay tube. Under the conditions of these experiments, the reaction was of zero order for at least 8 min. $K_m = 1.7 \times 10^{-4}$ M and $K_i = 8.93 \times 10^{-6}$ M.

**Kinetics of Inhibition of Acetyl-CoA Carboxylase by TPIA and CPIB** — The effects of acetyl-CoA, ATP, bicarbonate, and isocitrate concentrations on enzyme activity and on the inhibition by CPIB and TPIA was studied by Lineweaver-Burk analysis of the initial velocities. Figs. 2 to 9 show the results. With both drugs, the inhibition is competitive with respect to isocitrate and acetyl-CoA and noncompetitive with respect to ATP and bicarbonate. These results have been confirmed by kinetic analysis with the use of Dixon plots and in experiments with the use of the spectrophotometric assay (24) for acetyl-CoA carboxylase.

**Mixed Inhibition Studies** — If the hypolipidemic action of CPIB or TPIA or both is, in fact, attributable to inhibition of acetyl-CoA carboxylase in vivo, the question of whether the two drugs have independent or the same binding sites on the enzyme protein becomes important. Different binding sites might forecast a synergistic effect. This question is not answered by the kinetic analysis of the inhibition because both compounds are competitive for isocitrate and acetyl-CoA and noncompetitive for ATP and bicarbonate. Therefore, we investigated their interaction by means of mixed inhibition studies. The question of independent or identical sites of action was raised for the combinations of TPIA-CPIB and TPIA-palmityl-CoA. Palmityl-CoA was included in this study because it has been extensively studied as a possible feedback inhibitor of acetyl-CoA carboxylase activity (30). Recently, however, the physiological significance of such an inhibitor has been questioned (31).

Lineweaver-Burk analysis was performed in the absence of inhibitors, in the presence of each inhibitor separately, and then in the simultaneous presence of both members of the pair under consideration.

The slope to be expected from the simultaneous presence of TPIA and CPIB can be calculated from the ratio, $K_m V_{max}$, of the uninhibited system applying the factor

$$
\left(1 + \frac{I}{K_i} + \frac{I}{K_i}ight)
$$
Fig. 5. Lineweaver-Burk plots of the inhibition of acetyl-CoA carboxylase by CPIB with ATP as the varied substrate. Assay conditions are as in Fig. 2. The kinetic constants derived are $K_m = 1.9 \times 10^{-2} \text{M}$ for ATP and $K_i = 3.8 \times 10^{-8}$ for CPIB.

Fig. 6. Lineweaver-Burk analysis of the inhibition of acetyl-CoA carboxylase by TPIA with HCO$_3^-$ as the varied substrate. Other components of the reaction mixture were as in Fig. 2. The kinetic constants derived from these lines are $K_m = 8.7 \times 10^{-8} \text{M}$ for HCO$_3^-$ and $K_i = 1.61 \times 10^{-4} \text{M}$ for TPIA, and the inhibition is noncompetitive for HCO$_3^-$. For identical sites and

\[ \left(1 + \frac{i}{K_i} \right) \left(1 + \frac{i'}{K_i'} \right) \]

for different sites (32); $i$, $i'$ and $K_i$, $K_i'$ are the concentrations and the inhibition constants for TPIA and CPIB, respectively. The slope found for the pair TPIA-cloribrate from Fig. 10 is 0.83. The slope calculated for identical sites of action is 0.84, and the one calculated for different sites of action is 1.02. Therefore, the kinetic data obtained in the simultaneous presence of TPIA and CPIB support the conclusion that both agents affect one and the same site.

For the combination TPIA-palmityl-CoA, one inhibitor is competitive and the other noncompetitive with respect to acetyl-CoA. The equation (32) for such a system is

\[ \frac{1}{V} = \frac{i_t + 1}{V_{\text{max}}} + \frac{1}{S} \left( \frac{i_t + i_s + i_t + 1}{V_{\text{max}}} \right) \]

where $i_t$ is concentration of TPIA and $i_s$ is concentration of palmityl-CoA. The slope for the combined presence of TPIA
FIG. 9. Lineweaver-Burk analysis of the CPIB inhibition of acetyl-CoA carboxylase with varying isocitrate concentrations. Isocitrate and CPIB concentrations are as indicated; other conditions are as in Fig. 2. The inhibition is competitive for isocitrate with $K_m = 1.75 \times 10^{-3} \text{M}$ and $K_i = 3.36 \times 10^{-4} \text{M}$.

FIG. 10. Mixed inhibition study with TPIA and CPIB. Reaction mixtures contained the indicated concentrations of acetyl-CoA, other conditions being identical with those in Fig. 2. The experimentally found line for the mixed inhibition with TPIA plus CPIB is identical with the calculated line on the basis of identical sites of action of these inhibitors (see "Results"). The inhibitory constants, $K_i = 8.9 \times 10^{-4} \text{M}$ from Fig. 2 for TPIA and $K_i = 1.1 \times 10^{-3} \text{M}$ from Fig. 3 for CPIB, were used in the calculations.

and palmityl-CoA was calculated to be 2.11 by means of the above expression. The slope obtained by direct measurement of the combined inhibition appears in Fig. 11; its numerical value is 2.22. It is obvious that the two inhibitors should act on different sites of the enzyme, and their simultaneous presence produces an inhibition of mixed type (Fig. 11).

Heat Inactivation—Highly purified enzyme was used for these experiments. As shown in Fig. 12, when the enzyme was heated at 47° in the presence of isocitrate, the inactivation rate was relatively slow with a $t_\frac{1}{2}$ of 7.5 min. When the enzyme was heated in the absence of isocitrate, or in the presence of either inhibitor, a higher rate of inactivation occurred. A $t_\frac{1}{2}$ of 2.5 min was obtained when isocitrate was omitted, and $t_\frac{1}{2}$ of 3.7 min was found when 1.25 $\times 10^{-4} \text{M}$ TPIA was present. CPIB at 2.1 $\times 10^{-5} \text{M}$ had the same effect, reducing $t_\frac{1}{2}$ to 4.5 min (data not shown).

The activation of acetyl-CoA carboxylase by isocitrate or citrate was first shown to be caused by the aggregation of an inactive protomeric form by Vagelos, Alberts, and Martin (33). The presence of drugs in this experiment seems to have the same effect as the absence of isocitrate. Initial inactivation in the absence of isocitrate is rapid, suggesting the likelihood of a denaturation of the disaggregated enzyme. TPIA and CPIB act similarly; probably because they compete with isocitrate for the same site of the enzyme protein, and thus prevent the formation of the more heat-stable aggregates with isocitrate.

The same conclusion is supported from the kinetic analysis of experiments when isocitrate concentration was varied (see Figs. 8 and 9).
FIG. 12. Heat inactivation of acetyl-CoA carboxylase at 47°.
Incubation mixture contained 50 μmoles of Tris-HCl buffer (pH 7.5), 0.6 mg of bovine albumin, 8 μmoles of MgCl₂, 20 μmoles of isocitrate and 0.530 mg of purified acetyl-CoA carboxylase in a total volume of 1 ml. As indicated, in one tube, isocitrate was omitted, and in another, TPIA was added corresponding to a final concentration of 1.25 × 10⁻⁴ M. The tubes were heated at 47°, and aliquots were taken for assay at various points.

Sucrose Gradient Sedimentation—The sucrose gradient sedimentation patterns of acetyl-CoA carboxylase in the absence of isocitrate and the presence of isocitrate or isocitrate plus TPIA are shown in Fig. 13.

The enzyme is seen to aggregate in the presence of isocitrate, as shown by the increased sedimentation velocity in the presence of isocitrate. This aggregation seems to be prevented by the simultaneous presence of 7.5 × 10⁻⁴ M TPIA.

Gregolin et al. (24) have shown an excellent correlation between the molecular size of the carboxylase, in sucrose density gradients, and its state of activation. Under conditions closely approximating those of the carboxylation assay reaction mixture, but in the absence of isocitrate, the carboxylase is in the catalytically inactive form of the enzyme and sediments as a low molecular weight form. Addition of isocitrate promotes the formation of the active form which sediments as a high molecular weight form. Now it is shown that an inhibitor competing with isocitrate for the carboxylase does, indeed, prevent the aggregating effect of the isocitrate.

Arrhenius Plots—The effect of temperature on the activation of acetyl-CoA carboxylase has been studied previously (21). We have studied the effect of temperature on the TPIA- and CPIB-inhibited system as compared to the noninhibited system. Fig. 14 shows the results. As described previously (21), the plot has a biphasic character with pronounced temperature dependence at low temperatures. As the temperature increases, there is a transition point at about 21° where there is an abrupt change in slope. At this region, the temperature coefficient (Q₁₀) is about 2.0 and the energy of activation is 11.9 kcal for the noninhibited system. The presence of the inhibitor has no pronounced effect on the activation energy. ΔH calculated for the clofibrate inhibited system is 12.3 kcal, and for the TPIA-inhibited system it is 11.0 kcal. This suggests that the change in the free energy of activation seen in the inhibited system is attributable to a change in the entropy of activation (ΔF = ΔH - TΔS).

Changes in entropy may mean small conformational changes or displacement of water molecules from the ionic groups of the protein molecule in the presence of the drugs.
Inhibitors of Acetyl-CoA Carboxylase

The reactions of fatty acid synthesis from acetyl-CoA, in higher organisms, are catalyzed by acetyl-CoA carboxylase and the fatty acid synthetase multienzyme complex (34). Evidence from many laboratories (35-37) indicates that the regulation of fatty acid biosynthesis occurs at the level of acetyl-CoA carboxylase. It has been suggested that this enzyme is regulated by a feedback mechanism involving the end products, palmitoyl- and stearoyl-CoA (38), and that it is activated by tricarboxylic acid cycle intermediates, most notably citrate and isocitrate (21, 33). The carboxylation of acetyl-CoA has been considered to be the rate-limiting step of the over-all process of fatty acid synthesis. However, a recent report (26) suggests that acetyl-CoA carboxylase activity may not always be the limiting factor.

The experiments described in this report suggest that the effect of CPIB and TPIA as lipid-lowering agents in vivo may be exerted by inhibition of acetyl-CoA carboxylase. The activity of other enzymes tested is not affected by these compounds. We are considering whether or not there is a possibility that other enzymes might be affected, but it seems safe to assume at this time that TPIA and CPIB are not general enzyme inhibitors and that their inhibition on acetyl-CoA carboxylase is quite specific. Fatty acid synthetase activity is actually stimulated significantly in the presence of the drugs. This effect, however, is not likely to have any physiological significance inasmuch as malonyl-CoA formation is the rate-limiting step in the over-all process, and this step is strongly inhibited. The biotinyl moiety of the enzyme does not seem to be involved in the inhibition by TPIA or CPIB, because biotin cannot prevent the inhibition exerted by the compounds.

The levels of acetyl-CoA in the liver cell under different physiological conditions are not known. It can be supposed, as a general rule (39), that enzymes operate with substrate concentrations in the region of their apparent Michaelis constants. According to the reports of Wieland and Weiss (40) and Wieland et al. (41) the levels of acetyl-CoA have been estimated to be 15 to 20 mM moles per g, wet weight, of normal liver. From these considerations, and the kinetic data obtained from this study, it appears that at physiologically possible concentrations of the drugs, the inhibition of acetyl-CoA carboxylase could be quite pronounced and hence could significantly affect the over-all process of fatty acid synthesis.

The kinetic analysis of the data indicates that both compounds compete with acetyl-CoA and isocitrate for the same site on the enzyme.

The inactivation rate at 47° is high in the presence of the drugs or the absence of isocitrate. The reason is probably that the formation of active aggregates, in the presence of isocitrate, is prevented under these conditions and the subunits of the enzyme are more heat labile.

More direct evidence in this respect is provided by the sucrose gradient centrifugation experiments. The presence of 0.75 mM TPIA completely reverses the aggregation taking place when 20 mM isocitrate is present.

Binding of the drugs to the enzyme protein may cause small conformational changes sufficient to distort the shape of the subunits so that they cannot form a stable, active aggregate. Arrhenius plots suggest changes in the entropy of activation, which may be visualized as conformational changes of the enzyme protein, or as displacement of water molecules from the ionic groups of the enzyme.

Mixed inhibition studies support the conclusion that both drugs act on the same site of the enzyme, which is different from the binding site of palmitoyl-CoA.

Acknowledgments—The able assistance of Miss Hilda Hankin for the help in the interpretation of the data is gratefully acknowledged.

Results indicate that CPIB and TPIA may be regulating synthesis de novo of lipids by depressing acetyl-CoA carboxylase activity. It must be noted, however, that the present investigation is limited to the characteristics of the inhibition in vivo of acetyl-CoA carboxylase from chicken liver. Preliminary experiments (42) indicate that the use of acetyl-CoA carboxylase obtained from rat liver yields results which are essentially the same. However, rat liver enzyme needs preincubation with citrate for activity (43). If the drug is present at this activation stage, the inhibition is more pronounced than when the drug is added after initial incubation of the enzyme with citrate. This is in line with the heat inactivation experiments and supports the contention that the drugs interfere with the formation of the active aggregates of the enzyme, in the presence of citrate or isocitrate.

The effect of the inhibitors on hepatic acetyl-CoA carboxylase from other species, especially from human liver and particularly an evaluation of the significance of the inhibitory activities in vivo described here, is the subject of future studies.
in the purification of the enzyme is gratefully acknowledged. Special thanks are due to Dr. Hans Heymann for helpful suggestions in the preparation of this manuscript. I wish also to thank Dr. Daniel Lane for making available to me details of the purification of acetyl-CoA carboxylase before publication.

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Inhibition of Hepatic Acetyl Coenzyme A Carboxylase by Hypolipidemic Agents
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