

Regulation of rat liver hydroxymethylglutaryl coenzyme A reductase by a new class of noncompetitive inhibitors. Effects of dichloroacetate and related carboxylic acids on enzyme activity.

P W Stacpoole, H J Harwood Jr, C E Varnado

J Clin Invest. 1983;72(5):1575-1585. <https://doi.org/10.1172/JCI111116>.

Research Article

Dichloroacetate (DCA) markedly reduces circulating cholesterol levels in animals and in patients with combined hyperlipoproteinemia or homozygous familial hypercholesterolemia (FH). To investigate the mechanism of its cholesterol-lowering action, we studied the effects of DCA and its hepatic metabolites, glyoxylate and oxalate, on the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) obtained from livers of healthy, reverse light-cycled rats. Oral administration of DCA for 4 d decreased HMG CoA reductase activity 46% at a dose of 50 mg/kg per d, and 82% at a dose of 100 mg/kg per d. A 24% decrease in reductase activity was observed as early as 1 h after a single dose of 50 mg/kg DCA. The inhibitory effect of the drug was due to a fall in both expressed enzyme activity and the total number of reductase molecules present. DCA also decreased reductase activity when added to suspensions of isolated hepatocytes. With chronic administration, DCA inhibited $^3\text{H}_2\text{O}$ incorporation into cholesterol by 38% and into triglycerides by 52%. When liver microsomes were incubated with DCA, the pattern of inhibition of reductase activity was noncompetitive for both HMG CoA (inhibition constant $[K_i]$ 11.8 mM) and NADPH (K_i 11.6 mM). Inhibition by glyoxylate was also noncompetitive for both HMG CoA (K_i 1.2 mM) and NADPH (K_i 2.7 mM). Oxalate inhibited enzyme activity only at nonsaturating concentrations [...]

Find the latest version:

<https://jci.me/111116/pdf>



Regulation of Rat Liver Hydroxymethylglutaryl Coenzyme A Reductase by a New Class of Noncompetitive Inhibitors

EFFECTS OF DICHLOROACETATE AND RELATED CARBOXYLIC ACIDS ON ENZYME ACTIVITY

PETER W. STACPOOLE, H. JAMES HARWOOD, JR., and CAROL E. VARNADO, with the technical assistance of MARITA SCHNEIDER, *Department of Medicine, College of Medicine, Gainesville, Florida 32610*

A B S T R A C T Dichloroacetate (DCA) markedly reduces circulating cholesterol levels in animals and in patients with combined hyperlipoproteinemia or homozygous familial hypercholesterolemia (FH). To investigate the mechanism of its cholesterol-lowering action, we studied the effects of DCA and its hepatic metabolites, glyoxylate and oxalate, on the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) obtained from livers of healthy, reverse light-cycled rats. Oral administration of DCA for 4 d decreased HMG CoA reductase activity 46% at a dose of 50 mg/kg per d, and 82% at a dose of 100 mg/kg per d. A 24% decrease in reductase activity was observed as early as 1 h after a single dose of 50 mg/kg DCA. The inhibitory effect of the drug was due to a fall in both expressed enzyme activity and the total number of reductase molecules present. DCA also decreased reductase activity when added to suspensions of isolated hepatocytes. With chronic administration, DCA inhibited $^3\text{H}_2\text{O}$ incorporation into cholesterol by 38% and into triglycerides by 52%. When liver microsomes were incubated with DCA, the pattern of inhibition of reductase activity was noncompetitive for both HMG CoA (inhibition constant K_i 11.8 mM) and NADPH (K_i 11.6 mM). Inhibition by glyoxylate was also noncompetitive for both HMG CoA (K_i 1.2 mM) and NADPH (K_i 2.7 mM). Oxalate inhibited enzyme activity only at nonsaturating concentrations of NADPH (K_i 5.6 mM). Monochloroacetate, glycollate, and eth-

ylene glycol, all of which can form glyoxylate, also inhibited reductase activity. Using solubilized and 60-fold purified HMG CoA reductase, we found that the inhibitory effect of glyoxylate was reversible. Furthermore, the inhibition by glyoxylate was an effect exerted on the reductase itself, rather than on its regulatory enzymes, reductase kinase and reductase phosphatase. We conclude that the cholesterol-lowering effect of DCA is mediated, at least in part, by inhibition of endogenous cholesterol synthesis. The probable mechanisms are by inhibition of expressed reductase activity by DCA per se and by conversion of DCA to an active metabolite, glyoxylate, which noncompetitively inhibits HMG CoA reductase. These studies thus identify a new class of pharmacological agents that may prove useful in regulating cholesterol synthesis and circulating cholesterol levels in man.

INTRODUCTION

Hypercholesterolemia is a common disorder in man, but current therapy seldom provides satisfactory control. This is particularly so for patients with homozygous or heterozygous familial hypercholesterolemia (FH),¹ in whom high circulating levels of total and low density lipoprotein (LDL) cholesterol are associated with premature atherosclerosis (1). A major defect among patients with FH appears to be diminished

Received for publication 21 April 1983 and in revised form 6 July 1983.

¹ Abbreviations used in this paper: DCA, dichloroacetate; DPS, digitonin-precipitable sterols; FH, familial hypercholesterolemia; HMG CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase.

binding and uptake of LDL by many cells (2). The consequent decrease in the utilization of LDL cholesterol results in loss of feedback inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), the rate-limiting enzyme of cholesterol biosynthesis (3). Overproduction of cholesterol and reduced catabolism of LDL are thus considered to be the principal cause of hypercholesterolemia in this disease (1).

Pharmacologic intervention for patients with FH has traditionally relied on drugs, such as anionic resins, that enhance intestinal cholesterol excretion (4, 5), or those, such as nicotinic acid, that impair synthesis of triglyceride-rich precursors of LDL (6). Recently, however, competitive inhibitors of HMG CoA reductase, such as compactin (7) and mevinolin (8), have been developed and have been used in preliminary trials for treatment of heterozygous FH (9-11). Although these drugs are potent inhibitors of reductase *in vitro*, the competitive nature of the inhibition leads to a compensatory increase in enzyme synthesis (12), an effect that could lead to tachyphylaxis with chronic use. In addition, these drugs may not be effective in patients with homozygous FH (9), in whom high rates of endogenous cholesterol synthesis are thought to occur. There is need, therefore, for an improved treatment of the hypercholesterolemia associated with this disorder.

The pharmacologic and therapeutic effects of dichloroacetate (DCA) have been extensively explored during the past several years. DCA stimulates glucose uptake and utilization by peripheral tissues (13-15) and inhibits hepatic gluconeogenesis (16-18). It has been used to reduce hyperlactatemia in patients with lactic acidosis (19). DCA also exerts noteworthy effects on lipid and lipoprotein metabolism. In rats, the drug inhibits fatty acid and ketone body oxidation by peripheral tissues (13, 14) and is reported either to inhibit (20) or to stimulate (21, 22) fatty acid synthesis in liver without altering hepatocellular morphology (21, 23). In addition, DCA reduces circulating triglyceride and cholesterol concentrations in obese (24) and diabetic (23, 25) animals, but not in healthy animals (24, 25). In patients with phenotypes IIb or IV hyperlipoproteinemia, with or without type II diabetes, DCA decreases plasma cholesterol and triglyceride levels (26). Furthermore, in two patients with homozygous FH who lacked cell surface LDL receptor activity, the drug induced rapid and marked falls in circulating levels of total and LDL cholesterol, without altering high density lipoprotein cholesterol concentrations (27).

These results prompted us to explore the mechanism of the cholesterol-lowering effects of DCA. Since the drug reduces cholesterol levels in LDL receptor-neg-

ative FH patients, we reasoned it might suppress endogenous cholesterol synthesis. We now report that DCA significantly decreases cholesterol synthesis in rat liver by acting as a noncompetitive inhibitor of HMG CoA reductase. It also significantly reduces hepatic triglyceride formation. The inhibitory effect on reductase is probably mediated both by DCA *per se* and by hepatic conversion of the drug to an active metabolite, glyoxylate, which exerts a noncompetitive, but reversible, inhibition on the enzyme.

METHODS

Materials

Chemicals were from the following sources: DL-3-hydroxy-3-methyl-[3-¹⁴C]glutaric acid (55.1 Ci/mol), [4-¹⁴C]cholesterol (54 Ci/mol), ³H₂O (100 mCi/g), and Biofluor liquid scintillation fluid from New England Nuclear (Boston, MA); DL-[2-³H]mevalonic acid lactone (625 Ci/mol) from Amersham Corp. (Arlington Heights, IL); DL-HMG CoA reductase, digitonin, glyoxylate, oxalate, pyruvate, lactate, alanine, and all other amino acids, monochloroacetate, trifluoroacetate, and glycolic acid from Sigma Chemical Co. (St. Louis, MO); ethylene glycol, 2-chloropropionate, 3-chloropropionate from Eastman Kodak Co., (Rochester, NY); trichloroacetic acid and Scintilene liquid scintillation fluid from Fisher Scientific Co. (Orlando, FL); and sodium dichloroacetate from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Dr. S. Curry (Gainesville, FL) kindly established the purity and homogeneity of DCA by gas chromatography, nuclear magnetic resonance spectroscopy, infrared spectroscopy, and elemental analysis. Other chemicals were from previously listed sources (28-30).

Animals and drug treatment.

Male Sprague-Dawley rats (250-300 g) from the University of Florida breeding colony were fed Purina rat chow ad lib. (Ralston Purina Co., St. Louis, MO) and were exposed to 12 h of light (3:00 p.m. to 3:00 a.m.) and 12 h of darkness (3:00 a.m. to 3:00 p.m.) for at least 14 d before use. For both *in vivo* and *in vitro* experiments, rats were killed at 9:30 a.m., at the diurnal peak of reductase activity. For *in vivo* studies, drugs were dissolved in distilled water and administered by orogastric gavage either 1 h before each morning killing or each morning for 4 d to rats lightly anesthetized with ether. DCA concentrations of 50 and 100 mg/kg were used to reflect therapeutic and supratherapeutic doses used previously in humans (26, 27). Control rats received only distilled water.

Liver cell isolation and incubation

Isolated, morphologically intact, hepatic parenchymal cells were obtained in high yield by a modification (28) of the technique of Berry and Friend (31). Livers were perfused *in situ* with an oxygenated, calcium-free, Krebs-Henseleit original Ringer bicarbonate buffer (pH 7.4) containing 0.05% collagenase. After a 30-45 min perfusion, isolated cells were separated from debris, washed, and resuspended in fresh enzyme-free medium, as previously described (16, 28). At least 90% of the final cell preparation consisted of intact

parenchymal cells that excluded trypan blue stain. Cells were incubated in 25-ml polycarbonate Erlenmeyer flasks at a concentration of 5×10^6 cells in a volume of 6.0 ml. Flasks were gently shaken in a 95% O₂/5% CO₂ atmosphere at 37°C for 90 min. The incubation medium contained 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄·7H₂O, 25 mM NaHCO₃, 1 mM glucose, and 1.5% bovine serum albumin (pH 7.4).

Preparation of microsomal reductase

From hepatocytes. We isolated microsomes by previously described modifications (28, 29) of the method of Shapiro et al. (32). After incubation, 6 ml of cell suspension was transferred to 10-ml plastic centrifuge tubes and centrifuged at 50 g for 2 min. The supernatant was discarded and replaced with 3.0 ml of a hypotonic medium (buffer A) containing 1.5 mM Tris buffer, 50 mM NaCl, and 18 mM EDTA (pH 7.2). The cells were resuspended, transferred to Dounce homogenizers, and disrupted with 20 strokes of a loose fitting pestle. 7 ml of buffer B, containing 300 mM sucrose, 10 mM beta-mercaptoethanol, 10 mM EDTA, and 50 mM NaCl was added to each tube. The suspension was centrifuged for 10 min at 10,000 g, again for 10 min at 15,000 g to remove mitochondria and associated HMG CoA lyase activity, and finally for 60 min at 100,000 g. The supernatant liquid from the final centrifugation was decanted and the remaining microsomal pellet was frozen in dry ice/acetone and stored at -70°C for up to 3 wk. Before assay of HMG CoA reductase activity, pellets were resuspended in 300 µl of buffer C containing 50 mM imidazole, 50 mM NaCl, 5 mM dithiothreitol, and 1 mM EDTA (pH 7.5).

From whole livers. DCA-treated rats were anesthetized with pentobarbital, their livers were excised and equal portions from three to four animals were pooled. All subsequent operations were performed at 4°C. For the acute or chronic feeding studies, 4-5 g of liver was homogenized in 20 ml of buffer B with a motor-driven Potter-Elvehjem pestle. The homogenate was centrifuged, and the microsomes were isolated, frozen, and stored as described above. For the kinetic studies with microsomal reductase, pooled liver fractions were homogenized in buffer D (40 mM Tris [pH 7.5], 1 mM EDTA, 5 mM dithiothreitol), which also contained 70 mM KCl, with 10 strokes of a loose fitting Dounce pestle followed by 10 strokes of a tight fitting pestle. After centrifuging the homogenate for 10 min at 27,000 g, the precipitate was discarded and the supernatant was centrifuged for 90 min at 105,000 g. The supernatant was removed and the precipitate was suspended in 0.38 ml of buffer D containing 70 mM KCl/g wet wt liver by gentle homogenization with a motor-driven Potter-Elvehjem pestle. The suspension was divided into 2.0-ml portions and frozen in liquid N₂.

Preparation of solubilized reductase

Microsomal reductase was solubilized and purified 60-fold by the heat fractionation procedure described by Rogers et al. (33). Microsomes from the livers of 15 rats, killed at the middark period, were washed with 350 ml of buffer D, which also contained 50 mM KCl, and centrifuged at 105,000 g for 90 min. The washed microsomes were frozen overnight in liquid N₂. Microsomes were subsequently thawed and incubated in 18 ml of buffer D, containing 50% glycerol, for 1 h at room temperature. The microsomal suspension was diluted to 350 ml with buffer D, containing 1 M KCl, and centrifuged at 105,000 g for 90 min. The microsomal pellet

was discarded and the reductase contained in the supernatant was precipitated between 35 and 50% ammonium sulfate saturation. The precipitate was dissolved in buffer D, containing 1 M KCl and 30% glycerol, heated in a 60°C water bath for exactly 5 min, cooled to room temperature, and centrifuged at 20,000 g for 30 min. The resulting supernatant was retained as solubilized reductase, frozen in liquid N₂, and used within 1 wk. Under these conditions, solubilized reductase retains full activity.

Assay of enzyme activity

Assay of microsomal HMG CoA reductase. HMG CoA reductase activity was measured essentially as described by Hunter and Rodwell (34). Samples to be assayed, in a total volume of 50 µl, were mixed with 25 µl of a cofactor-substrate solution that contained 2.25 µmol glucose-6-phosphate, 0.26 µmol NADP⁺, 0.2 U of glucose-6-phosphate dehydrogenase, 3.8 µmol EDTA, 18,000 cpm of DL-[2-³H]mevalonolactone (629 Ci/mol) as an internal standard and either 25 nmol of RS-[3-¹⁴C]HMG CoA (2.5 cpm/pmol) or 60 nmol of RS-[3-¹⁴C]HMG CoA (12-15 cpm/pmol). After incubation of up to 30 min at 37°C, the reaction was stopped by addition of 10 µl of 6 M HCl. 30-µl portions were applied to silica gel thin-layer chromatography (TLC) sheets ruled into 16 channels of 15 × 10 cm. Sheets were dried and developed in toluene/acetone, 1:1 (vol/vol) to separate mevalonolactone from HMG CoA (*R*_f for mevalonolactone = 0.59). The region of the TLC channel corresponding to *R*_f 0.5 to *R*_f 1.0 was cut from the sheet and counted in 4.0 ml of Scintilene fluor.

For determination of the Michaelis constant (*K*_m) of HMG CoA reductase for NADPH, at saturating concentrations of HMG CoA, NADP⁺ was omitted from the cofactor-substrate solution and was added directly to the assay mixture at the desired final concentration. Reductase activity was measured after a 15-min incubation at 37°C. To estimate the *K*_m of reductase for HMG CoA at saturating concentrations of NADPH, enzyme activity was measured essentially as described by Langdon and Counsell (35). [¹⁴C]HMG CoA was omitted from the cofactor-substrate solution and was added directly to the assay mixture at the desired final concentration. Reductase activity was measured in a 2.9-min incubation at 37°C. For determination of the effects of DCA, its analogues or its metabolites on reductase activity, microsomes and drugs were incubated together for either 0 or 20 min at 37°C before measurement of enzyme activity.

Assay of glucose-6-phosphate dehydrogenase. Assays contained, in a final volume of 3 ml, 8 mg NADPH, 26 mg glucose-6-phosphate, 70 µmol KCl and either buffer D containing 70 mM KCl (control), or buffer D containing 70 mM KCl and either 18 µmol DCA, 18 µmol oxalate, or 3, 6, or 18 µmol glyoxylate. Glucose-6-phosphate dehydrogenase, at a concentration of 6.25 units in 25 µl of buffer D containing 70 mM KCl, was added at zero time and the solution was mixed. The rate of increase of absorbance at 25°C was measured at 340 nm against a water blank. Neither DCA, glyoxylate, or oxalate affected enzyme activity at the concentrations tested.

Measurement of ³H₂O incorporation into digitonin-precipitable sterols

Paired control and DCA-treated rats received an intraperitoneal injection of 5 mCi ³H₂O in a volume of 0.2 ml at 8:30 a.m. (30 min before the diurnal peak for reductase ac-

tivity). 1 h later, animals were anesthetized with an intraperitoneal injection of pentobarbital. The abdominal cavity was opened and 0.5 ml blood was obtained from the inferior vena cava for determination of $^3\text{H}_2\text{O}$ specific activity, as described by Jeske and Dietschy (36). Next, a cannula was inserted into the portal vein. The inferior vena cava was cut below the level of the renal veins and the liver was perfused with 60 ml saline (25°C) at a rate of 30 ml/min. The liver was excised and two 2-g portions, comprising equal amounts from the three major lobes, were obtained. The samples were extracted into chloroform/methanol, 2:1 (vol/vol) by homogenization in a VirTis Co. (Gardiner, NY) model 23 homogenizer (4°C, 5 min). The homogenate was filtered through Whatman No. 1 filter paper (Whatman Laboratory Products Inc., Clifton, NJ) to remove solid residue. [^{14}C]Cholesterol (30,000 cpm) was added to the filtrate as an internal standard and the solution was evaporated to dryness under air. We included the internal standard to minimize random differences in the rate of sterol biosynthesis resulting from losses during isolation of digitonin-precipitable sterols. We found that, although recovery of [^{14}C]cholesterol varied <10% in each experiment, recoveries from experiment to experiment usually ranged from 60 to 100%. Inclusion of the internal standard permitted correction of these interexperimental differences. This correction resulted in a precision from experiment to experiment that closely approximated that for each individual experiment. In addition, use of an internal standard permitted assessment of the actual rate of sterol biosynthesis. This is emphasized by the somewhat lower values for the rate of sterol biosynthesis obtained by investigators using the same basic technique but without an internal standard (2.06 $\mu\text{mol}/\text{h}$ per g [37]; 1.8 $\mu\text{mol}/\text{h}$ per g [36]).

5 ml of 96% ethanol and 10 ml of 5 M NaOH were added to the dry residue. The mixture was sealed, heated in an 80°C water bath for 3 h, and then cooled to room temperature and stored overnight in the dark. The next day, 20 ml petroleum ether and 20 ml 50% ethanol were added to each sample. Samples were transferred to a 125-ml separatory funnel and mixed for 10 min. The petroleum ether phase, containing nonsaponifiable lipids, was removed. The aqueous phase was washed with two successive 10-ml portions of petroleum ether. The three petroleum ether extracts (40 ml) were washed free of alkali with 50 ml of 50% ethanol. The ethanol wash was added to the original aqueous solution (containing the saponifiable lipids) and was retained for estimation of the rate of triglyceride synthesis. The washed petroleum ether extract was diluted to a volume of 40 ml with petroleum ether, and two 16-ml aliquots were dried under air at 60°C. The dried residues were dissolved in 4 ml of 95% ethanol/acetone, 1:1. The solution was mixed with 2 ml of a 0.5% digitonin solution in 50% ethanol and stored overnight at room temperature in the dark. The next day, the samples were centrifuged for 15 min at 2,400 rpm at 20°C and the supernatant was discarded. The digitonin precipitate was washed with 6 ml diethyl ether/acetone, 1:1 (vol/vol), followed by 12 ml of anhydrous diethyl ether. The precipitate was dried under air, redissolved in 1 ml methanol, mixed with 15 ml Biofluor liquid scintillation fluid, and counted.

Measurement of $^3\text{H}_2\text{O}$ incorporation into triglyceride

The combined aqueous phase and 50% ethanol wash were acidified with ~5 ml of 12 M HCl (final pH < 2). The resulting solution was extracted with three successive 10-ml

portions of petroleum ether. The extracts were pooled, dried at 60°C under air, dissolved in Biofluor, and counted.

Protein assay

Microsomal protein was estimated by the method of Bradford (38), with bovine serum albumin as a standard.

Calculations

The specific activity of plasma water, expressed as ^3H counts per minute per nanomole of H_2O , was calculated as described by Jeske and Dietschy (36) in the following relationship:

$$\text{sp act} \left(\frac{^3\text{H} \text{ cpm}}{\text{nmol water}} \right) = \frac{(\text{cpm } ^3\text{H}/\text{ml plasma})(1.09)}{(5.56 \times 10^7 \text{ nmol H}_2\text{O}/\text{ml H}_2\text{O})(0.92 \text{ ml H}_2\text{O}/\text{ml plasma})}.$$

The term 1.09 corrects the specific activity of plasma water, determined at 1 h after $^3\text{H}_2\text{O}$ injection, to the mean specific activity of body water present throughout the 1-h incubation (36). The 0.92 term corrects the calculation for the percentage of plasma that is water (36, 37). The rate of triglyceride synthesis by the liver, expressed as micromoles of $^3\text{H}_2\text{O}$ incorporated into triglycerides per gram wet weight of liver per hour, was calculated by dividing the ^3H counts per minute measured in the saponifiable lipids fraction by the product of the liver sample weight (in grams), the time of incubation (in hours), and the specific activity of plasma water (^3H counts per minute per micromole of H_2O). The rate of cholesterol synthesis by the liver, expressed as micromoles of $^3\text{H}_2\text{O}$ incorporated into digitonin-precipitable sterols (DPS) per gram wet weight of liver per hour, was calculated as above by dividing the total $^3\text{H}_2\text{O}$ incorporated into DPS by the product of the weight of the liver sample (in grams), the time of incubation (in hours), and the specific activity of the plasma water (^3H counts per minute per micromole of water). The total $^3\text{H}_2\text{O}$ incorporated into DPS was calculated by using the following relationship:

Total ^3H cpm into DPS

$$= [(^3\text{H}_2\text{O} \text{ in the digitonin precipitate})$$

$$- (\text{spill})(^{14}\text{C} \text{ cpm in the digitonin precipitate})]$$

$$\left(\frac{\text{total } ^{14}\text{C cholesterol added}}{^{14}\text{C cpm in the digitonin precipitate}} \right).$$

The spill term represents the fraction of ^{14}C measured as ^3H . The (spill) (^{14}C cpm) term corrects the ^3H cpm measured in the digitonin precipitate for this spillover. The total [^{14}C]cholesterol added divided by the ^{14}C measured in the digitonin precipitate corrects the calculation both for losses incurred during isolation and for use of only two-fifths of the petroleum ether extracts in determining ^3H -labeled digitonin-precipitable sterols.

Reductase activity, expressed as picomoles of mevalonate formed from HMG CoA and NADPH per minute of incubation at 37°C, was calculated as described by Shapiro et al. (32).

RESULTS

Table I shows the effect of DCA on hepatic HMG CoA reductase activity in rats receiving the drug as a single dose 1 h before death or daily for 4 d. 1 h after a single oral drug dose of 50 mg/kg, enzyme activity decreased 24% ($P < 0.001$). Treatment for 4 d with DCA inhibited enzyme activity 46% ($P < 0.001$) at a dose of 50 mg/kg per d, and 82% ($P < 0.01$) at a dose of 100 mg/kg per d. Reductase activity was also markedly reduced when isolated hepatocytes were incubated with DCA. The inhibitory effect of chronic DCA administration on HMG CoA reductase was paralleled by a 38% ($P < 0.001$) fall in hepatic cholesterol synthesis from $^3\text{H}_2\text{O}$ (Table II). In addition, the drug also significantly decreased (52%, $P < 0.01$) $^3\text{H}_2\text{O}$ incorporation into hepatic triglycerides.

DCA is not metabolized by muscle tissue of the rat (14). However, DCA is decarboxylated and oxidized by rat liver to glyoxylate, oxalate, and CO_2 (39, 40). To determine whether the inhibition of reductase activity by DCA observed *in vivo* and in isolated cells was due to the drug itself, its metabolites, or both, we studied the effect of DCA, glyoxylate, and oxalate on reductase, using microsomes to which the test drug was added for 20 min at 37°C before addition of the cofactor-substrate solution, or to which the test drug and cofactor-substrate solution were added simultaneously but separately to the microsomes. As shown in Fig. 1, preincubation of microsomes with glyoxylate led to a dose-dependent decrease in HMG CoA re-

TABLE I
Inhibition of HMG CoA Reductase Activity in Livers of Rats Treated with DCA or in Rat Hepatocytes Incubated with DCA

| Whole livers | | Hepatocytes | |
|--------------|---|-------------|---|
| DCA | HMG CoA reductase activity pmol/min/mg protein | DCA | HMG CoA reductase activity pmol/min/mg protein |
| None | 505±121 | None | 1,400±140 |
| 50 mg/kg/d | 272±76 | 1.0 mM | 670±150 |
| 100 mg/kg/d | 90±4 | | |

In the chronic drug administration studies, rats received water ($n = 13$), 50 mg/kg DCA ($n = 13$), or 100 mg/kg DCA ($n = 7$) once daily for 4 d. Rats were killed at 9:30 a.m. on the 5th d, 24 h after the last orogastric feeding, and the livers from each group were pooled. Equal portions from each pool of livers were homogenized and microsomes were prepared, as described in Methods. In the *in vitro* experiments, isolated hepatocytes obtained from three rats were divided equally into flasks containing no added DCA or 1.0 mM DCA and incubated at 37°C for 90 min. For both whole liver and hepatocyte studies, HMG CoA reductase activity was assayed in duplicate, as described in Methods. Values are means±SEM.

TABLE II
Inhibition of $^3\text{H}_2\text{O}$ Incorporation into Cholesterol and Triglycerides in Livers of Rats Treated with DCA

| DCA | $^3\text{H}_2\text{O}$ incorporation into | |
|------------|---|---------------|
| | Cholesterol | Triglycerides |
| None | 9.2±0.5 | 4.4±0.6 |
| 50 mg/kg/d | 5.7±0.4 | 2.1±0.2 |

Rats received by orogastric tube saline ($n = 5$) or 50 mg/kg DCA ($n = 5$) once daily for 4 d. All rats were killed at 9:30 a.m. on the 5th day, 24 h after the last administration. 1 h before death, animals were given 5 mCi of $^3\text{H}_2\text{O}$ i.p. The rate of $^3\text{H}_2\text{O}$ incorporation into DPS and nondigitonin-precipitable sterols were measured as described in Methods. Values are means±SEM.

ductase activity. In contrast, preincubation with DCA or oxalate had no consistent effect on the enzyme. The inhibitory effect of glyoxylate was seen with both fresh

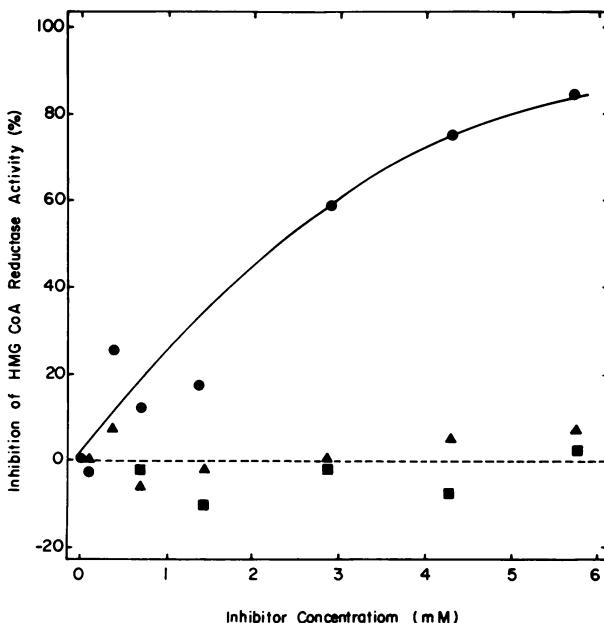


FIGURE 1 Dose-dependent inhibition of HMG CoA reductase by glyoxylate. Microsomal reductase (5 μl , 140 μg protein) was mixed with glyoxylate, oxalate, or DCA at 1.5 times the indicated final concentrations in a volume of 45 μl of buffer D containing 70 mM KCl. After a 20-min incubation at 37°C, 25 μl of reductase substrate-cofactor solution was added and reductase activity was measured in a 5-min incubation at 37°C. The glyoxylate, oxalate, and DCA concentrations indicated are the concentrations present during the 5-min incubation. Data are for individual analyses. Shown is the percent inhibition of reductase activity as a function of glyoxylate (●), DCA (■), or oxalate (▲) concentrations.

TABLE III
Inhibition of Rat Liver HMG CoA Reductase by Glyoxylate Is
Not Due to Altered Reductase Kinase or Reductase
Phosphatase Activity

| Preincubation | Additions to preincubation medium | Inhibition of reductase activity % |
|---------------|-----------------------------------|------------------------------------|
| — | Glyoxylate | 44 |
| + | Glyoxylate | 85 |
| + | Glyoxylate + NaF | 88 |
| + | Glyoxylate + EDTA | 90 |
| + | Glyoxylate + NaF + EDTA | 92 |

Microsomal reductase (5 μ l, 300 μ g protein, 1.26 nmol/min/mg) was mixed with 30 μ l of 10 mM glyoxylate (controls received 30 μ l H₂O) and 5 μ l of either H₂O, 250 mM sodium fluoride, 250 mM EDTA, or 250 mM sodium fluoride and 250 mM EDTA. After a 20-min incubation at 37°C, samples to which EDTA was omitted during the first incubation received 5 μ l of 250 mM EDTA or 5 μ l H₂O (control). All samples received 20 μ l of a reductase cofactor-substrate solution and were incubated for 5 min at 37°C. HMG CoA reductase was assayed in duplicate, as described in Methods.

and frozen microsomes and was present, albeit to a lesser extent, even if glyoxylate was added without preincubation. A modest inhibitory effect of DCA on reductase (25% at 4 mM DCA) was noted only when fresh microsomes were preincubated with the drug. Oxalate had no effect on enzyme activity, regardless of whether the microsomes were fresh or whether a preincubation step was used.

HMG CoA reductase is modulated, in part, by a phosphorylation-dephosphorylation mechanism (41, 42). Reductase kinase, an ADP-requiring protein kinase (30), converts HMG CoA reductase into an inactive, phosphorylated form, while a Ca²⁺-dependent phosphoprotein phosphatase regenerates the active enzyme (40, 41). In theory, therefore, an agent that stimulates reductase kinase or inhibits reductase phosphatase might indirectly inhibit HMG CoA reductase. To explore these possibilities, microsomes were preincubated with glyoxylate in the presence or absence of sodium fluoride (to inhibit reductase phosphatase), EDTA (to inhibit reductase kinase), or both. As shown in Table III, the inhibitory effect of glyoxylate on re-

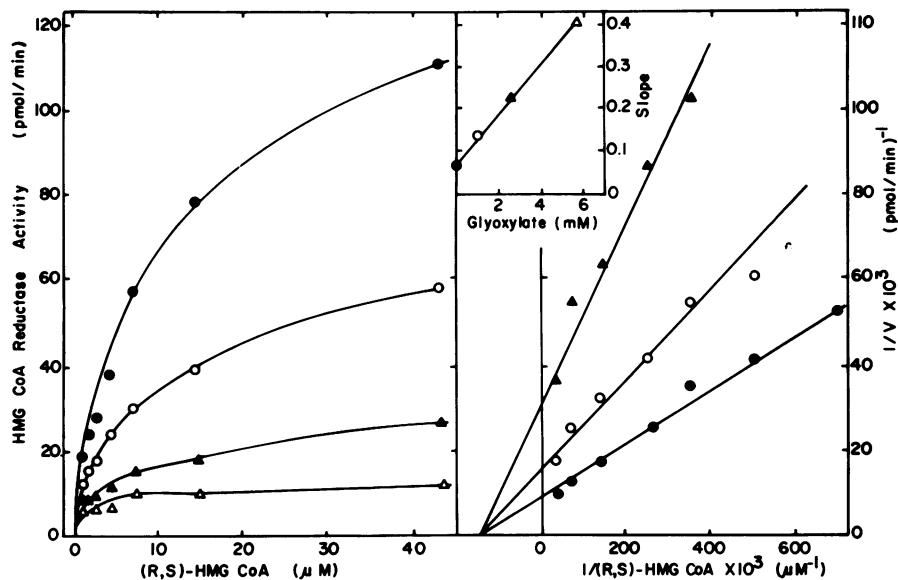


FIGURE 2 Noncompetitive inhibition of HMG CoA reductase by glyoxylate with respect to HMG CoA. Microsomal reductase (100 μ l, 6.4 mg protein) was mixed with either 800 μ l of buffer D containing 70 mM KCl, 800 μ l of 10 mM glyoxylate, 352 μ l of 10 mM glyoxylate plus 448 μ l buffer D containing 70 mM KCl, or 144 μ l glyoxylate plus 656 μ l buffer D containing 70 mM KCl and incubated for 20 min at 37°C. 45- μ l portions of this mixture were then combined, in a final volume of 70 μ l of buffer D containing 70 mM KCl, with 10 μ l of reductase substrate-cofactor solution and [¹⁴C]HMG CoA (sp act 12.3 cpm/pmol) at the indicated final concentrations. Reductase activity was measured in a 2.9-min incubation at 37°C. Concentrations of glyoxylate present during reductase assay were 0 mM (●), 1 mM (○), 2.5 mM (▲), and 5.7 mM (△). Data are for duplicate analyses. (Left) Reductase activity as a function of HMG CoA concentration. (Right) Reciprocal velocity as a function of reciprocal HMG CoA concentration. (Inset) Secondary plot of the slope as a function of glyoxylate concentration. The K_m of reductase for HMG CoA was 3.0 μ M for the S-isomer (6.0 μ M for R,S mixture). The K_i for glyoxylate with respect to HMG CoA was 1.25 mM.

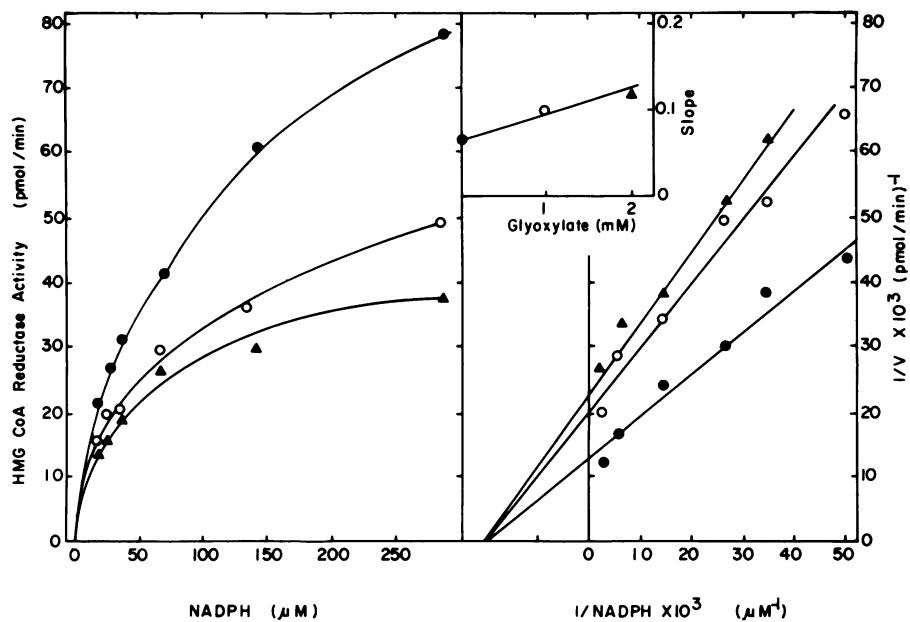


FIGURE 3 Noncompetitive inhibition of HMG CoA reductase by glyoxylate, with respect to NADPH. Microsomal reductase (100 μ l, 4.5 mg protein) was mixed with either 800 μ l buffer D containing 70 mM KCl or 288 μ l of 10 mM glyoxylate plus 512 μ l buffer containing 70 mM KCl and incubated for 20 min at 37°C. 45- μ l portions of this mixture were then combined in a final volume of 70 μ l of buffer D containing 70 mM KCl, with 15 μ l of reductase substrate-cofactor solution and NADPH at the indicated final concentrations. Reductase activity was measured in a 15-min incubation at 37°C. Concentrations of glyoxylate present during reductase were 0 mM (●), 1 mM (○), 2 mM (▲). Data are for duplicate analyses. (Left) Reductase activity as a function of NADPH concentration. (Right) Reciprocal velocity as a function of reciprocal NADPH concentration. (Insert) Secondary plot of the slope as a function of glyoxylate concentration. The K_m of reductase for NADPH was 51 μ M. The K_i for glyoxylate with respect to NADPH was 2.7 mM.

ductase activity was not altered by the presence of sodium fluoride or EDTA. Thus, glyoxylate acts directly on HMG CoA reductase, rather than on its constituent phosphorylating or dephosphorylating enzymes.

The pattern of inhibition of HMG CoA reductase activity by glyoxylate was noncompetitive with respect to both HMG CoA (inhibition constant K_i) 1.2 mM; Fig. 2) and NADPH (K_i 2.7 mM; Fig. 3). Since irreversible inhibition as well as reversible, noncompetitive inhibition results in similar double reciprocal plots (43), we examined the reversibility of enzyme inhibition by glyoxylate, using microsomal reductase, solubilized and purified 60-fold. A secondary plot of maximum velocity (V_{max}) vs. enzyme concentration revealed the noncompetitive inhibition by glyoxylate was reversible (Fig. 4). DCA was also a noncompetitive, although less potent, inhibitor of the reductase, (K_i 11.8 mM for HMG CoA and K_i 11.6 mM for NADPH). Oxalate was an uncompetitive inhibitor only at nonsaturating concentrations of NADPH (K_i 5.6 mM).

We next asked whether the inhibitory actions of

DCA and glyoxylate on reductase were due to a change in expressed (unphosphorylated) enzyme activity, total (unphosphorylated plus phosphorylated) activity, or both. The results presented in Table IV show that a single dose of 50 mg/kg DCA or glyoxylate is followed in 1 h by approximately a 20% fall in total reductase activity. In contrast, the proportion of enzyme in an unphosphorylated form was suppressed 43% by DCA but only 8% by glyoxylate. Thus, an early effect of DCA, before its conversion to glyoxylate, is to decrease the amount of active reductase present.

Various analogues of DCA and/or precursors of glyoxylate were tested subsequently for their effect on microsomal or partially purified HMG CoA reductase (Table V). Only glyoxylate inhibited reductase in the absence of preincubation. In addition, only glyoxylate and its precursors, monochloroacetate, glycolate and ethylene glycol inhibited the enzyme after preincubation. Other DCA analogues that do not form glyoxylate—such as 2-chloropropionate, 3-chloropropionate, *n*-dichloroacetylserine, lactate, and alanine—all failed to inhibit reductase activity, with or without

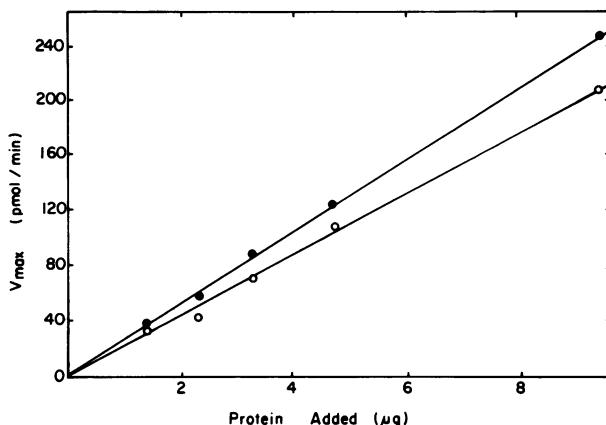


FIGURE 4 Reversible inhibition of HMG CoA reductase by glyoxylate. Solubilized, 60-fold purified reductase was mixed, in the indicated amounts, with either 215 μ l of water (●) or 215 μ l of 10 mM glyoxylate (○) and buffer D containing 70 mM KCl to a final volume of 900 μ l. After a 20-min incubation at 37°C, 45 μ l of the above mixture was added to 25 μ l of reductase assay solution containing either 0, 20, 29, 43, 114, or 286 μ M NADPH, and mixed. Reductase activity was measured after a 15-min incubation at 37°C. Shown is V_{max} , calculated from a plot of $1/V$ vs. $1/S$ (where V is velocity and S is substrate) as a function of solubilized reductase protein added.

preincubation. Trifluoroacetate and trichloroacetate had no effect on microsomal reductase without preincubation, but stimulated activity 22 and 12%, respectively, after preincubation.

DISCUSSION

The present data demonstrate that DCA, a potent cholesterol-lowering drug in animals (24, 25) and man (26, 27), is a noncompetitive inhibitor of HMG CoA reductase obtained from rat liver. The decline in enzyme activity, which occurs both *in vivo* and *in vitro* in response to DCA, is paralleled by a suppression of hepatic cholesterolgenesis. In addition, hepatic triglyceride synthesis is also markedly reduced by chronic, oral administration of the drug. It seems reasonable to conclude, therefore, that the inhibitory effect of DCA on hepatic HMG CoA reductase activity and cholesterol synthesis is a principal mechanism by which the drug lowers cholesterol levels *in vivo*. In addition, the suppressive effect of DCA on hepatic triglyceride formation may also account, at least in part, for the ability of the drug to reduce circulating triglyceride concentrations. The precise mechanisms by which DCA alters hepatic triglyceride synthesis is unknown.

The inhibitory effect of DCA on reductase activity appears to be mediated, at least in part, by its hepatic

conversion to glyoxylate. This is substantiated by the following observations. (a) The inhibitory constants for DCA are an order of magnitude greater than for glyoxylate, (b) DCA inhibits reductase activity *in vitro* only when it is incubated with microsomes for a period of time before addition of reductase substrates and cofactors, and (c) only those analogues of DCA that can be metabolized to glyoxylate are inhibitors of reductase activity. Preincubation of microsomes with these drugs is required in order to elicit an inhibitory effect on reductase, presumably to allow for their metabolism to glyoxylate.

Both DCA and glyoxylate are noncompetitive inhibitors of HMG CoA reductase. The pattern of inhibition is reversible with respect to NADPH for glyoxylate and, probably, for DCA. The inhibitory effect of glyoxylate is exerted on reductase itself, since it persists when the regulatory enzymes, reductase kinase and reductase phosphatase are inhibited. Although the acute feeding studies and *in vitro* experiments indicate that the inhibitory effects of DCA and glyoxylate are due to a fall in expressed enzyme activity, the marked suppression of reductase observed with the chronic feeding experiments and the decrease in total reductase activity observed with acute administration suggest that these drugs also reduce the total number of HMG CoA reductase molecules present. Such a change may be due to a decreased rate of enzyme synthesis, an enhanced rate of enzyme degradation, or both. The marked fall in the amount of active reductase present after acute DCA administration, compared with the modest change seen after glyoxylate treatment, suggests

TABLE IV
Inhibition of HMG CoA Reductase Activity in Livers of Rats Treated with a Single Dose of DCA or Glyoxylate

| Treatment | HMG CoA reductase activity | | |
|-----------------------|----------------------------|-----------------|-------|
| | +NaF (Ra) | -NaF (Rt) | Ra/Rt |
| nmol/min/mg protein | | | |
| None | 1.09 \pm 0.03 | 1.32 \pm 0.02 | 0.83 |
| DCA (50 mg/kg) | 0.62 \pm 0.09 | 1.00 \pm 0.07 | 0.62 |
| Glyoxylate (50 mg/kg) | 1.00 \pm 0.02 | 1.09 \pm 0.01 | 0.92 |

Rats each received, by orogastric tube, H₂O ($n = 3$), 50 mg/kg DCA ($n = 3$) or 50 mg/kg glyoxylate ($n = 3$). All rats were killed at 9:30 a.m., 1 h after orogastric administration. Four portions from each liver were pooled, homogenized in either Buffer D containing 50 mM NaF to inhibit phosphatase activity (Ra) or Buffer D containing 50 mM NaCl (Rt), and microsomes were prepared, as described in Methods. Microsomes were resuspended and assayed in the same buffer in which they were isolated. HMG CoA reductase activity was assayed in triplicate, as described in Methods. Data are means \pm SEM.

TABLE V
*Inhibition of Microsomal or Solubilized, Partially Purified Rat Liver HMG CoA Reductase
 by Analogs of DCA or Precursors of Glyoxylate*

| DCA analogue or glyoxylate precursor | | | Inhibition of microsomal reductase | | Inhibition of purified reductase | |
|---|-----|-------------------|------------------------------------|----------------------|----------------------------------|----------------------|
| X | Y | Trivial name | 0-min preincubation | 20-min preincubation | 0-min preincubation | 20-min preincubation |
| % | | | | | | |
| O HC— | —OH | Glyoxylate | 42 | 82 | 19 | 70 |
| CH ₂ Cl— | —OH | Monochloroacetate | 0 | 40 | 0 | 53 |
| HOCH ₂ — | —OH | Glycolate | 0 | 33 | 0 | 0 |
| O CH ₃ C— | —OH | Pyruvate | 0 | 10 | 0 | 0 |
| HO—CH ₂ —CH ₂ —OH | | Ethylene glycol | 0 | 18 | 0 | 0 |

Rats were killed at the middark period and microsomes were isolated as described in Methods. Microsomal reductase (5 μ l, 320 μ g protein, 1.11 nmol/min/mg) was mixed with 45 μ l of 10 mM DCA analogue or glyoxylate precursor or 45 μ l H₂O (control). Solubilized reductase (5 μ l, 9.25 μ g protein, 24.8 nmol/min per mg) was mixed with 35 μ l of 10 mM DCA analogue or glyoxylate precursor or 35 μ l H₂O (control). After a 0- or 20-min incubation at 37°C, all samples received 25 μ l of reductase substrate-cofactor solution and were incubated for 5 min at 37°C. HMG CoA reductase activity was assayed in duplicate, as described in Methods.

that a second mode of action of DCA exists that is independent of its conversion to glyoxylate. This may involve an effect of DCA per se on reductase kinase, reductase phosphatase, or both. In this regard, it is noteworthy that DCA alters the activity of two other protein kinases, pyruvate dehydrogenase kinase (44) and α -ketoacid dehydrogenase kinase (45). Thus, it is possible that DCA decreases expressed reductase activity by stimulating the activity of reductase kinase.

Although competitive inhibitors of HMG CoA reductase appear to be effective in reducing hypercholesterolemia in man (9–11), the nature of the inhibition leads to a marked increase in intracellular concentrations of reductase molecules (12). This effect has a theoretical disadvantage in that progressively higher doses of the inhibitor may be required to overcome the increase in reductase synthesis and prevent the development of tachyphylaxis. Indeed, in states in which HMG CoA reductase activity is already markedly elevated, such as in homozygous FH, competitive inhibitors of the enzyme do not appear to be clinically effective (9). On the other hand, noncompetitive, but reversible, inhibitors of reductase may not lead to an increase in enzyme synthesis and thus may retain their efficacy with chronic use. Furthermore, noncompetitive inhibitors may also be effective in reducing cholesterol

synthesis and circulating cholesterol levels in patients with homozygous FH. This premise is substantiated by the observation that chronic administration of DCA leads to marked and sustained decreases in serum total and LDL cholesterol concentrations in two receptor-negative homozygous FH patients (27), one of whom was found subsequently to have an elevated rate of cholesterol synthesis (46).

DCA is not without potential toxicity. One patient who received the drug for ~4 mo developed a polyneuropathy that resolved after treatment stopped (27). Chronic administration of DCA to animals in doses far exceeding those used clinically also induces a reversible peripheral neuropathy (47–49). Oxalate, an end product of DCA metabolism (39, 40), is a known neurotoxin (50, 51) and may be responsible for the neuropathic changes associated with DCA. The clinical efficacy of the drug at doses lower than that causing neurotoxicity, however, has not been tested. Our finding that DCA and glyoxylate represent a new pharmacologic class of potent, noncompetitive inhibitors of HMG CoA reductase suggests that analogues or derivatives of these agents, possessing similar effects on cholesterol and triglyceride metabolism but devoid of serious toxicity, may be important adjuncts in the treatment of hypercholesterolemia and other lipoprotein disorders in man.

ACKNOWLEDGMENTS

We thank Drs. R. Henneberg and V. W. Rodwell for helpful advice.

This work was supported by grant HL 27807 from the National Institutes of Health and by a grant from the American Diabetes Association.

REFERENCES

1. Frederickson, D. S., J. L. Goldstein, and M. S. Brown. 1978. The familial hyperlipoproteinemias. In *The Metabolic Basis of Inherited Disease*. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill, Inc., New York. 604-655.
2. Goldstein, J. L., and M. S. Brown. 1979. The low-density lipoprotein pathway and its relation to atherosclerosis. *Annu. Rev. Biochem.* 46:897-930.
3. Rodwell, V. W., J. L. Nordstrom, and J. J. Mitschelen. 1976. Regulation of HMG-CoA reductase. *Adv. Lipid Res.* 14:1-74.
4. Moore, R. B., C. A. Crane, and I. D. Franz, Jr. 1968. Effect of cholestyramine on fecal excretion of intravenously administered cholesterol-4-¹⁴C and its degradation products in a hypercholesterolemic patient. *J. Clin. Invest.* 47:1664-1671.
5. Montafis, C. D., and N. B. Myant. 1969. The metabolism of cholesterol in two hypercholesterolaemic patients with cholestyramine. *Clin. Sci.* 37:443-454.
6. Grundy, S. M., H. Y. I. Mok, L. Zech, and M. Berman. 1981. Influence of nicotinic acid on metabolism of cholesterol and triglycerides in man. *J. Lipid Res.* 22:24-36.
7. Endo, A., Y. Tsujita, M. Kuroda, and K. Tanzawa. 1977. Inhibition of cholesterol synthesis *in vitro* and *in vivo* by ML-236 A and ML-236 B, competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Eur. J. Biochem.* 77:31-36.
8. Alberts, A. W., J. Chen, J. Kuron, V. Hunt, J. Huff, C. Hoffman, J. Rothrock, M. Lopez, H. Joshua, E. Harris, A. Patchett, R. Monaghan, S. Currie, E. Stapley, G. Albers-Schonberg, O. Hensens, J. Hirshfield, K. Hoogsteen, J. Liesch, and J. Springer. 1980. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl coenzyme A reductase and a cholesterol-lowering agent. *Proc. Natl. Acad. Sci. USA*. 77:3957-3961.
9. Yamamoto, A., S. Hiroshi, and A. Endo. 1980. Therapeutic effects of ML-236B in primary hypercholesterolemia. *Atherosclerosis*. 35:259-266.
10. Mabuchi, H., T. Haba, R. Tatami, S. Miyamoto, Y. Sakai, T. Wakasugi, A. Watanabe, J. Koizumi, and R. Takeda. 1981. Effects of an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase on serum lipoproteins and ubiquinone-10 levels in patients with familial hypercholesterolemia. *N. Engl. J. Med.* 305:478-482.
11. Tobert, J. A., G. Hitzenberger, W. R. Kukovetz, I. B. Holmes, and K. H. Jones. 1982. Rapid and substantial lowering of human serum cholesterol by mevinolin (MK-803), an inhibitor of hydroxymethylglutaryl-coenzyme A reductase. *Atherosclerosis*. 41:61-65.
12. Kita, T., M. S. Brown, and J. L. Goldstein. 1980. Feedback regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in livers of mice treated with mevinolin, a competitive inhibitor of the reductase. *J. Clin. Invest.* 66:1094-1100.
13. Stacpoole, P. W., and J. M. Felts. 1970. Diisopropylammonium dichloroacetate (DIPA) and sodium dichloroacetate (DCA): effect on glucose and fat metabolism in normal and diabetic tissue. *Metab. Clin. Exp.* 19:71-78.
14. McAllister, A., S. P. Allison, and P. J. Randle. 1973. Effects of dichloroacetate on the metabolism of glucose, pyruvate, acetate, 3-hydroxybutyrate and palmitate in rat diaphragm and heart muscle *in vitro* and on extraction of glucose, lactate, pyruvate and free fatty acids by dog heart *in vivo*. *Biochem. J.* 134:1067-1081.
15. Diamond, M. P., J. H. Suhre, Jr., P. E. Williams, W. W. Lacy, and A. D. Cherrington. 1982. Contribution of the liver and extrasplanchic tissues to the hypoglycemic action of dichloroacetate in the conscious dog. *Diabetes*. 31:326-332.
16. Stacpoole, P. W. 1977. Effect of dichloroacetate on gluconeogenesis in isolated rat hepatocytes. *Metab. Clin. Exp.* 26:107-116.
17. Demaugre, F., J.-P. Leroux, and P. Cartier. 1978. The effects of pyruvate concentration, dichloroacetate and α -cyano-4-hydroxycinnamate on gluconeogenesis, ketogenesis and [3-hydroxybutyrate]/[3-oxobutyrate] ratios in isolated rat hepatocytes. *Biochem. J.* 172:91-96.
18. Diamond, M. P., P. E. Williams, W. W. Lacy, and A. D. Cherrington. 1981. Effect of dichloroacetate on gluconeogenesis *in vivo* in the presence of a fixed gluconeogenic substrate supply to the liver. *Metab. Clin. Exp.* 30:880-885.
19. Stacpoole, P. W., E. M. Harman, S. H. Curry, T. G. Baumgartner, and R. I. Misbin. 1983. Treatment of lactic acidosis with dichloroacetate. *N. Engl. J. Med.* 309:390-396.
20. Misbin, R. I. 1979. Effects of dichloroacetate on lipid metabolism in isolated rat liver cells. *Diabetes*. 28:265-271.
21. Anderson, J. W., D. Karounos, and T. Yoneyama. 1975. Dichloroacetate-induced changes in liver of normal and diabetic rats. *Exp. Biol. Med.* 149:814-821.
22. Crabb, D. W., J. P. Mapes, R. W. Boersma, and R. A. Harris. 1976. Effect of dichloroacetate on carbohydrate and lipid metabolism of isolated hepatocytes. *Arch. Biochem. Biophys.* 173:658-665.
23. Felts, J. M., I. Staprans, and P. W. Stacpoole. 1976. The effect of sodium dichloroacetate (DCA) on plasma triglycerides of streptozotocin diabetic rats. *Diabetes*. 25(Suppl.1):363.
24. Hayek, A., and W. F. Woodside. 1980. Short-term influences of dichloroacetate on genetically hyperlipemic rats. *Metab. Clin. Exp.* 29:120-124.
25. Ribes, G., G. Valette, and M.-M. Loubatieres-Mariani. 1979. Metabolic effects of sodium dichloroacetate in normal and diabetic dogs. *Diabetes*. 28:852-856.
26. Stacpoole, P. W., G. W. Moore, and D. M. Kornhauser. 1978. Metabolic effects of dichloroacetate in patients with diabetes mellitus and hyperlipoproteinemia. *N. Engl. J. Med.* 298:526-530.
27. Moore, G. W., L. L. Swift, D. Rabinowitz, O. B. Crofford, J. A. Oates, and P. W. Stacpoole. 1979. Reduction of serum cholesterol in two patients with homozygous familial hypercholesterolemia by dichloroacetate. *Atherosclerosis*. 33:285-293.
28. Stacpoole, P. W., C. E. Varnado, and D. P. Island. 1982. Stimulation of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase activity by *o,p*-DDD. *Biochem. Pharmacol.* 31:857-860.
29. Harwood, Jr., H. J., and V. W. Rodwell. 1982. HMG-CoA reductase kinase: measurement of activity by methods that preclude interference by inhibitors of HMG-

CoA reductase activity or by mevalonate kinase. *J. Lipid Res.* 23:754-761.

30. Harwood, Jr., H. J. 1983. Allosteric activation of HMG-CoA reductase kinase (RK) by ADP. *Fed. Proc.* 42:2246.
31. Berry, M. N., and D. S. Friend. 1969. High-yield preparation of isolated rat liver parenchymal cells. *J. Cell Biol.* 43:506-520.
32. Shapiro, D. J., J. L. Nordstrom, J. J. Mitschelen, V. W. Rodwell, and R. T. Schimke. 1974. Microassay for 3-hydroxy-3-methylglutaryl CoA reductase in rat liver and in L-cell fibroblasts. *Biochim. Biophys. Acta.* 370:369-377.
33. Rogers, D. H., S. R. Panini, and H. Rudney. 1980. Rapid, high-yield purification of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Anal. Biochem.* 101:107-111.
34. Hunter, C. F., and V. W. Rodwell. 1980. Regulation of vertebrate liver HMG-CoA reductase via reversible modulation of its catalytic activity. *J. Lipid Res.* 21:399-405.
35. Langdon, R. B., and R. E. Counsell. 1976. Determination of the Michaelis-Menten constant for β -hydroxy- β -methylglutaryl coenzyme A reductase. Demonstration of a substrate affinity 10-fold greater than previously reported. *J. Biol. Chem.* 251:5820-5823.
36. Jeske, D. J., and J. M. Dietschy. 1980. Regulation of rates of cholesterol synthesis *in vivo* in the liver and carcass of the rat measured using [3 H]water. *J. Lipid Res.* 21:364-376.
37. Lakshmanan, M. R., and R. L. Veech. 1977. Measurement of rate of rat liver sterol synthesis *in vivo* using tritiated water. *J. Biol. Chem.* 252:4667-4673.
38. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
39. Demaugre, F., C. Cepanec, and J.-P. Leroux. 1978. Characterization of oxalate as a catabolite of dichloroacetate responsible for the inhibition of gluconeogenesis and pyruvate carboxylation in rat liver cells. *Biochem. Biophys. Res. Commun.* 85:1180-1185.
40. Harris, R. A., and D. W. Crabb. 1978. Inhibition of hepatic gluconeogenesis by dichloroacetate. *Arch. Biochem. Biophys.* 189:364-371.
41. Nordstrom, J. L., V. W. Rodwell, and J. J. Mitschelen. 1977. Interconversion of active and inactive forms of rat liver hydroxymethylglutaryl-CoA reductase. *J. Biol. Chem.* 252:8924-8934.
42. Beg, Z. H., J. A. Stonik, and H. B. Brewer, Jr. 1978. 3-Hydroxy-3-methylglutaryl coenzyme A reductase: regulation of enzymatic activity by phosphorylation and dephosphorylation. *Proc. Natl. Acad. Sci. USA.* 75:3678-3682.
43. Segel, I. H. 1976. *Biochemical Calculations*. John Wiley & Sons, Inc., New York. Second ed. 256-257.
44. Whitehouse, S., R. H. Cooper, and P. J. Randle. 1974. Mechanism of activation of pyruvate dehydrogenase by dichloroacetate and other halogenated carboxylic acids. *Biochem. J.* 114:761-774.
45. Paxton, R., and R. A. Harris. 1982. Isolation of rabbit liver bran chain α -ketoacid dehydrogenase and regulation by phosphorylation. *J. Biol. Chem.* 257:14433-14439.
46. Stacpoole, P. W., S. M. Grundy, L. L. Swift, H. L. Greene, A. E. Slonim, and I. M. Burr. 1981. Elevated cholesterol and bile acid synthesis in an adult patient with homozygous familial hypercholesterolemia. Reduction by a high glucose diet. *J. Clin. Invest.* 68:1166-1171.
47. Katz, R., C. N. Tai, R. M. Diener, R. F. McConnell, and D. E. Semonick. 1981. Dichloroacetate, sodium: 3-month oral toxicity studies in rats and dogs. *Toxicol. Appl. Pharmacol.* 57:273-287.
48. Spencer, P. S., M. C. Bischoff, and P. W. Stacpoole. 1981. Differential neurotoxicity of dichloroacetate and 2,5-hexanedione: implications for the pathogenesis of gamma-diketone neuropathy. *Toxicologist*. 1:51.
49. Yount, E. A., S. Y. Felton, B. L. O'Connor, R. G. Peterson, R. S. Powell, M. N. Yum, and R. A. Harris. 1982. Comparison of the metabolic and toxic effects of 2-chloropropionate and dichloroacetate. *J. Pharmacol. Exp. Ther.* 222:501-507.
50. Bilbao, J. M., H. Berry, J. Marotta, and R. C. Ross. 1976. Peripheral neuropathy in oxalosis. A case report with electron microscopic observations. *Can. J. Neurol. Sci.* 3:63-67.
51. Hodgkinson, A. 1977. *Oxalic acid in biology and medicine*. Academic Press, Inc., New York. 214-255.