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CLOFIBRATE, INHIBITOR OF INTESTINAL CHOLESTEROGENESIS*†

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Abstract—Clofibrate (chlorophenoxyisobutyrate) effectively inhibited cholesterol synthesis from acetate by the hamster small intestine. Intestinal cholesterogenesis measured *in vivo* decreased to 54 and 68 per cent of control in drug-treated refed and starved animals. Cholesterogenesis by intestinal slices from treated hamsters decreased to 24 per cent of control. Hepatic cholesterogenesis was also decreased under all experimental conditions. Intestinal and hepatic cholesterol concentration decreased significantly with Clofibrate treatment. Cholesterol synthesis in biotin-deficient hamsters was not reduced, indicating that an alternate pathway for cholesterol synthesis via carboxylation to malonyl CoA was not quantitatively significant as a site of drug action. D-Thyroxine, similar to Clofibrate, inhibited hepatic cholesterogenesis from acetate, but did not affect intestinal cholesterogenesis. These results support the concept of different mechanisms of control of hepatic and intestinal cholesterogenesis.

CLOFIBRATE (chlorophenoxyisobutyrate) is a potent hypolipidemic drug currently in clinical use. It influences various aspects of lipid and lipoprotein synthesis, transport and metabolism,[1,2] but its mechanism of action is not entirely clear.

For many years, the liver was regarded as the principal endogenous source of the circulating cholesterol pool.[3,4] Inhibition of hepatic cholesterogenesis by Clofibrate has been demonstrated in the intact rat or in liver slices, and might explain the hypocholesteremic effect of the drug.[5–7] Recently, intestinal cholesterol synthesis has been emphasized as a significant contributor to the circulating cholesterol pool.[8–10] For this reason, we have investigated the effects of Clofibrate treatment on intestinal cholesterogenesis from acetate, particularly since a previous study[5] had noted no inhibitory effect of the drug in rats.

A possible thyromimetic action of the drug[11] was explored in additional experiments comparing intestinal and hepatic cholesterogenesis in animals given D- and L-thyroxine. To investigate an alternate biotin-dependent pathway of cholesterol biosynthesis in intestine,[12] which might be affected by Clofibrate, cholesterol biosynthesis was quantified in biotin-deficient animals.

MATERIALS AND METHODS

Male golden hamsters (Lakeview), weighing about 100 g, were used for all experi-

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ments. They were kept in the laboratory for 2–3 weeks before experiments and were fed Purina rat chow *ad lib.* (0·06% cholesterol).*

Clofibrate (*chlorophenoxyisobutyryl ethyl ester*). Clofibrate† was added to the diet to a concentration of 0·2%, and fed to 26 hamsters for 14 days.

D- *And* L-*thyroxine*. D- Or L-thyroxine,‡ 0·125 mg dissolved in 0·025 N sodium hydroxide, 0·25 ml, was injected subcutaneously thrice weekly for seven injections to two groups of nine hamsters each. The last injection was administered 24 hr before experiments. Nine control animals were handled similarly and given injections of the same volume of buffer containing sodium hydroxide.

Avidin. Four hamsters were fed 40 per cent raw egg white (Nutritional Biochemical Company, Cleveland, Ohio) incorporated into Purina rat chow for 4 weeks.

Refed animals. Food was withdrawn overnight for 15 hr. In the morning, animals had free access to food for 2 hr prior to killing or acetate feeding. The latter group was fed until killed.

Animals starved 24 *hr.* Twenty-four hr before acetate injection, food was withdrawn and water was provided *ad libitum.*

Experimental methods

Cholesterol biosynthesis in vitro. Animals were killed by decapitation about noonday. Blood was collected in tubes containing 10 mg ethylenediaminetetraacetate, sodium salt. The entire small intestine was removed, its lumen rinsed with saline, measured and weighed. The jejunum was everted and cut into rings 5 mm wide. Duplicate portions of intestinal rings weighing about 300 mg were incubated with 5 ml Krebs–Ringer bicarbonate buffer, pH 7·4, containing, per milliliter, 1 μc sodium [^{14}C]2-acetate (New England Nuclear Corp., Boston, Mass.), 2 μmoles sodium acetate and 2 mg glucose. Liver was removed, weighed and sliced into 0·5 mm slices with a Stadie–Riggs tissue slicer. Two portions of liver weighing approximately 1 g each were incubated with 10 ml Krebs–Ringer bicarbonate buffer, pH 7·4, containing, per ml, 0·3 μc sodium [^{14}C]2-acetate, 1·0 μmole sodium acetate and 2 mg glucose. Tissues were incubated for 1 hr in a Dubnoff metabolic shaker bath at 37° in an atmosphere of 95% O_2 and 5% CO_2.

Cholesterol biosynthesis in vivo. Between 8 a.m. and 10 a.m., sodium [^{14}C]2-acetate, 15 μc (specific activity, 0·25 μc/mg), dissolved in 0·5 ml of 0·89% sodium chloride solution, was injected via stomach tube into hamsters lightly anesthetized with ether. Five hr later, the animals were killed by decapitation. Blood was collected, the intestine and the liver were removed and weighed. Jejunum (10 cm) and approximately 1 g liver were weighed.

Radioactivity and chemical analyses

Intestinal and liver lipids were extracted by homogenizing tissues in a Waring blender with chloroform–methanol, 2:1 (v/v), 18 ml/g of tissue. Eight ml KH_2PO_4, 0·15 M, was added for phase separation. Sodium sulfate (1 g) was added to the separated chloroform phase for dehydration. Lipid extracts were evaporated to dryness *in vacuo* at 40°. Cholesterol was isolated from a portion of the lipid extract by thin-layer

* Lipids in rat chow were extracted and cholesterol was measured with the methods used for quantifying cholesterol in tissues.

† Obtained from Dr. Jerome Noble of Ayerst Laboratories, New York, N.Y.

‡ Obtained from Dr. W. L. Warner of Travenol Laboratories, Morton Grove, Ill.

chromatography (TLC) on Silica gel G. A two-solvent developing system[13] was used: diethyl ether–benzene–ethanol–acetic acid, 44:55:2·2:0·22 (v/v), followed by hexane–diethyl ether–acetic acid, 90:10:1 (v/v). Cholesterol was identified by comparison to standards visualized with iodine spray. The Silica gel "spot" was scraped into radioactivity counting vials. Radioactivity was measured in a Beckman LS 100 liquid scintillation spectrometer after addition of Beckman "Cocktail D" (naphthalene, 200 g, and diphenyloxazole, 10 g, per 2 l. of reagent grade dioxane). Radioactivity of cholesterol esters was not used in calculations, since all the ^{14}C radioactivity was located in the fatty acid moiety.[14] Radioactivity was expressed as counts per minute $\times 10^{-3}$ per gram of tissue and as specific activity (counts per minute $\times 10^{-3}$ per milligram of cholesterol). Recoveries of cholesterol in lipid extracts averaged 98 per cent. Mean recoveries of radioactivity applied to TLC plates were 97 per cent.

Cholesterol concentration was determined by gas–liquid chromatography (GLC). Cholesterol and cholesterol esters, separated by thin-layer chromatography, were visualized by spraying with Rhodamine-G solution. The appropriate Silica gel "spots" were eluted with diethyl ether. Cholesterol esters were saponified with ethanolic KOH and the free cholesterol was extracted into petroleum ether. Trimethyl silyl ether derivatives were prepared. Cholestane was used as an internal standard.[15] Measurements were carried out in a Barber–Colman series 5000 gas–liquid chromatograph, with hydrogen flame detector, using a column of 3% QF_1 on Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pa.). The column bath temperature was 235°. Argon was used as a carrier gas at 45 ml/min. Peak areas were measured by an electronic integrator (Kent Chromolog 2). Cholesterol concentration was expressed as milligrams per gram of tissue. Mean recoveries of cholesterol were 96 per cent.

Cholesterol was extracted from plasma with chloroform–methanol, 2:1 (v/v); phase separation was achieved by adding 0·9% saline.[16] Total cholesterol was determined chemically.[17] Results were expressed as milligrams per gram (tissue) or milligrams per 100 ml (plasma).

Experimental modifications

In vitro. Experiments were performed in 14 refed hamsters in three groups: untreated, 4; Clofibrate, 6; Avidin, 4. Data from each group were averaged to yield mean values and standard errors of the means. Comparisons of mean values were performed using Student's *t*-test.

In vivo. Cholesterol biosynthesis was quantified in 26 hamsters given no drug treatment and divided into two groups of nutritional regimens: refed, 5; starved 24 hr, 21. Sixteen hamsters fed Clofibrate were divided into two nutritional groups: refed, 4; starved 24 hr, 12. D- and L-thyroxine-treated hamsters were all starved 24 hr before experiments (nine animals in each group).

RESULTS

Intestinal biosynthesis of cholesterol

Intestinal cholesterol biosynthesis from acetate was reduced significantly as were the concentration and the specific activity of intestinal cholesterol in all Clofibrate-treated hamsters.

Cholesterol radioactivity in intestinal slices of Clofibrate-treated refed hamsters was reduced significantly to one-quarter the mean value in control animals (Table 1). Cholesterol concentration of small intestine was decreased significantly by one-sixth. Specific activity of intestinal cholesterol was one-third that obtained in untreated animals. In hamsters given Clofibrate, intestinal cholesterol biosynthesis from acetate, measured *in vivo*, was reduced significantly both in refed and starved animals in comparison to control hamsters on similar nutritional regimens (Table 2). Incorporation of radioactivity into intestinal cholesterol in the Clofibrate refed group was reduced to 68 per cent of the value in control refed animals. Radioactivity of intestinal cholesterol of Clofibrate-treated starved animals was 58 per cent of the value of starved controls. Intestinal cholesterogenesis from acetate was similar in untreated starved hamsters and refed animals.

TABLE 1. INTESTINAL AND LIVER CHOLESTEROGENESIS *in vitro* (EFFECTS OF CLOFIBRATE)*

	Intestine		Liver	
	Untreated	Clofibrate	Untreated	Clofibrate
Number of hamsters	4	6	4	6
Radioactivity (counts/min $\times 10^{-3}$ /g)	55·4 ±5·75	14·3†	11·7 ±4·10	7·00 ±1·98
Specific activity (counts/min $\times 10^{-3}$ mg)	25·2 ±4·72	7·43‡ ±0·524	8·93 ±2·36	6·47 ±0·564
Concentration (mg/g)	2·20 ±0·034	1·93§ ±0·104	1·31 ±0·056	1·08§ ±0·058

* Values represent mean ± 1 S.E.M. Untreated hamsters were fed Purina rat chow *ad lib.* All animals were starved overnight for 15 hr. Food was given for 2 hr in the morning before sacrifice. Hamsters in the Clofibrate group were given 0·2% Clofibrate in chow for 2 weeks. Animals were sacrificed at noon. Intestinal slices, 300 mg, were incubated for 1 hr in 5 ml Krebs–Ringer bicarbonate buffer, pH 7·4, containing 5 μc sodium [^{14}C]2-acetate at 37° in a metabolic shaker bath. Liver slices 1 g, were incubated in 10 ml Krebs–Ringer bicarbonate buffer containing 3 μc sodium [^{14}C]2-acetate.

† $P < 0{\cdot}01$, comparing Clofibrate group to untreated by Student's *t*-test.

‡ $0{\cdot}01 < P < 0{\cdot}02$, comparing Clofibrate group to untreated by Student's *t*-test.

§ $0{\cdot}02 < P < 0{\cdot}05$, comparing Clofibrate group to untreated by Student's *t*-test.

Hepatic biosynthesis of cholesterol

Hepatic cholesterol synthesis from acetate was decreased with Clofibrate treatment in all experimental conditions. Hepatic cholesterogenesis was decreased to 60 per cent of control in liver slices of refed animals treated with Clofibrate. The decrease was not statistically significant because of the great variation in rate of cholesterol synthesis in the control group (Table 1). Hepatic cholesterol concentration decreased significantly by 25 per cent with Clofibrate treatment. Specific activity of liver cholesterol was reduced to two-thirds the value in control animals. Incorporation of acetate into cholesterol by liver slices of control refed hamsters was about 40 per cent that obtained with intestinal slices from these animals.

TABLE 2. INTESTINAL CHOLESTEROGENESIS *in vivo* (EFFECTS OF CLOFIBRATE)*

	Refed		Starved 24 hr	
	Untreated	Clofibrate	Untreated	Clofibrate
Number of hamsters	5	4	21	12
Radioactivity (counts/min × 10^{-3}/g)	19·6 ±0·583	10·5† ±1·82	19·4 ±1·38	13·3‡ ±2·42
Specific activity (counts/min × 10^{-3}/mg)	9·67 ±0·668	6·641† ±0·230	9·04 ±0·703	7·72‡ ±0·505
Concentration (mg/g)	2·03 ±0·096	1·58‡ ±0·150	2·20 ±0·137	1·73 ±0·189

* Refed animals were managed as in Table 1. Other groups of hamsters were starved 24 hr prior to the feeding of acetate and until killed (see Table 1 for Clofibrate treatment). All animals were given 15 μc sodium [^{14}C]2-acetate between 8 and 10 a.m. by stomach tube and were sacrificed 5 hr later.

† $P < 0{\cdot}01$, comparing Clofibrate group to corresponding untreated group by Student's *t*-test.

‡ $0{\cdot}02 < P < 0{\cdot}05$, comparing Clofibrate group to corresponding untreated group by Student's *t*-test.

Hepatic cholesterol biosynthesis from acetate in intact animals was reduced significantly in refed and starved hamsters given Clofibrate (Table 3). Incorporation of radioactivity into hepatic cholesterol in the Clofibrate refed group was reduced to 60 per cent of the values in control refed animals. Radioactivity of hepatic cholesterol of Clofibrate-treated starved animals was 30 per cent of the value of starved untreated animals. Hepatic cholesterogenesis from acetate measured *in vivo* in untreated animals was similar in refed and starved hamsters. Starvation without drug did not affect liver cholesterol concentration. Hepatic cholesterol concentration decreased by 30 per cent in fed and starved groups fed Clofibrate. With Clofibrate, hepatic cholesterol specific activity decreased by one-third in refed animals and by one-half in starved animals compared with corresponding control animals.

D- *And* L-*thyroxine*

In contrast to Clofibrate inhibition of intestinal cholesterogenesis from acetate, D- and L-thyroxine increased intestinal cholesterol biosynthesis by one-half ($P < 0{\cdot}01$) and one-fifth respectively (Fig. 1). Specific activity of intestinal cholesterol increased significantly ($P < 0{\cdot}01$) by 80 per cent ($10{\cdot}2 \pm 0{\cdot}180$ counts/min × 10^{-3}/mg) and 28 per cent ($11{\cdot}6 \pm 0{\cdot}147$ counts/min × 10^{-3}/mg) with administration of D- and L-thyroxine, respectively, compared to control animals (see Table 2). Animals injected with buffer did not differ from untreated control hamsters, so data were pooled. Intestinal cholesterol concentration decreased significantly by one-fifth ($P < 0{\cdot}02$) in D-thyroxine-treated hamsters ($1{\cdot}69 \pm 0{\cdot}159$ mg/g), and was unchanged in L-thyroxine-treated animals ($1{\cdot}94 \pm 0{\cdot}130$ mg/g).

There was a dissociation of the effects of D- and L-thyroxine on hepatic cholesterogenesis. D-Thyroxine treatment, similar to Clofibrate, significantly ($P < 0{\cdot}01$) decreased hepatic cholesterol radioactivity by one-half (Fig. 1) in contrast to its stimulatory

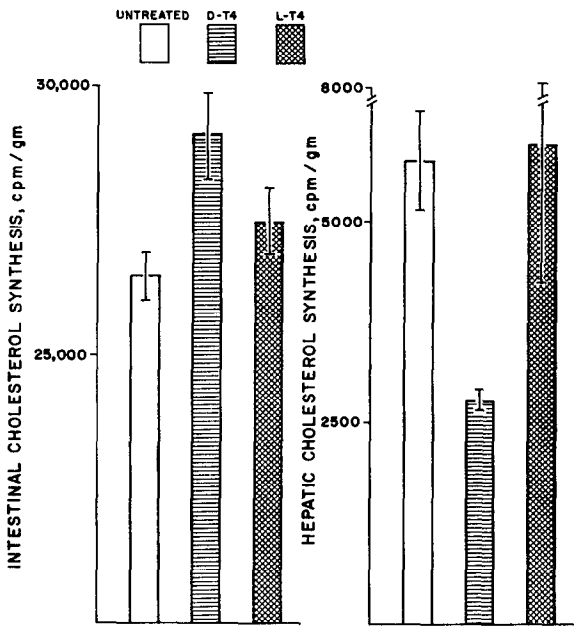

FIG. 1. Effects of D- and L-thyroxine on intestinal and hepatic cholesterogenesis from acetate measured *in vivo*. Hamsters were starved 24 hr and received sham injections or were given D- or L-thyroxine, 0·125 mg twice weekly for 2 weeks. The heights of the bars represent mean values of radioactivity of intestinal or hepatic cholesterol per gram of tissue. Vertical lines represent ± 1 S.E.M. D-T_4 = D-thyroxine; L-T_4 = L-thyroxine.

TABLE 3. LIVER CHOLESTEROGENESIS *in vivo* (EFFECTS OF CLOFIBRATE)*

	Refed		Starved 24 hr	
	Untreated	Clofibrate	Untreated	Clofibrate
Number of hamsters	5	4	21	12
Radioactivity (counts/min × 10^{-3}/g)	3·95 ±0·185	2·36† ±0·201	5·76 ±0·620	1·70† ±0·263
Specific activity (counts/min × 10^{-3}/mg)	2·63 ±0·201	1·98‡ ±0·312	3·03 ±0·432	1·34† ±0·296
Concentration (mg/g)	1·50 ±0·247	1·20 ±0·077	1·88 ±0·185	1·27§ ±0·104

* See Table 1 for feeding and drug regimens and administration of [^{14}C]acetate.
† $P < 0·01$, comparing Clofibrate group to corresponding untreated group by Student's *t*-test.
‡ $0·02 < P < 0·05$, comparing Clofibrate group to corresponding untreated group by Student's *t*-test.
§ $0·01 < P < 0·02$, comparing Clofibrate group to corresponding untreated group by Student's *t*-test.

effect on intestinal cholesterogenesis. Specific activity of hepatic cholesterol was decreased to one-half of the untreated value (Table 3) with D-thyroxine (1·89 ± 0·18

counts/min $\times$ 10^{-3}/mg) ($P < 0{\cdot}02$). Hepatic cholesterol concentration was reduced significantly by one-quarter ($1{\cdot}47 \pm 0{\cdot}06$ mg/g; $P < 0{\cdot}05$). L-Thyroxine administration did not affect hepatic cholesterol radioactivity (Fig. 1); hepatic cholesterol concentration decreased by one-quarter ($1{\cdot}52 \pm 0{\cdot}20$ mg/g), resulting in a significant increase by one-quarter ($P < 0{\cdot}02$) in hepatic cholesterol specific activity ($40{\cdot}06 \pm 0{\cdot}22$ counts/min $\times$ 10^{-3}/mg).

Avidin

Intestinal cholesterol biosynthesis from acetate was similar in Avidin-treated animals and control hamsters (Fig. 2). Intestinal cholesterol concentration decreased to $1{\cdot}89 \pm 0{\cdot}17$ mg/g with Avidin treatment. Specific activity of intestinal cholesterol increased by one-third to $33{\cdot}7 \pm 3{\cdot}50$ counts/min $\times$ 10^{-3}/mg. Hepatic cholesterol biosynthesis was doubled in Avidin-treated animals (Fig. 2). Hepatic cholesterol concentration did not differ significantly from control ($1{\cdot}27 \pm 0{\cdot}045$ mg/g). Specific activity of hepatic cholesterol was doubled by Avidin treatment ($17{\cdot}0 \pm 4{\cdot}62$ counts/min $\times$ 10^{-3}/mg). Mean values for acetate incorporation did not differ significantly in Avidin-treated animals compared to controls. Large variances observed in both groups because of small numbers of animals may have obscured biologically significant differences.

Plasma cholesterol concentration

Plasma cholesterol was reduced with Clofibrate treatment to 70 per cent of the plasma cholesterol value of the control in the refed groups (Table 4). A lesser, significant reduction to 84 per cent of control was observed with Clofibrate treatment in

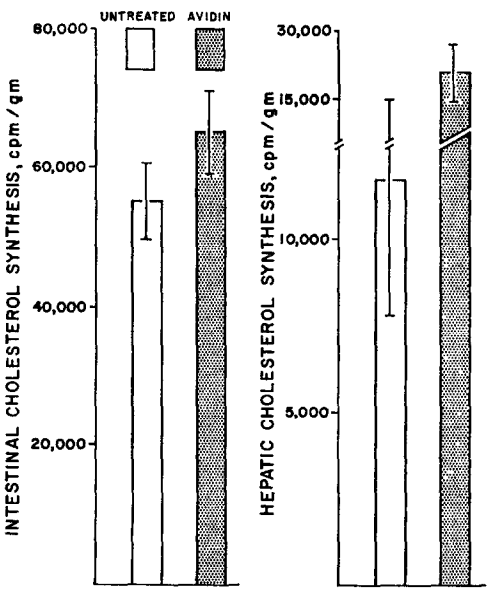

FIG. 2. Cholesterol synthesis from [^{14}C]acetate by intestinal and liver slices from groups of refed hamsters given no treatment, or fed *ad libitum* 40% raw egg white in Purina chow for 4 weeks.

animals starved 24 hr. Starvation for 24 hr did not affect the plasma cholesterol concentration.

TABLE 4. EFFECT OF NUTRITIONAL STATE AND CLOFIBRATE ON PLASMA AND LIVER CHOLESTEROL CONCENTRATIONS AND LIVER WEIGHT*

Category	No. of hamsters	Plasma cholesterol (mg/100 ml)	Liver cholesterol (mg/liver)	Liver wt. (g)	Body wt. (g)	Liver wt./ body wt. (%)
Refed						
Untreated	9	97·0 ±3·1	5·56 ±0·31	3·95 ±0·33	107 ±6·2	4·09 ±0·25
Clofibrate	10	70·0† ±4·7	5·23 ±0·41	4·17 ±0·36	102 ±0·7	4·29 ±0·17
Starved 24 hr						
Untreated	21	96·0 ±6·0	7·08 ±0·55	3·37 ±0·08	113 ±1·1	3·16 ±0·08
Clofibrate	12	84·5‡ ±3·5	6·92 ±0·63	5·59† ±0·47	129 ±7·0	4·13† ±0·19

* See footnotes to Tables 1 and 2 for details of Clofibrate treatment and nutritional regimens.
† $P < 0·01$, comparing Clofibrate to untreated group by Student's *t*-test.
‡ $0·02 < P < 0·05$, comparing Clofibrate to untreated group by Student's *t*-test.

Liver weights and cholesterol concentration

Liver weight increased significantly with Clofibrate treatment both as absolute values and calculated as the proportion of body weight (Table 4). Liver weight, expressed as per cent of body weight, decreased by one-quarter with starvation in untreated animals and was unchanged with Clofibrate. Cholesterol concentration per whole liver was similar in Clofibrate-treated refed or starved hamsters compared to controls.

Starvation for 24 hr resulted in a 4 per cent loss of body weight in control hamsters and a 5 per cent loss of weight in Clofibrate-treated hamsters.

DISCUSSION

Clofibrate decreased plasma cholesterol,[18,20] except in animals on high fat diets.[21,22] Hepatic cholesterol concentration also decreased with Clofibrate.[5,18] These effects were demonstrated in this study in hamsters. Liver weights increased with Clofibrate,[5,18] including this study in hamsters, with cholesterol content of the liver unchanged.

Inhibition of hepatic cholesterol synthesis from acetate *in vitro* and *in vivo* was reported in the rat,[5] and confirmed[6,7] and demonstrated in this study in hamsters. Decreased acetate incorporation into cholesterol may represent: alterations in uptake of acetate, increased metabolic dilution by precursor pools, or a change in equilibrium between cholesterol pools. We have no information on these possible effects of Clofibrate and have assumed that acetate incorporation into cholesterol represents biosynthesis. Inhibition of cholesterol synthesis in man was suggested by demonstrating decreased fractional turnover of circulating cholesterol in human subjects given the

drug.[23] The drug also decreased by 41 per cent the conversion of [^{14}C]acetate, administered intravenously, to plasma cholesterol.[24]

Avoy *et al.*[5] postulated that Clofibrate inhibited HmG CoA reductase activity (mevalonate:NADP oxidoreductase (acylating CoA), EC 1.1.1.34), which catalyzed the irreversible reduction of β-hydroxy-β-methylglutaryl CoA to mevalonic acid, since conversion of mevalonate to cholesterol was not inhibited. The drug did not interfere with ketone body formation.[5] Direct evidence that Clofibrate decreased hepatic microsomal HMG CoA reductase has been presented recently.[25]

Factors modifying hepatic cholesterogenesis did not necessarily affect intestinal cholesterol synthesis similarly. Cholesterol feeding decreased hepatic cholesterol synthesis,[3,4,26,27] but intestinal biosynthesis of cholesterol was either unaffected or decreased slightly.[28,29] Fasting, another potent inhibitor of hepatic cholesterogenesis,[10] questionably or slightly inhibits intestinal cholesterogenesis.[29] Cayen[30] reported marked inhibition by fasting of cholesterol synthesis by rat intestinal slices, and that cholesterol feeding inhibited intestinal cholesterol synthesis in the rat stimulated by cholestyramine feeding. Cholesterol feeding and starvation inhibit hepatic cholesterogenesis by decreasing levels of hepatic HMG CoA reductase.[31,32]

X-irradiation,[33] injection of Triton,[26] and vitamin B_6 deficiency[34] stimulate hepatic but not intestinal cholesterol synthesis. The one factor influencing cholesterol synthesis in both tissues is biliary drainage[35] or cholestyramine,[36] which increases cholesterol synthesis 2- to 8-fold, or bile refeeding or perfusion,[37] which decreases cholesterol synthesis. Bile salts inhibit both hepatic[38] and intestinal[10] HMG CoA reductase.

Our experiments indicate that Clofibrate acts similarly in liver and intestine to decrease cholesterol synthesis. Recently, it was reported that Clofibrate treatment in hypercholesteremic human subjects receiving cholestyramine increased the ratio of specific activities of jejunal mucosal to plasma cholesterol, indicating a decrease in mucosal synthesis of cholesterol with Clofibrate.[39]

Clofibrate inhibition of acetyl CoA carboxylase (acetyl CoA:carbon dioxide ligase; EC 6.4.1.2) has been reported.[40] This biotin-containing enzyme catalyzes the synthesis of malonyl CoA by carboxylation of acetyl CoA, an alternate pathway of cholesterol synthesis in pigeon liver.[12] This alternate pathway in either liver or intestine might provide a locus for Clofibrate action, or define different mechanisms of cholesterol synthesis in liver and intestine. Biotin deficiency did not reduce cholesterol synthesis in either tissue, negating both hypotheses.

A thyromimetic action, especially on the liver, has also been postulated for Clofibrate.[11] Thyroxine and hyperthyroidism stimulate cholesterol synthesis from acetate by liver 2- to 3-fold[41–43] and also enhance intestinal cholesterogenesis.[41] Increase of hepatic HMG CoA reductase activity by thyroxine was described recently.[44] In our experiments, both thyroxine isomers stimulated intestinal cholesterogenesis. L-Thyroxine enhanced hepatic cholesterol specific activity. D-Thyroxine decreased hepatic cholesterol synthesis, similar to Clofibrate. These results suggest: different mechanisms of control of hepatic and intestinal cholesterogenesis as yet undefined; differences between D- and L-thyroxine in effects on cholesterol synthesis; similarities in effects on the liver of Clofibrate and D-thyroxine.

Cholesterol synthesis in hamsters was considerably greater in intestine than liver, in contrast to rat liver slices which incorporate acetate into cholesterol at double the rate in intestine.[29] Hamsters incorporated three times more intraperitoneal acetate into

intestinal cholesterol than into liver sterol. Rats treated similarly incorporated acetate into cholesterol 50 per cent more actively in liver.[14] With oral acetate, intestinal synthesis in the rat is four times that of liver;[45] in hamsters, cholesterol synthesis in intestine was seven times that of liver. Only the guinea pig exceeds this rate of intestinal cholesterogenesis.[27] Cholesterol synthesis in rat ileum exceeds that of jejunum.[29] In hamsters, cholesterol synthesis from intraperitoneal acetate was equal in upper and lower small intestine (10·5 and 11·8 counts/min $\times 10^{-3}$/g respectively). Starvation did not influence hamster hepatic cholesterol synthesis. Hamsters are exquisitely sensitive to cholesterol feeding with cholesterol biosynthesis suppressed to zero, and plasma and liver cholesterol increase greatly.[27] Hamsters fed a glucose diet, rather than laboratory chow, synthesized cholesterol at an increased rate;[46,47] in the mouse, cholesterol synthesis decreased.[48] These data suggest that the cholesterol in laboratory chow may suppress cholesterol synthesis; release of this inhibition by removal of the chow (starvation) may nullify the suppressive effect of starvation, with no net change in cholesterol synthesis.

Diurnal variation in levels of hepatic HMG CoA reductase levels occurs in rats[38] and mice.[49] Although we did not measure enzyme levels in liver or intestine, all experiments were done in the early afternoon, with presumably comparable enzyme levels.

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