Serum Lipid-Lowering Properties of 6-Chloro-9-[2-(6-methyl-3-pyridyl)ethyl]-1,2,3,4-tetrahydrocarbazole-2-carboxylic Acid

COLIN DALTON ˣ and WILLIAM R. POOL

Abstract □ 6-Chloro-9-[2-(6-methyl-3-pyridyl)ethyl]-1,2,3,4-tetrahydrocarbazole-2-carboxylic acid hydrochloride lowered serum cholesterol, triglyceride, phospholipid, and free fatty acid levels in normal rats. The compound appeared to have a low toxicity and to be well tolerated in mice, adult and neonatal rats, and rabbits. At hypolipidemic doses, it caused a slight hepatomegaly associated with an increase in liver phospholipid and protein content. Liver *N*-demethylase and catalase activities were increased by the drug treatment. This acid had no *in vitro* antilipolytic activity and did not affect serum desmosterol–cholesterol ratios. The results indicate that it has a mechanism of action similar to clofibrate.

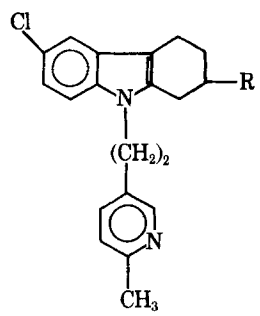
Keyphrases □ Tetrahydrocarbazole, substituted—effect on serum lipid levels in rats, toxicity in rats, mice, and rabbits □ Lipid levels, serum—effect of substituted tetrahydrocarbazole, rats □ Toxicity—substituted tetrahydrocarbazole, rats, mice, and rabbits □ Antihyperlipidemic activity—substituted tetrahydrocarbazole evaluated, rats

Interest in pharmacological agents that lower blood lipid levels is primarily based upon data that implicate elevated blood lipid levels in the etiology of coronary disease. Clofibrate[1], ethyl 2-(4-chlorophenoxy)-2-methylpropanoate, is currently marketed as an active hypolipidemic agent. It is being extensively tested in an international primary prevention trial of ischemic heart disease (1) and for its long-term influence on morbidity and mortality in patients with a history of clinical heart disease (2). This report concerns a new compound, 6-chloro-9-[2-(6-methyl-3-pyridyl)ethyl] -1,2,3,4 -tetrahydrocarbazole- 2-carboxylic acid hydrochloride (I) (3), which is structurally unrelated to clofibrate but has serum lipid-lowering properties.

EXPERIMENTAL

Treatment of Animals—Male albino rats[2], 180–200 g, were housed in individual stainless steel cages with wire screen bottoms. The cages were maintained at 23°. All animals were allowed food[3] and tap water *ad libitum*.

Drugs were suspended or dissolved in an aqueous suspending vehicle (sodium chloride, 9 g/liter; carboxymethylcellulose, 5 g/liter; polysorbate 80, 3.9 g/liter; and benzyl alcohol, 8.6 ml/liter, in distilled water) and administered at the doses indicated in 5 ml of vehicle/kg. Control rats received an equal volume of the vehicle. Routes and times of drug administration are given under *Results*.



I: R = COOH
II: R = CH_2OH

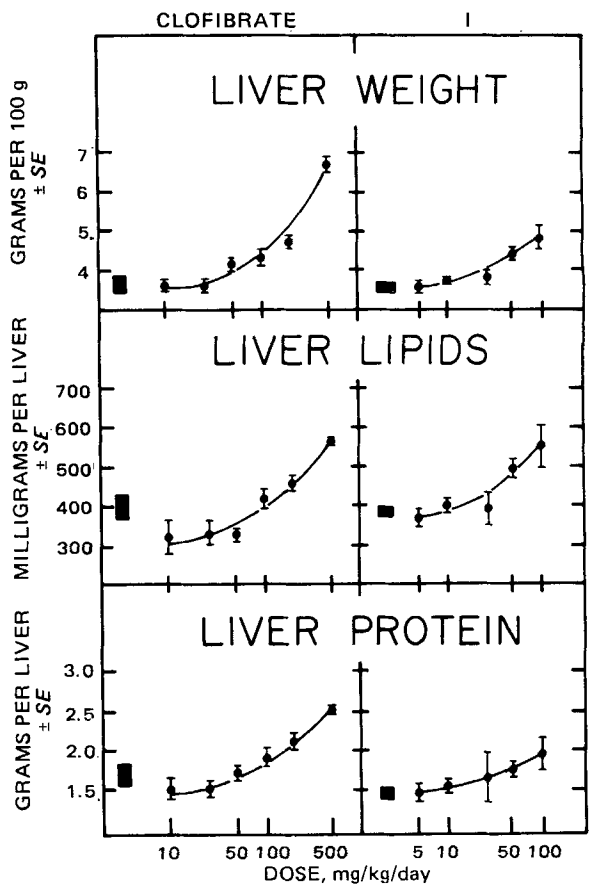


Figure 1—*Effect of hypolipidemic agents on rat liver weight and liver lipid and protein content. Key:* ■, *control.*

The animals were fasted overnight and sacrificed by decapitation. Body, liver, adrenal, and epididymal fat pad weights were recorded. Separate portions of liver were used for the preparation of subcellular fractions, analysis of lipids, and determination of *N*-demethylase activity.

Hypertriglyceridemic Rats—The test compounds were suspended in 5% gum acacia solution and administered orally for 11 days by intubation each day, except on the weekend when the compounds were administered as a dietary admix. Six animals were used for each dose level. Two groups of 10 control animals each were untreated. A 10% fructose solution was substituted for normal drinking water for the last 20 hr of the test. One control group continued to receive normal drinking water. On Day 12, the animals were sacrificed for serum triglyceride determination (4).

Preparation of Subcellular Fractions—A 10% liver homogenate in 0.25 *M* sucrose was prepared using six strokes of a motor-driven glass–plastic[4] homogenizer at 4°. Mitochondria were isolated according to the method of Schneider (5). Samples were centrifuged[5] at 800×*g* at

[1] Atromid-S, Ayerst Laboratories, New York, N.Y.
[2] Charles River Breeding Laboratories.
[3] Pelleted Purina Rat Chow.
[4] Teflon (du Pont).
[5] International refrigerated centrifuge model PR-6.

Table I—Effect of I on Rat Serum Lipid Content[a]

Dose, mg/kg/day	Cholesterol, mg % ± *SE*	Triglycerides, mg % ± *SE*	Phospholipids, mg % ± *SE*	Free Fatty Acids, μEq/liter ± *SE*
0	77 ± 3	79 ± 10	118 ± 5	819 ± 26
50	60 ± 5*	48 ± 8*	83 ± 3***	650 ± 29**
100	60 ± 5*	47 ± 10*	90 ± 6**	607 ± 25***

[a] The significance of the difference between the treated and the control groups is designated by the *p* values: * <0.05, ** <0.01, and *** <0.001; *n* = six to 12 rats per group.

Table II—Effect of I on Rat Body and Organ Weight

Dose, mg/kg/day	Increase in Body Weight, g ± *SE*	Adrenal Weight, mg ± *SE*	Fat Pad Weight, g ± *SE*	Liver Weight, g ± *SE*
0	59.4 ± 2.1	46 ± 3	1.5 ± 0.09	9.1 ± 0.2
50	56.5 ± 7.5	43 ± 2	1.3 ± 0.16	10.8 ± 0.7
100	60.1 ± 5.2	45 ± 2	1.2 ± 0.12	11.7 ± 0.6[a]

[a] The significance of the difference between the treated and the control groups is designated by the *p* value: <0.01.

Table III—Acute Toxicity of I

Species	Route of Administration	LD_{50} ± *SE* mg/kg
Mice	Oral	13,000 ± 1120
Mice	Intraperitoneal	410 ± 20
Mice	Subcutaneous	>2000
Adult rats	Oral	>4000
Neonatal rats	Oral	3700 ± 240
Rabbits	Oral	880 ± 200

Table IV—Effect of I on Serum Triglyceride Levels in Hypertriglyceridemic Rats

Dose, mg/kg po	Serum Triglycerides, mg % ± *SE*	Change from Control, %
Control	223 ± 10	—
1	244 ± 23	+9
20	193 ± 12	−13
50	185 ± 25	−17
100	151 ± 11[a]	−32

[a] $p < 0.01$.

2° for 10 min to remove nuclei and unbroken cells. The supernate was collected, and the residue was washed twice with isotonic sucrose. Washes were combined with the supernate, and the residue was discarded. The combined supernates were then centrifuged[6] at 8500×*g* at 2° for 10 min to sediment the mitochondria. The supernate was collected and the mitochondrial pellet was washed twice with isotonic sucrose.

Microsomes were prepared by centrifuging[7] the combined supernates from the mitochondrial fraction of 105,000×*g* at 2° for 75 min. The mitochondrial and microsomal pellets were resuspended in isotonic sucrose and rehomogenized gently with a glass–plastic[4] homogenizer to a final tissue equivalent of 150 mg/ml.

Analytical Procedures—Serum was analyzed for cholesterol (6), triglycerides (4), phospholipids (7), and free fatty acids (8). The protein content of the fractions was determined by the biuret reaction conducted on an automated analyzer[8]. Lipids were extracted with chloroform–methanol (2:1 v/v), using a modification of the Folch *et al.* procedure (9), determined gravimetrically, and analyzed for phospholipid content (7).

Determination of Hepatic *N*-Demethylase Activity—A 20% liver homogenate in 0.01 *M* phosphate buffer containing 1.15% KCl was prepared by homogenizing in a high speed blender[9] three times for 10 sec each. The homogenate was centrifuged[10] in 50-ml polyethylene tubes at 10,000×*g* for 30 min at 2°, and the supernate was removed with a syringe and either analyzed immediately or frozen overnight. *N*-Demethylase activity was assayed using an automated adaptation (10) of the method of Axelrod (11), utilizing ethylmorphine as the substrate.

GLC of Sterols—Aliquots of rat serum were saponified with 0.5 *N* ethanolic potassium hydroxide by heating for 5 min at 90°, and water was added after cooling. The nonsaponifiable fraction was extracted four times with 3 ml of petroleum ether (bp 30–60°), and cholestane was added to the extracts as an internal standard. The extracts were combined, brought to dryness at 70° under nitrogen flow, and dissolved in 0.1 ml of chloroform[11].

Isothermal GLC was performed on a gas chromatograph equipped with flame-ionization detectors using a 1.8-m (6-ft) × 4-mm glass column, 3% OV-17 on Gas Chrom Q, at 240° with a helium carrier flow rate of 90 ml/min. All peak area measurements were made in triplicate by polar planimetry and were quantitated by internal standardization with cholestane (12).

***In Vitro* Lipolytic Activity**—An isolated fat cell suspension was prepared according to Rodbell (13) and incubated in plastic vials in a total volume of 1 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) containing 4% bovine plasma albumin. Theophylline (5×10^{-4} *M*) or levarterenol (5×10^{-7} *M*) was used to stimulate lipolysis. Compound I was dissolved in ethanol and added to yield the concentrations indicated. The control samples were incubated with an equal volume of ethanol. The cell suspension was incubated for 1 hr at 37°. The reaction was terminated, and glycerol was extracted by the addition of 4 ml of water to the incubation vial followed by mechanical shaking for 5 min.

The aqueous extract was used directly for the enzymatic glycerol analysis (14). Separate aliquots of the fat cell suspension were taken for triglyceride analysis (4), and the results were expressed as micromoles of glycerol released per gram of triglyceride per hour.

Catalase Determination—Livers from freshly sacrificed rats were homogenized in a glass–plastic[4] homogenizer at 4° with 1.25 m*M* phosphate buffer. The homogenate was allowed to stand for 30 min at 4°, rehomogenized, and adjusted to a final volume of 50 ml. The absorbance at 240 nm was measured[12] at room temperature. A 0.01-ml aliquot of the liver homogenate was added to 3 ml of hydrogen peroxide–phosphate buffer, and the time required for a decrease in absorbance from 0.55 to 0.50 was determined.

Catalase activity is expressed as units per milligram of wet weight of liver. A catalase unit is defined as the amount of enzyme that liberates half of the peroxide oxygen from a hydrogen peroxide solution of any concentration in 100 sec at 25° (15).

Acute Toxicity—CF-1 mice (17–25 g), Wistar-strain adult (130–175 g) and neonatal (6–9 g) rats, and albino rabbits (1.9–2.7 kg) were used. Compound I was ground in a mortar and suspended in 5% gum acacia solution or aqueous suspending vehicle and was administered orally, intraperitoneally, or subcutaneously. Eight to 10 animals were used per

[6] Sorvall automatic refrigerated centrifuge model RC2-B.
[7] Beckman model L-2 ultracentrifuge.
[8] Technicon Corp. methodology N-14-b.
[9] Waring.
[10] Sorvall centrifuge with SS-34 head.
[11] M.C.B. spectroquality.
[12] Beckman DU spectrophotometer.

Table V—Effect of Hypolipidemic Drugs on Liver Lipid Content[a]

	Cholesterol		Triglycerides		Phospholipids	
	mg/g	mg/Liver	mg/g	mg/Liver	mg/g	mg/Liver
Control	4.1 ± 0.4	36 ± 1	5.9 ± 0.8	52 ± 8	31 ± 1	278 ± 1
Clofibrate	2.9 ± 0.1**	34 ± 2	5.7 ± 1.4	68 ± 17	35 ± 1	406 ± 2*
I	3.6 ± 0.1**	39 ± 1	3.8 ± 0.4**	42 ± 5	35 ± 3	380 ± 2**

[a] The significance of the difference between the treated and the control groups is designated by the p values: * <0.01, and ** <0.001.

dose level. The animals were observed for 5 days, and total mortality was reported. The LD_{50} was calculated by the method of Miller and Tainter (16). Some of the treated neonates were missing and were presumed to have been cannibalized after dying. The missing rats were counted as dead rats in calculating the LD_{50}.

The statistical significance of the data was determined by the Student t test and is designated as a p value.

RESULTS

Oral administration of I for 2 weeks to rats lowered serum concentrations of cholesterol, triglycerides, phospholipids, and free fatty acids (Table I). Maximum lipid lowering was observed after dosing with 50 mg/kg, and no further decrease was found at 100 mg/kg. Rats treated with I gained weight at the same rate as placebo controls, indicating that appetite was not affected and that the drug was well tolerated (Table II). Drug-tested rats looked and behaved like normal, healthy animals.

Acute toxicity determinations in mice, rats, and rabbits indicated that I had low oral, intraperitoneal, and subcutaneous toxicity (Table III). The high LD_{50} ratio for oral *versus* intraperitoneal routes of administration indicated a low bioavailability for high oral doses of I. Such decreased absorption after large oral dosage may explain the apparent lack of a dose–response curve for serum lipid lowering. A female dog receiving the compound in gelatin capsules tolerated oral doses up to 320 mg/kg without symptoms. Normal hematological and serum clinical chemical values were observed in this dog[13].

The low toxicity during 2 weeks of drug administration was corroborated by normal adrenal and epididymal fat pad weights (Table II). In common with other hypolipidemic agents (17), I increased rat liver weight (Table II). Compound I also caused a dose-related decrease in serum triglyceride levels in rats made hypertriglyceridemic by adding fructose to their drinking water (Table IV).

Compound I was studied in comparison with clofibrate to compare their mechanisms of action. Both compounds caused a dose-related increase in liver weight and in liver lipid and protein content (Fig. 1). Examination of the composition of the liver from rats treated with various hypolipidemic agents revealed no change in DNA or water content, indicating that neither hyperplasia nor edema could explain the liver enlargement (17). Significant increases in liver RNA were found. Analysis of the different lipid classes in the hepatomegalic livers, induced by two hypolipidemic drugs, revealed an apparent decrease in cholesterol and triglyceride concentrations when expressed on a milligrams per gram of liver basis. However, this result represented no change in the total cholesterol or triglyceride content of the liver (Table V). Liver phospholipid content was strikingly increased in clofibrate- and I-treated rats.

It was of interest to determine the subcellular localization of the changes in liver composition. Oral administration of 250 mg/kg of clofi-

Table VI—Effect of Hypolipidemic Agents on Liver Microsomal *N*-Demethylase Activity[a]

Dose, mg/kg/day	*N*-Demethylase Activity, μmoles/g/min	
	I	Clofibrate
0	7.4 ± 0.5	5.9 ± 0.9
10	9.8 ± 1.0	6.4 ± 1.1
25	11.2 ± 0.5	7.9 ± 1.9
100	11.6 ± 0.9*	9.1 ± 1.1
200	—	14.0 ± 0.8**

[a] The significance of the difference due to the drugs is designated by the p values: * <0.01, and ** <0.001.

Table VII—Effect of Hypolipidemic Drugs on Serum Cholesterol and Desmosterol Composition

Treatment	Dose, mg/kg/day	Cholesterol, %	Desmosterol, %
Control	—	99+	Trace
Triparanol	50	38.9	61.1
I	50	95.1	4.9
Clofibrate	250	99+	Trace

Table VIII—Effect of I and Clofibrate on Liver Catalase Activity[a]

Drug	Dose, mg/kg	Catalase Activity, units/mg
Control	—	6.1 ± 0.4
I	100	10.0 ± 1.1*
Clofibrate	350	7.2 ± 0.4**

[a] The significance of the difference due to drugs is designated by the p values: * <0.05, and ** <0.01.

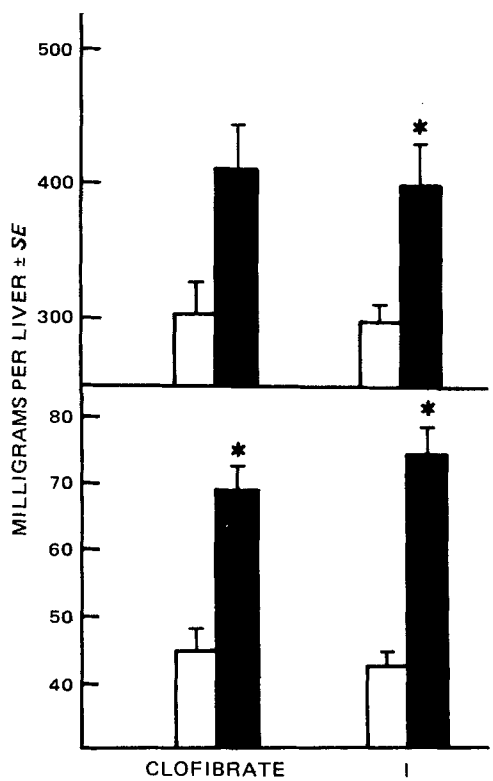


Figure 2—*Effect of chronic administration of hypolipidemic agents on liver mitochondrial protein (top) and phospholipid content (bottom). The significance of the difference between the treated and the control groups, designated by the *, was a p value of <0.05. Key: □, control; and ■, treated.*

[13] Data on file, Hoffmann–La Roche.

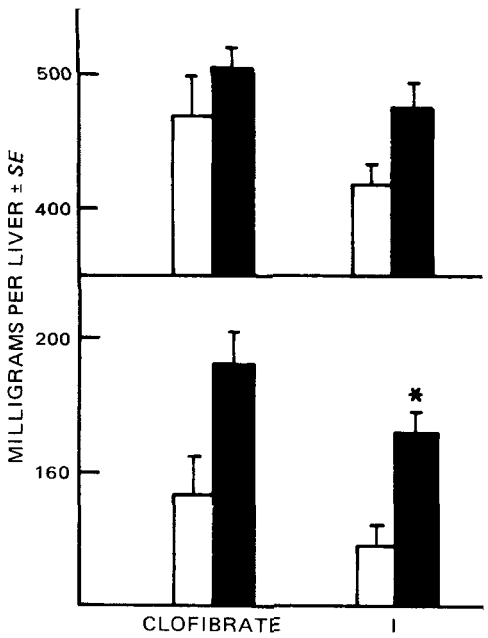


Figure 3—*Effect of chronic administration of antihyperlipidemic drugs on liver microsomal protein (top) and phospholipid content (bottom). The significance of the difference between the treated and the control groups, designated by the *, was a p value of <0.05. Key: □, control; and ■, treated.*

brate or 50 mg/kg of I for 9 days increased rat liver mitochondrial phospholipid and protein content (Fig. 2). A slight, but not statistically significant, increase in liver microsomal protein and phospholipid content was also obtained in rats treated with clofibrate (250 mg/kg/day), and a significant increase in microsomal phospholipid content was found upon treatment with I (50 mg/kg/day) (Fig. 3). Both agents induced liver microsomal drug-metabolizing enzymes, as shown by the ability of these enzymes to *N*-demethylate a test substrate, ethylmorphine (Table VI).

Triparanol, an effective lipid-lowering drug in experimental animals, is known to interfere with cholesterol synthesis by blocking the enzymatic step immediately prior to cholesterol formation, thus leading to an accumulation of desmosterol. During comparative tests, I and clofibrate had only minimal effects on serum desmosterol–cholesterol ratios, whereas serum sterols derived from triparanol-treated rats contained approximately 60% desmosterol (Table VII).

One characteristic finding in animals that have ingested clofibrate is a reversible increase in liver size, accompanied by the appearance of numerous microbodies (peroxisomes) detectable with an electron microscope (18, 19). The enzyme, catalase, is a useful peroxisome marker, since it is a major constituent of peroxisomes. Increased catalase activity in clofibrate-treated rats correlated with an increase in the number of microbodies. An increase in liver size and increased catalase activity were confirmed in clofibrate-treated rats and also found in I-treated rats (Table VIII).

Incubation of I at different concentrations had no effect on the basal rate of glycerol release from rat-isolated fat cells and did not alter the increased rates of glycerol release induced by incubation with optimal concentrations of levarterenol (0.5 μM) or theophylline (0.5 mM) (Table IX).

Table IX—Effect of I on Basal, Levarterenol, and Theophylline-Induced Lipolysis in Isolated Fat Cells

Drug	Lipolysis, μmoles of Glycerol/g of Fat Cells/hr ± *SE*		
	Control	I (1 mM)	I (0.1 mM)
	7.7 ± 0.2	7.8 ± 0.1	7.8 ± 0.8
Levarterenol, 0.5 μM	8.9 ± 0.2	9.6 ± 0.2	9.7 ± 0.1
Theophylline, 0.5 mM	21.5 ± 0.8	22.0 ± 0.5	24.1 ± 1.0

DISCUSSION

Data presented here demonstrate that I is an effective serum lipid-lowering compound in normal and hypertriglyceridemic rats. The effect of I on serum lipids resembles that of clofibrate (20), lifibrate[14] (III) (21), and nafenopin[15] (IV) (22). The chemical structure of I is quite different from III and IV, both of which structurally resemble clofibrate. All four compounds, however, have similar lipid-lowering activities. Serum lipid lowering occurred after a 50-mg/kg po regimen of I, III, or IV, whereas a 250-mg/kg regimen of clofibrate was required for consistent lipid-lowering activity (17). The low toxicity gives I a high therapeutic index so that I might be considered as a possible prophylactic agent for the prevention of hyperlipidemic "risk factor" of atherosclerosis.

Compound I was selected as one of many substituted tetrahydrocarbazoles with hypolipidemic activity (3) and studied in detail. Its analog, 6-chloro-9-[2-(2-methyl-5-pyridyl)ethyl]-1,2,3,4-tetrahydrocarbazole-2-methanol hydrochloride (II), showed similar marked hypolipidemic activity to I in rats[13]. Spectrofluorometric methods for the detection of I and II in biological systems were developed (23) and applied to a biopharmaceutical and pharmacokinetic evaluation of these substituted tetrahydrocarbazoles[16].

Many explanations have been offered for the serum lipid-lowering mechanism of clofibrate. These include an enhancement of thyroid hormone activity produced by competition for binding sites on plasma proteins (24), inhibition of cholesterol (25) and fatty acid (26) biosynthesis, increased hepatic cholesterol oxidation (27), decreased rates of release of hepatic lipoproteins (28), increased peripheral removal of lipoproteins (29), and increased neutral sterol excretion (30). More recently, Dalton *et al.* (17), in attempting to explain the mechanism of action of clofibrate, proposed that the demand for new membrane constituents by the livers of drug-treated animals restricts the production of triglycerides in order to favor that of phospholipids. Thus, the formation and transport of triglyceride-rich lipoproteins into circulating blood are reduced.

The observation that I had only minimal effects on the normal serum cholesterol–desmosterol ratio indicates that the cholesterol-lowering mechanism of action of I does not concern inhibition of the terminal stage of cholesterol biosynthesis. In this regard, it differs from triparanol and is, therefore, unlikely to give rise to an undesirable accumulation of desmosterol after chronic administration. These experiments, however, would not detect an effect of I on earlier stages of cholesterol biosynthesis.

It has been suggested (31, 32) that inhibition of free fatty acid mobilization could be indirectly responsible for serum lipid lowering in experimental animals. Compound I had no direct lipolytic or antilipolytic activity in isolated rat fat cells, indicating that the cholesterol-lowering mechanism of this compound is probably not associated with an inhibition of free fatty acid mobilization. An indirect inhibition of mobilization of free fatty acids was also ruled out, since an acute depletion of free fatty acids was not observed in fasted rats following drug administration.

An increase in liver size and the number of liver microbodies was reported in experimental animals treated with clofibrate (18, 19). Rats treated with I or clofibrate had both increased liver sizes and catalase activities, an enzyme specifically associated with peroxisomes. However, electron microscope studies to detect changes in liver peroxisomal numbers were not completed. In addition, chronic administration of either I or clofibrate caused an accumulation of phospholipids and protein but not of triglycerides or cholesterol in the liver. This result is in agreement with the findings of others with clofibrate (20, 33, 34) and clearly separates this hepatomegalic condition from the pathological types of fatty liver that have high levels of triglyceride and cholesterol esters associated in fat droplets. The increased amounts of phospholipids and proteins are probably associated with membranes of subcellular organelles. Upon cessation of drug administration, the hepatomegaly and ultrastructural changes induced by clofibrate were readily reversible (18, 19, 35).

These data might indicate that the primary event after administration of either clofibrate or I is an induction of enzyme protein resulting from

[14] SaH 42-348, Sandoz Pharmaceuticals, East Hanover, N.J.
[15] Su-13437, Ciba Pharmaceutical Co., Summit, N.J.

[16] S. A. Kaplan, D. E. Maynard, A. Maggio, V. Mattaliano, C. Abruzzo, and S. Cotler, to be published.

a functional enlargement of the liver. The induction of rat liver microsomal enzymes after clofibrate and I treatment is in agreement with a similar observation in rats treated with clofibrate and III (36). Clofibrate and I also increased mitochondrial protein and phospholipid content, effects that are difficult to link with drug metabolism. Our observation, however, is consistent with the increase in number (37) and protein content of the mitochondria (38) produced after clofibrate administration. These intracellular structural changes and the broad influence of clofibrate and related drugs on mitochondrial enzyme activity (39–41) are difficult to explain but may be related to the protein binding capacity of these drugs (33).

REFERENCES

(1) M. F. Oliver, in "Atherosclerosis: Proceedings of the 2nd International Symposium," R. J. Jones, Ed., Springer-Verlag, New York, N.Y., 1970, p. 582.

(2) J. Stamler, in *ibid.*, p. 586.

(3) L. Berger and A. J. Corraz, U.S. pat. 3,862,953 (Jan. 28, 1975).

(4) G. Kessler and H. Lederer, in "Automation in Analytical Chemistry," L. T. Skeggs, Jr., Ed., Mediad, New York, N.Y., 1966, p. 341.

(5) W. Schneider, *J. Biol. Chem.*, **176,** 259 (1948).

(6) W. D. Block, K. J. Jarrett, and J. B. Levine, in "Automation in Analytical Chemistry," L. T. Skeggs, Jr., Ed., Mediad, New York, N.Y., 1966, p. 345.

(7) R. Whitely and H. Alburn, *Ann. N. Y. Acad. Sci.*, **130,** 634 (1965).

(8) C. Dalton and C. Kowalski, *Clin. Chem.*, **13,** 744 (1967).

(9) J. Folch, M. Lees, and G. H. S. Stanley, *J. Biol. Chem.*, **226,** 497 (1957).

(10) C. Dalton and D. DiSalvo, *Technicon Quart.*, **3,** 20 (1972).

(11) J. Axelrod, *J. Pharmacol. Exp. Ther.*, **117,** 322 (1956).

(12) P. C. Barbato, G. R. Umbreit, and R. J. Leibrand, "Applications Laboratory Report 1005," Hewlett-Packard, Avondale, Pa., 1966, p. 1.

(13) M. Rodbell, *J. Biol. Chem.*, **239,** 375 (1964).

(14) J. P. Mallon and C. Dalton, *Anal. Biochem.*, **40,** 174 (1971).

(15) H. Luck, in "Methods of Enzymatic Analysis," H. U. Bergmeyer, Ed., Academic, New York, N.Y., 1965, p. 885.

(16) L. C. Miller and M. L. Tainter, *Proc. Soc. Exp. Biol. Med.*, **57,** 261 (1944).

(17) C. Dalton, W. C. Hope, H. R. Hope, and H. Sheppard, *Biochem. Pharmacol.*, **23,** 685 (1974).

(18) D. Svoboda and D. Azarnoff, *J. Biol. Chem.*, **30,** 442 (1966).

(19) D. Svoboda, H. Grady, and D. Azarnoff, *J. Cell Biol.*, **35,** 127 (1967).

(20) M. M. Best and C. H. Duncan, *J. Lab. Clin. Med.*, **64,** 634 (1964).

(21) A. R. Timms, L. A. Kelly, R. S. Ho, and J. H. Trapold, *Biochem. Pharmacol.*, **18,** 1861 (1969).

(22) R. Hess and W. L. Bencze, *Experientia*, **24,** 418 (1968).

(23) J. A. F. de Silva, N. Strojny, F. Rubio, J. C. Meyer, and B. A. Koechlin, *J. Pharm. Sci.*, **66,** 353 (1977).

(24) J. M. Thorp, *J. Atheroscler. Res.*, **3,** 351 (1963).

(25) D. R. Avoy, E. A. Swyryd, and R. G. Gould, *J. Lipid Res.*, **6,** 369 (1965).

(26) M. E. Maragoudakis, *J. Biol. Chem.*, **244,** 5005 (1969).

(27) D. Kritchevsky and S. A. Tepper, *Fed. Proc.*, **27,** 822 (1968).

(28) R. G. Gould, E. A. Swyryd, D. Avoy, and B. Coan, "Progress in Biochemical Pharmacology," vol. 2, D. Steinberg, Ed., S. Karger, New York, N.Y., 1967, p. 345.

(29) W. G. Ryan and T. B. Schwartz, *J. Lab. Clin. Med.*, **64,** 1001 (1964).

(30) S. M. Grundy, E. A. Ahrens, G. Salen, and E. Quintao, *J. Clin. Invest.*, **48,** 33a (1969).

(31) L. A. Carlson and E. R. Nye, *Acta Med. Scand.*, **179,** 453 (1966).

(32) C. Dalton, C. Kowalski, J. Mallon, and C. Marschhaus, *J. Atheroscler. Res.*, **8,** 265 (1968).

(33) J. M. Thorp and W. S. Waring, *Nature*, **194,** 948 (1962).

(34) R. G. Gould, E. A. Swyryd, B. J. Coan, and D. R. Avoy, *J. Atheroscler. Res.*, **6,** 555 (1966).

(35) R. Hess, W. Straubli, and W. Reiss, *Nature*, **208,** 856 (1965).

(36) R. A. Salvador, S. Haber, S. Atkins, B. W. Gommi, and R. M. Welch, *Life Sci.*, **9,** 397 (1970).

(37) G. E. Paget, *J. Atheroscler. Res.*, **3,** 729 (1963).

(38) C. K. R. Kurup, H. N. Aithal, and T. Ramasarma, *Biochem. J.*, **116,** 773 (1970).

(39) D. S. Platt and B. L. Cockrill, *Biochem. Pharmacol.*, **15,** 927 (1966).

(40) *Ibid.*, **16,** 2257 (1967).

(41) U. Schacht and E. Granzer, *Biochem. Pharmacol.*, **19,** 2963 (1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 1, 1976, from the *Departments of Pharmacology and Toxicology, Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110.*

Accepted for publication May 18, 1976.

The authors thank Dr. L. O. Randall for support, Mr. L. Berger for chemical analogs, and Robert Scism, Diane DiSalvo, William C. Hope, Theordore C. Van Trabert, Janie B. Quinn, Dorothy Hane, and Helen Reich for assistance.

[x] To whom inquiries should be directed.