Antihyperlipidemic Activity of *o*-Chlorobenzylsulfonamide in Rodents

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Abstract □ *o*-Chlorobenzylsulfonamide was observed to be a potent hypolipidemic agent in rodents, reducing serum cholesterol levels 71% and triglyceride levels 49% at 60 mg/kg/d. The agent was effective both in normal and hyperlipidemic mice and was active both orally and intraperitoneally. *o*-Chlorobenzylsulfonamide significantly inhibited phosphatidate phosphohydrolase activity and marginally inhibited *sn*-glycerol-3-phosphate acyl transferase activity both *in vitro* and *in vivo*. The lowering of serum cholesterol levels appeared to be correlated with the accelerated excretion of cholesterol *via* the bile into the feces, with a marginal reduction of cholesterol absorption from the intestine. Reduced concentrations of lipids, *e.g.*, cholesterol, triglycerides, and neutral lipids, were observed in the liver, small intestine, and blood lipoprotein fractions of rats.

Keyphrases □ *o*-Chlorobenzylsulfonamide—antihyperlipidemic activity, rodents, cholesterol, triglycerides □ Antihyperlipidemic agents—potential, *o*-chlorobenzylsulfonamide, activity in rodents, cholesterol, triglycerides

Recently a series of *N*-benzoylsulfamates, *N*-benzylsulfamates, and benzylsulfonamides were shown to be potent hypolipidemic agents in mice (1). These agents were examined for hypolipidemic activity because of their structural resemblance to saccharin, which has been shown to lower serum cholesterol levels 33% and serum triglyceride levels 49% in mice (2) at a dose of 20 mg/kg/d for 16 d. *o*-Chlorobenzylsulfonamide was observed to lower serum cholesterol levels 27% and serum triglyceride levels 48% after dosing for 16 d at 20 mg/kg/d in mice (1). This series of compounds was nonmutagenic and demonstrated no acute toxicity or impaired liver or kidney functions in male mice (1). Since the saccharin moiety has been associated with potential carcinogenicity, and consequently may not be a desirable hypolipidemic agent, we decided to investigate the effects of *o*-chlorobenzylsulfonamide on lipid metabolism to assess the possibility of this compound being a more feasible therapeutic agent.

EXPERIMENTAL

Source of Compound—The sodium salt of *o*-chlorobenzylsulfonamide was prepared as described previously (1).

Antihyperlipidemic Screens in Normal Rodents—The sodium salt of *o*-chlorobenzylsulfonamide was suspended in 1% carboxymethylcellulose–water and administered to male CF_1 mice (~25 g) intraperitoneally for 15 d or male Holtzman rats (~350 g) orally by an intubation needle for 14 d. On days 9 and 14 or 15, blood was obtained by tail vein bleeding, and the serum was separated by centrifugation for 3 min. The serum cholesterol levels were determined by a modification of the Liebermann–Burchard reaction (3). Serum was also collected on day 14 or 15 and the triglyceride content was determined by a commercial kit[1].

Testing in Atherogenic Mice—Male CF_1 mice (~25 g) were placed on a commercial diet[2] which contained butterfat (400 g), cellulose[3] (60 g), cholesterol (53 g), choline dihydrogen citrate (4 g), salt mixture oil[4] (40 g), sodium cholate (20 g), sucrose (223 g), vitamin-free casein (200 g), and total vitamin supplement for 10 d. After the cholesterol and triglyceride levels were assayed and observed to be elevated, the mice were administered test drugs at 20 mg/kg/d ip for an additional 12-d period. Serum cholesterol and triglyceride levels were measured after 12 d of drug administration.

Animal Weights and Food Intake—Periodic animal weights were obtained during the experiments and expressed as a percentage of the animal's weight on day 0. After dosing for 14 d with test drugs, selected organs were excised, trimmed of fat, and weighed.

Toxicity Studies—The acute toxicity (LD_{50} value) (4) was determined in male CF_1 mice by administering test drugs intraperitoneally from 100 mg to 2 g/kg as a single dose. The number of deaths were recorded over a 7-d period for each group.

Enzymatic Studies—*In vitro* enzymatic studies were determined using 10% homogenates of male CF_1 mouse liver with 2.5–10 μmol of the test drugs. *In vivo* enzymatic studies were determined using 10% homogenates of liver from male CF_1 mice obtained after administering the agents for 15 d at a dose ranging from 10–60 mg/kg/d ip. The liver homogenates for both *in vitro* and *in vivo* studies were prepared in 0.25 mM EDTA [(ethylenedinitrilo)tetraacetic acid].

Acetyl-CoA synthetase (5) and adenosine triphosphate-dependent citrate lyase (6) activities were determined spectrophotometrically at 540 nm as the hydroxamate of acetyl CoA formed after 30 min at 37°C. Mitochondrial citrate exchange was determined by the procedure of Robinson *et al.* (7, 8) using sodium [^{14}C]bicarbonate (41 mCi/mmol) incorporated into mitochondrial [^{14}C]citrate after isolating rat mitochondria (9000×*g* for 10 min) from the homogenates. The exchange of the [^{14}C]citrate was determined after incubating the mitochondrial fraction, which was loaded with labeled citrate and test drugs, for 10 min. Radioactivity was measured in the mitochondrial and supernatant fractions in scintillation fluid[5] and expressed as a percentage. Cholesterol side-chain oxidation was determined by the method of Kritchevsky and Tepper (9) using [26-^{14}C]cholesterol (50 mCi/mmol) and mitochondria isolated from rat liver homogenates. After an 18-h incubation at 37°C with the test drugs, the generated $^{14}CO_2$ was trapped in the center well in {2-[2-(*p*-1,1,3,3-tetramethylbutylcresoxy)ethoxy]ethyl}dimethylbenzylammonium hydroxide[6] and counted[5]. 3-Hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase) was measured using [1-^{14}C]acetate (56 mCi/mmol) and a postmitochondrial supernatant (9000×*g* for 20 min) incubated for 60 min at 37°C (10). The digitonide derivative of cholesterol was isolated and counted (11). Acetyl-CoA carboxylase activity was measured by the method of Greenspan and Lowenstein (12). Initially, the subunits of the enzyme had to be polymerized for 30 min at 37°C, and then the assay mixture containing sodium [^{14}C]bicarbonate (41.0 mCi/mmol) was added and incubated for 30 min at 37°C with test drugs. Fatty acid synthetase activity was determined by the method of Brady *et al.* (13) using [2-^{14}C]malonyl CoA (37.5 mCi/mmol), which was incorporated into newly synthesized fatty acids that were extracted with ether and counted[5]. *sn*-Glycerol-3-phosphate acyl transferase activity was determined with glycerol-3-phosphate [2-^{3}H] (7.1 Ci/mmol) and the microsomal fraction of the liver homogenates (14). The reaction was terminated after 10 min, and the lipids were extracted with chloroform–methanol (1:2) containing 1% concentrated HCl and counted. Phosphatidate phosphohydrolase activity was measured as the inorganic phosphate released after 30 min from phosphatidic acid by the method of Mavis *et al.* (15). The released inorganic phosphate after development with ascorbic acid and ammonium molybdate was determined at 820 nm.

Liver, Small Intestine and Fecal Lipid Extraction—In male CF_1 mice that had been administered test drugs for 15 d, the liver, small intestine, and fecal materials (24-h collection) were removed and a 10%

[1] Hycel Triglyceride Test Kit; Fisher Scientific Co.
[2] Basal Atherogenic Test Diet; U.S. Biochemical Corp.
[3] Celufil.
[4] Wesson.

[5] Fisher Scintiverse in a Packard Scintillation Counter.
[6] Hyamine Hydroxide; New England Nuclear.

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Table I—Effects of *o*-Chlorobenzylsulfonamide on Serum Cholesterol and Triglycerides in Mice and Rats [a]

Compound	Mouse [b]			Rat [c]		
	Serum Cholesterol		Serum Triglyceride	Serum Cholesterol		Serum Triglyceride
	Day 9	Day 15	Day 15	Day 9	Day 14	Day 14
Control (1% Carboxymethylcellulose)	100 ± 5[d]	100 ± 6[e]	100 ± 6[f]	100 ± 9[g]	100 ± 7[h]	100 ± 8[i]
o-Chlorobenzylsulfonamide						
10 mg/kg	77 ± 4[j]	70 ± 2[j]	89 ± 6	—	—	—
20 mg/kg	90 ± 3[k]	70 ± 6[j]	48 ± 3[j]	84 ± 7	71 ± 6[j]	51 ± 6[j]
40 mg/kg	86 ± 4[j]	62 ± 3[j]	45 ± 4[j]	—	—	—
60 mg/kg	70 ± 3[j]	56 ± 5[j]	29 ± 2	—	—	—

[a] Expressed as percentage of control (mean ± *SD*). [b] Administered intraperitoneally as a suspension of the sodium salt in 1% carboxymethylcellulose. [c] Administered orally as a suspension of the sodium salt in 1% carboxymethylcellulose. [d] 125 mg%. [e] 122 mg%. [f] 137 mg/dL. [g] 75 mg%. [h] 78 mg%. [i] 110 mg/dL. [j] $p \leq 0.001$. [k] $p \leq 0.005$.

Table II—*In Vitro* Enzymatic Inhibition by *o*-Chlorobenzylsulfonamide of Liver Lipid-Synthesized Enzymes [a]

	Inhibition % of Control			
	Control	50 μM	100 μM	200 μM
Mitochondrial citrate exchange	100 ± 10[b]	88 ± 3	88 ± 5	87 ± 3
Citrate lyase	100 ± 9[c]	100 ± 8	100 ± 7	100 ± 4
Acetyl-CoA synthetase	100 ± 8[d]	100 ± 9	100 ± 8	100 ± 6
HMG-CoA reductase	100 ± 7[e]	80 ± 7	80 ± 6[k]	100 ± 8
Cholesterol side-chain oxidation	100 ± 8[f]	—	58 ± 5[k]	—
Acetyl-CoA carboxylase	100 ± 6[g]	70 ± 6[k]	86 ± 5	103 ± 7
Fatty acid synthetase	100 ± 7[h]	76 ± 5[k]	80 ± 6[k]	70 ± 7[k]
Phosphatidate phosphohydrolase	100 ± 7[i]	22 ± 3[k]	22 ± 5[k]	24 ± 4[k]
Acyl transferase	100 ± 8[j]	78 ± 6[k]	73 ± 7[k]	61 ± 5[k]

[a] Mean ± *SD*; the compound was administered as a suspension of the sodium salt in 1% carboxymethylcellulose. [b] 30.8 ± 3.1 mg% exchange in mitochondrial citrate. [c] 30.5 ± 2.74 mg if citrate hydrolyzed/g of wet tissue/30 min. [d] 28.5 ± 3.14 mg of acetyl CoA formed/g of wet tissue/30 min. [e] 384,900 ± 26,943 dpm of cholesterol formed/g of wet tissue/60 min. [f] 6980 ± 558 dpm of CO_2 formed/g of wet tissue/18 h. [g] 32,010 ± 1921 dpm/g of wet tissue/30 min. [h] 37,656 ± 2635 dpm/g of wet tissue/30 min. [i] 16.70 ± 1.16 μg Pi/g of wet tissue/15 min. [j] 537,800 ± 43,024 dpm of triglyceride formed/g of wet tissue/10 min. [k] $p \leq 0.001$.

homogenate in 0.25 M sucrose plus 0.001 M EDTA was prepared. A 2-mL aliquot of the homogenate was extracted by the methods of Folch *et al.* (16) and Bligh and Dyer (17) and the milligrams of lipid were determined. The lipid was taken up in methylene chloride and the cholesterol level (3), triglyceride levels[7], neutral lipid content (18), and phospholipid content (19) were determined.

[^{3}H]Cholesterol Distribution in Rats—Male Holtzman rats (~350 g) were administered test agents orally for 14 d. On day 13, 10 μCi of [^{3}H]cholesterol was administered intraperitoneally to mice and orally to rats, and feces were collected after 24 h. Twenty-four hours after cholesterol administration, the major organs were excised and samples of blood, chyme, and urine were obtained. Homogenates (10%) of the tissues were prepared, combusted[8], and counted[5]. Some tissue samples were plated on filter paper[9], dried, and digested for 24 h in base[6] at 40°C and counted[5]. Results were expressed as dpm per total organ.

Cholesterol Absorption Study—Male Holtzman rats (~400 g) were administered test drug intraperitoneally for 14 d at 20 mg/kg/d. On day 13, 10 μCi of [1,2-^{3}H]cholesterol (40.7 Ci/mmol) was administered to the rat orally. Twenty-four hours later, blood was collected and the serum separated by centrifugation (20). Both the serum and the precipitate were counted[5].

Bile Cannulation Study—Male Holtzman rats (~400 g) were treated with test drugs at 20 mg/kg/d orally for 14 d. The rats were anesthetized with chlorpromazine[10] at 25 mg/kg followed after 30 min by pentobarbital[11] (22 mg/kg ip). The duodenum of the small intestine was isolated and ligatures were placed around the pyloric sphincter and distally to a site approximately one-third of the way down the duodenum. Sterile isotonic saline was injected into the sectioned off duodenal segment. The saline expanded the duodenum and the common bile duct. Once the bile duct was identified, a loose ligature was placed around it, an incision was made, and plastic tubing[12] was introduced into the duct. Once past the ligature, the tubing was tied in place, and the ligatures around the duodenum were removed. Once bile was freely moving down the cannulated tube, [1,2-^{3}H]cholesterol (40.7 mCi/mmol) was injected subcutaneously into the rats. The bile was collected over the next 6 h and measured (in milliliters). Aliquots were counted[5] as well as analyzed for cholesterol content (3).

[7] Bio-Dynamics/bmc Triglyceride Kit.
[8] Packard Tissue Oxidizer.
[9] Whatman No. 1.
[10] Thorazine, chlorpromazine hydrochloride; Smith, Kline & French Laboratories.
[11] Nembutal, sodium pentobarbital; Abbott Laboratories.
[12] PE-10 Intramedic polyethylene tubing.

Plasma Lipoprotein Fractions—Male Holtzman rats (~400 g) were administered test drugs at 20 mg/kg/d for 14 d. On day 14, blood was collected from the abdominal aorta. Serum was separated from whole blood by centrifugation at 3500 rpm. Aliquots (3 mL) were separated by density gradient ultracentrifugation into the chylomicrons, very low-density lipoproteins, high-density lipoproteins, and low-density lipoproteins according to the methods of Hatch and Lees (21) and Havel *et al.* (22). Each of the fractions was analyzed for cholesterol (3), triglyceride[7], neutral lipids (18), phospholipids (19), and protein levels (23).

RESULTS

Data in the tables are expressed as the mean percent of control ± the standard deviation. The probable significant level (p) between each test group and the control group was determined by the Student's t test.

o-Chlorobenzylsulfonamide was found to effectively lower serum cholesterol and triglyceride levels in rats and mice (Table I). In mice, the agent lowered serum cholesterol levels after intraperitoneal administration in a dose-dependent manner with 60 mg/kg/d affording 44% reduction, the maximum effect observed. Serum triglyceride levels were also reduced in a dose-dependent manner, with 60 mg/kg/d resulting in 71% reduction on day 15. After 14 d of oral administration at 20 mg/kg/d to rats, *o*-chlorobenzylsulfonamide reduced serum cholesterol levels 29% and serum triglyceride levels 49%, comparable to the same reductions observed in mice at 20 mg/kg/d. Subsequent to inducing a hyperlipidemic state in mice, in which case serum cholesterol levels were elevated 183% (354 mg%) above normal control values (125 mg%), the agent reduced serum cholesterol levels to 76% (220 mg%) above normal serum cholesterol levels. Serum triglyceride levels were elevated 168% (367 mg/dL) above the control value (137 mg/dL) by the high lipid diet; however, drug administration reduced the serum triglyceride levels to 40% (192 mg/dL) above control triglyceride values. Whereas the agent did not lower serum lipids absolutely to the normal value in male CF_1 mice, a significant reversal of the induced hyperlipidemic state was observed by drug administration for 12 d. The LD_{50} value for the agent was 400 mg/kg.

In vitro mouse liver enzymatic studies (Table II), with *o*-chlorobenzylsulfonamide at 50–200 μM final concentration, showed that citrate lyase and acetyl CoA synthetase activities were not inhibited by the agent. Mitochondrial citrate exchange and HMG-CoA reductase activity were marginally inhibited by the agent (0–20%). Acetyl-CoA carboxylase activity was reduced 30% at 50 μM, and fatty acid synthetase was inhibited 20–30%. *sn*-Glycerol-3-phosphate acyl transferase activity was reduced in a dose-dependent manner, with 200 μM affording 39% inhibition. Phosphatidate phosphohydrolase activity was markedly inhibited

Table III—Effects of *o*-Chlorobenzylsulfonamide on *In Vivo* CF_1 Mouse Liver Enzyme Activities Involved in Lipid Synthesis After 15 d of Intraperitoneal Administration [a]

Compound	Citrate Lyase	Acetyl-CoA Synthetase	HMG-CoA Reductase
Control (1% Carboxymethylcellulose)	100 ± 10	100 ± 8	100 ± 8
o-Chlorobenzylsulfonamide			
10 mg/kg	90 ± 6	84 ± 7	103 ± 3
20 mg/kg	92 ± 4	85 ± 6	101 ± 5
40 mg/kg	96 ± 4	78 ± 7[b]	101 ± 8
60 mg/kg	97 ± 7	100 ± 9	97 ± 6

	Acetyl-CoA Carboxylase	Fatty Acid Synthetase	*sn*-Glycerol-3-P Acyl Transferase	Phosphatidate Phosphohydrolase
Control (1% Carboxymethylcellulose)	100 ± 7	100 ± 8	100 ± 6	100 ± 7
o-Chlorobenzylsulfonamide				
10 mg/kg	85 ± 9	94 ± 7	78 ± 7[b]	54 ± 5[b]
20 mg/kg	87 ± 4[c]	92 ± 7	73 ± 4[b]	32 ± 4[b]
40 mg/kg	93 ± 3	77 ± 6[b]	77 ± 5[b]	28 ± 2[b]
60 mg/kg	93 ± 5	87 ± 8	90 ± 6	28 ± 3[b]

[a] Expressed as percentage of control (mean ± *SD*). The compound was administered as a suspension of the sodium salt in 1% carboxymethylcellulose. [b] $p \leq 0.001$. [c] $p \leq 0.005$.

Table IV—Effects of *o*-Chlorobenzylsulfonamide on Lipid Distribution of Rat Organs After 14 d of Administration at 20 mg/kg [a]

	Amount of Lipid, mg	Cholesterol	Neutral Lipids	Triglycerides	Phospholipids	Protein
Liver						
Control	100 ± 6	100 ± 7[b]	100 ± 4[c]	100 ± 5[d]	100 ± 8[e]	100 ± 8[f]
Treated	87 + 3	73 ± 4*	55 ± 5*	64 ± 4*	84 ± 4**	102 ± 6
Small Intestine						
Control	100 ± 5	100 ± 7[g]	100 ± 5[h]	100 ± 6[i]	100 ± 8[j]	100 ± 5[k]
Treated	31 ± 4*	40 ± 3*	41 ± 6*	20 ± 3*	101 ± 7	84 ± 6
Feces						
Control	100 ± 8	100 ± 8[l]	100 ± 6[m]	100 ± 7[n]	100 ± 5[o]	100 ± 7[p]
Treated	70 ± 6	116 ± 9	77 ± 5*	89 ± 5	165 ± 9*	96 ± 6
Blood Lipoproteins						
Chylomicrons						
Control		100 ± 9[q]	100 ± 8[r]	100 ± 6[s]	100 ± 10[t]	100 ± 7[u]
Treated		71 ± 6*	48 ± 4*	55 ± 6*	102 ± 6	101 ± 6
Very low-density lipoprotein						
Control		100 ± 8[v]	100 ± 9[w]	100 ± 7[x]	100 ± 8[y]	100 ± 8[z]
Treated		100 ± 9	49 ± 5*	37 ± 3*	122 ± 3*	87 ± 8
Low-density lipoprotein						
Control		100 ± 9[aa]	100 ± 7[bb]	100 ± 8[cc]	100 ± 7[dd]	100 ± 8[ee]
Treated		85 ± 7	46 ± 6*	86 ± 4	189 ± 11*	100 ± 6
High-density lipoprotein						
Control		100 ± 8[ff]	100 ± 9[gg]	100 ± 4[hh]	100 ± 6[ii]	100 ± 8[jj]
Treated		73 ± 5*	43 ± 2*	52 ± 5*	126 ± 7*	100 ± 6
Rat Bile						
Control		100 ± 7[kk]	100 ± 4[ll]	100 ± 6[mm]	100 ± 4[nn]	100 ± 8[oo]
Treated		129 ± 5*	108 ± 3**	126 ± 7*	122 ± 8*	166 ± 12*

[a] Expressed as percentage of control (mean ± *SD*); asterisks indicate statistical significance at $p \leq 0.001$ double asterisks at $p \leq 0.010$. The compound was administered as a suspension of the sodium salt in 1% carboxymethylcellulose. [b] 24.03 mg/g. [c] 44.11 mg/g. [d] 6.37 mg/g. [e] 7.19 mg/g. [f] 4.5 mg/g. [g] 7.82 mg/g. [h] 6.98 mg/g. [i] 1.12 mg/g. [j] 2.06 mg/g. [k] 42 mg/g. [l] 28.47 mg/g. [m] 33.94 mg/g. [n] 1.86 mg/g. [o] 1.39 mg/g. [p] 6.99 mg/g. [q] 337 mg/mL. [r] 67 mg/mL. [s] 420 mg/mL. [t] 149 mg/mL. [u] 3 mg/mL. [v] 190 mg/mL. [w] 98 mg/mL. [x] 22 mg/mL. [y] 26 mg/mL. [z] 50 mg/mL. [aa] 210 mg/mL. [bb] 10 mg/mL. [cc] 45 mg/mL. [dd] 41 mg/mL. [ee] 0.681 mg/mL. [ff] 544 mg/mL. [gg] 620 mg/mL. [hh] 27 mg/mL. [ii] 153 mg/mL. [jj] 5.677 mg/mL. [kk] 118 mg%. [ll] 5 mg/dL. [mm] 170 mg/mL. [nn] 1.75 mg/mL. [oo] 1.29 mg/ml.

(76–78%) by *o*-chlorobenzylsulfonamide from 50–200 μM. Cholesterol side-chain oxidation was reduced 52% at 100 μM.

In vivo enzymatic studies (Table III) in mice showed similar patterns of inhibition by *o*-chlorobenzylsulfonamide. Citrate lyase, HMG CoA reductase, and acetyl CoA carboxylase activities were essentially unaffected by drug administration. Acetyl-CoA synthetase and fatty acid synthetase activities were inhibited 22 and 23%, respectively, at 40 mg/kg/d after 15 d of drug administration. Acyl transferase activity was inhibited 22–27% at doses from 20–60 mg/kg/d.

Phosphatidate phosphohydrolase activity was inhibited 72% at 10 and 20 mg/kg/d, 68% at 40 mg/kg/d, and 46% at 60 mg/kg/d. The inhibition of phosphatidate phosphohydrolase activity was of a magnitude to account for the degree of reduction of serum triglyceride levels observed after *o*-chlorobenzylsulfonamide treatment. However, the enzymes involved in cholesterol synthesis were essentially unaffected by the agent. Thus, it seemed important to examine the effect of the agent on cholesterol distribution and excretion (Tables IV and V).

o-Chlorobenzylsulfonamide markedly accelerates the movement of cholesterol through the normal excretion routes, *i.e.*, high values of [^{3}H]cholesterol content were observed in fecal and urine collections. Both kidney and urine showed increases (16% and 40%, respectively) of [^{3}H]-cholesterol content after drug administration. The small intestine (115%), large intestine (71%), chyme (77%), and feces (185%) all demonstrated increases of [^{3}H]cholesterol content in the respective organs above that of the control. Studies of the actual lipid content of the liver and small intestine of the rats showed a decrease in cholesterol, triglycerides, and neutral lipid levels compared with the control. The reduction of small intestine lipids was greater than that observed in liver. Feces showed an increase in cholesterol and phospholipid levels, but a reduction in triglyceride and neutral lipid levels. The bile cholesterol, triglyceride, and phospholipid levels were increased, with neutral lipid levels being affected the least. Marginal inhibition (20%) by the agent of cholesterol absorption from the intestine after oral administration of [^{3}H]cholesterol was observed.

The lipoprotein fractions were also examined for cholesterol content, which was lower in the chylomicron, low-density, and high-density fractions. All four fractions were reduced in triglyceride and neutral lipid content; however, phospholipid content was elevated in all four fractions after drug administration. Protein content of the lipoprotein fractions was not altered by drug administration.

Table V—Rat Organ Weights and Radioactivity Distribution of 10 μCi of [^{3}H]Cholesterol After 14 d of Dosing with *o*-Chlorobenzylsulfonamide at 20 mg/kg/d[a]

	Organ Weight		dpm/Total Organ	
	Control	Treated	Control	Treated
Liver	14.466	12.667	47429	46375
Brain	1.966	1.966	5877	5292
Spleen	0.667	0.767	2315	6339
Lung	2.600	1.700	7069	7073
Kidney	3.367	3.134	8170	9482
Heart	1.334	1.200	4004	4338
Stomach	2.967	2.433	11375	7077
Small intestine	10.167	6.333	41656	89703
Large intestine	4.100	3.566	14104	24116
Chyme	2.567	7.583	9226	16309
Feces	2.634	5.033	8349	23779
			dpm/mL	
Urine			281	393
Bile			1016	1257
Serum			648	523

[a] Administered as a suspension of the sodium salt in 1% carboxymethylcellulose.

DISCUSSION

o-Chlorobenzylsulfonamide appears to be active in a safe therapeutic range. Whereas it was more effective in lowering serum triglycerides, significant effects were also observed for serum cholesterol. The agent was active orally and intraperitoneally in both normal and hyperlipidemic mice and was shown to be a more potent hypolipidemic agent than clofibrate. Clofibrate at 150–200 mg/kg lowers rat cholesterol levels 15% maximally and serum triglyceride levels 25% (24). *o*-Chlorobenzylsulfonamide did not suppress *de novo* cholesterol synthesis in a manner similar to clofibrate at HMG CoA reductase, the regulatory site. However, like clofibrate, *o*-chlorobenzylsulfonamide did accelerate cholesterol excretion *via* the bile and feces. The lipids removed from the blood compartment were not deposited in the major organs. This can be observed from three different types of studies: (*a*) there was no increase in organ weight, (*b*) lipid content of the organs examined was reduced, and (*c*) the [^{3}H]cholesterol distribution studies showed lower content in the treated animals than the normal tissue.

The reduction of serum triglyceride levels can be explained by the observed reduction of phosphatidate phosphohydrolase and *sn*-glycerol-3-phosphate acyl transferase activities by *o*-chlorobenzylsulfonamide. There appears to be a positive correlation between the two events, since clofibrate (14), saccharin (2), and phthalimide (25) all reduce the activity of these two enzymes and reduce serum triglyceride levels in rodents. Both enzymes play a major regulatory role in the synthesis of triglycerides.

The effects of *o*-chlorobenzylsulfonamide on the lipid content of lipoprotein fractions were varied. Nevertheless, a marked reduction of triglyceride content of chylomicrons and very low-density lipoproteins (*i.e.*, the lipoproteins which have the highest triglyceride content), as well as minor reduction of low- and high-density lipoprotein triglyceride content, were observed after drug treatment. Cholesterol content was marginally reduced (29–15%) in the chylomicron and high-density fractions. Whereas it is difficult to extrapolate observations of lipid reduction of rat lipoprotein fractions by an agent to human lipoproteins, these studies do indicate that *o*-chlorobenzylsulfonamide has the capability to modulate lipid content of lipoprotein fractions and may have the potential to affect the lipoprotein lipid levels of humans.

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