The Hypolipidemic Activity of N-(p-Chlorobenzoyl)-sulfamate in Rodents and its Effect on Lipid Metabolism

I. H. Hall[1], S. D. Wyrick[1], P. J. Voorstad[1], and A. Dubey[2]

Received: April 2, 1983; accepted: July 8, 1983.

Abstract: N-(p-Chlorobenzoyl)-sulfamate was observed to produce potent hypolipidemic activity in rodents at 10 to 60 mg/kg/day. Liver phosphatidate phosphohydrolase and *sn*-glycerol-3-phosphate acyl transferase activities were suppressed by the agent *in vitro* and *in vivo,* and ATP dependent citrate lyase and acetyl CoA carboxylase activities were reduced *in vivo.* N-(p-Chlorobenzoyl)-sulfamate reduced cholesterol, neutral lipids, and triglycerides in the liver and increased excretion of cholesterol and neutral lipids in the bile and feces. Drug treatment reduced the neutral lipid and triglyceride content of serum chylomicrons, VLDL and LDL, and cholesterol content was reduced in the chylomicron and HDL fractions.

Introduction

Recently, a series of aryl substituted N-benzoyl- and N-benzylsulfamic acid sodium salts and benzylsulfonamide sodium salts were synthesized and shown to possess hypolipidemic (1, 2) activity in rodents. The compounds structurally resemble saccharin (2), except that the cyclic imide ring of saccharin has been opened. Saccharin has been observed to lower serum cholesterol and triglyceride levels significantly in rodents (2). N-(p-Chlorobenzoyl)-sulfamic acid sodium salt at 20 mg/kg/day, i.p., lowered serum cholesterol levels 48 % and serum triglyceride levels 45 % after 16 days dosing (1). The agents were observed to be negative in the Ames test, demonstrated no acute toxicity or impaired liver and kidney function, and the compounds appeared chemically stable. Thus, an investigation of the effects of N-(p-chlorobenzoyl)-sulfamate on lipid synthesis and distribution was undertaken.

Methods and Procedure

Source of Compound

As previously reported, N-(p-chlorobenzoyl)-sulfamic acid sodium salt was prepared by the sulfonation of p-chlorobenzamide with pyridine sulfurtrioxide complex in pyridine (1). The product was isolated as the ammonium salt followed by conversion to the sodium salt monohydrate by ion exchange chromatography.

[1] Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514
[2] Chemistry and Life Science Group, Research Triangle Institute, Research Triangle Park, NC 27709

Hypolipidemic Activity in Normal Rodents

N-(p-Chlorobenzoyl)-sulfamate sodium salt was suspended in 1 % carboxymethylcellulose-water and administered to CF_1 male mice (~ 25 g) intraperitoneally for 15 days, or to Holtzman male rats (~ 350 g) orally by an intubation needle for 14 days. On days 9 and 14–15, blood was obtained by tail vein bleeding and the serum 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 14–15, and the triglyceride content was determined by a commercial kit (Fisher, Hycel Triglyceride Test Kit).

Testing in Hyperlipidemic Mice

CF_1 male mice (~ 25 g) were placed on a commercial diet (U.S. Biochemical Corporation Basal Atherogenic Test Diet) which contained butterfat (400 g), celufil (cellulose) (60 g), cholesterol (53 g), choline dihydrogen citrate (4 g), salt mixture oil (Wesson) (40 g), sodium cholate (20 g), sucrose (223 g), vitamin free casein (200 g), and total vitamin supplement for 10 days. After the cholesterol and triglyceride levels were assayed and observed to be elevated, the mice were administered test drug at 20 mg/kg/day, intraperitoneally for an additional 12-day period. Serum cholesterol and triglyceride levels were measured after 12 days of administration of the drug.

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 days with test drug, selected organs were excised, trimmed of fat and weighed.

Toxicity Studies

The acute toxicity (LD_{50} values) (4) was determined in CF_1 male 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-day period for each group.

Enzymatic Studies

In vitro enzymatic studies were determined using 10 % homogenates of CF_1 male mouse liver with 2.5 to 10 μmoles of test drug. *In vivo* enzymatic studies were determined using 10 % homogenates of liver from CF_1 male mice obtained after administering the agent for 15 days at a dose ranging from 10 to 60 mg/kg/day intraperitoneally. The liver homogenates

for both *in vitro* and *in vivo* studies were prepared in 0.25 M sucrose + 0.001 M EDTA (ethylenedinitrilo-tetraacetic acid). Acetyl coenzyme A synthetase (5) and adenosine triphosphate dependent citrate lyase (6) activities were determined spectrophotometrically at 540 nm as the hydroxamate of acetyl coenzyme A formed after 30 min at 37° C. Mitochondrial citrate exchange was determined by the procedure of Robinson *et al.* (7, 8) using ^{14}C sodium bicarbonate (41 mCi/mmol) incorporated into mitochondrial ^{14}C-citrate after isolating rat mitochondria (9000 g x 10 min) from the homogenates. The exchanges of the ^{14}C-citrate were determined after incubating the mitochondrial fraction which was loaded with labeled citrate and test drug for 10 min. Then the radioactivity was measured in the mitochondrial and supernatant fractions in scintillation fluid (Fisher Scintiverse in a Packard Scintillation Counter) and expressed as a percentage of ^{14}C-citrate exchange. 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 18 h incubation at 37° C with 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 (Hyamine Hydroxide, New England Nuclear) and counted. Cholesterol biosynthesis was measured using 1-^{14}C-acetate (56 mCi/mmol) and a postmitochondrial supernatant (9000 g x 20 min) incubated for 60 min at 37° C (10). The digitonide derivative of cholesterol was isolated and counted (11). Acetyl coenzyme A carboxylase activity was measured by the method of Greenspan and Lowenstein (12). Initially, the enzyme had to be polymerized for 30 min at 37° C; 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-coenzyme A (37.5 mCi/mmol) which was incorporated into newly synthesized fatty acids that were extracted with ether and assayed for ^{14}C content. sn-Glycerol-3-phosphate acyl transferase activity was determined with glycerol-3-phosphate [L-2-$^{3}H(N)$] (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 % conc. HCl and assayed for ^{3}H content. 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 CF_1 male mice that had been administered test drugs for 15 days, the liver, small intestine, and fecal materials (24 h collection) were removed and a 10 % homogenate in 0.25 M sucrose + 0.001 M (ethylendinitrilo)tetraacetic acid was prepared. An aliquot (2 ml.) of the homogenate was extracted by the methods of Folch *et al.* (16) and Bligh and Dyer (17), and the lipid was weighed. The lipid was taken up in methylene chloride and the cholesterol level (3), triglyceride levels (Bio-Dynamics/bmc Triglyceride Kit), neutral lipid content (18), and phospholipid content (19) were determined.

^{3}H-Cholesterol Distribution in Rats

Holtzman male rats (~ 350 g) were administered test agent for 14 days orally. On day 13, 10 μCi ^{3}H-cholesterol was administered intraperitoneally in mice and orally in 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 %) were prepared of the tissues which were combusted in a Packard Tissue Oxidizer and assayed for ^{3}H content. Some tissue samples were plated on Whatman filter paper # 1, dried and digested for 24 h in Hyamine Hydroxide (New England Nuclear) at 40° C. Results were expressed as dpm per total organ.

Cholesterol Absorption Study

Holtzman male rats (~ 400 g) were administered test drug orally for 14 days at 20 mg/kg/day. On day 13, 10 μCi of 1,2-$^{3}H(N)$-cholesterol (40.7 Ci/mmole) was administered to the rat orally. Twenty-four hours later, the blood was collected and the serum separated by centrifugation (20). Both the serum and the precipitate were assayed for ^{3}H content.

Bile Cannulation Study

Holtzman male rats (~ 400 g) were treated with test drugs at 20 mg/kg/day orally for 14 days. The control and treated rats were anesthetized with chlorpromazine · HCl 25 mg/kg followed in 30 min by sodium pentobarbital (22 mg/kg) intraperitoneally. The duodenum section of the small intestine was isolated, 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 ligated duodenum 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 made and plastic tubing (PE-10 Intramedia Polyethylene Tubing) 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-$^{3}H(N)$-cholesterol (40.7 Ci/mmol) was injected intravenously into the rats. The bile was collected over the next 6 h and measured (ml). Aliquots were assayed for ^{3}H content and cholesterol content (3).

Plasma Lipoprotein Fractions

Holtzman male rats (~ 400 g) were administered test drugs at 20 mg/kg/day orally for 14 days. 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 according to the methods of Hatch and Lees (21) and Havel *et al.* (22) into the chylomicrons, very low density lipoproteins, high density lipoproteins and low density lipoproteins. Each of the fractions was analyzed for cholesterol (3), triglyceride (Biodynamics/bmc Triglyceride Kit), neutral lipids (18), phospholipids (19), and protein levels (23).

Data are expressed in the Tables I–V as 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.

Results

N-(p-Chlorobenzoyl)-sulfamate was shown to be an effective hypolipidemic agent. Serum cholesterol levels of mice were reduced optimally at 20 mg/kg/day after 15 days by 47 %. Serum triglyceride levels were lowered 54 % at 20 mg/kg and 61 % at 40 mg/kg/day. The serum lipid levels of rats were

Table I. The Effect of N-(p-Chlorobenzoyl)-sulfamate on Serum Cholesterol and Triglycerides in Mice and Rats.

	Mouse (I.P.)			% Control	Rat (Orally)	
Compound (N = 6)	**Serum Cholesterol**		**Serum Triglyceride**	**Serum Cholesterol**		**Serum Triglyceride**
N-(p-Chlorobenzoyl)-sulfamate	**9th X ± S.D.**	**15th Day X ± S.D.**	**15th Day X ± S.D.**	**9th Day X ± S.D.**	**14th Day X ± S.D.**	**14th Day X ± S.D.**
10 mg/kg	78 ± 6[a]	69 ± 5[a]	66 ± 6[a]	—	—	—
20 mg/kg	69 ± 6[a]	53 ± 6[a]	46 ± 5[a]	82 ± 8	66 ± 6[a]	60 ± 7[a]
40 mg/kg	64 ± 5[a]	62 ± 3[a]	39 ± 5[a]	—	—	—
60 mg/kg	61 ± 2[a]	75 ± 6[a]	62 ± 7[a]	—	—	—
Clofibrate 150 mg/kg	88 ± 7	87 ± 5	75 ± 5	—	—	—
1% Carboxymethylcellulose	100 ± 6 (125 mg%)	100 ± 7 (122 mg%)	100 ± 6 (137 mg/dl)	100 ± 7 (75 mg%)	100 ± 6 (78 mg%)	100 ± 6 110 mg/dl)

[a] $p \leq 0.001$

Table II. In Vitro Inhibition by N-(p-Chlorobenzoyl)-sulfamate of Liver Enzyme Activities.

		Percent Control			Standard Agents				
Enzyme Assay (N = 6)	**Control**	**μM Concentration of Drug N-(p-Chlorobenzoyl) sulfamate**			**Aceto-zamide**	**Saccharin**	**Clofibrate**	**1, 3, 5-benzene-tricarb-oxylicacid**	**Tere-phtalic**
	X̄ ± S.D.	**50 μM X̄ ± S.D.**	**100 μM X̄ ± S.D.**	**200 μM X̄ ± S.D.**	**50 μM X̄ ± S.D.**	**50 μM X̄ ± S.D.**	**100μM X̄ ± S.D.**	**100 μM X̄ ± S.D.**	**100 μM X̄ ± S.D.**
Mitochondrial Citrate Exchange	100 ± 10[c]	86 ± 8	90 ± 5	90 ± 4	—	7.5	—	42 ± 4	—
Citrate Lyase	100 ± 9[d]	100 ± 9	100 ± 8	99 ± 9	66 ± 7	65 ± 6	—	—	—
Acetyl CoA Synthetase	100 ± 8[e]	96 ± 8	80 ± 7[b]	74 ± 6a	66 ± 4	61 ± 7	—	—	—
Cholesterol Biosynthesis	100 ± 7[f]	118 ± 9	117 ± 11	108 ± 7	—	71 ± 7	85 ± 4	—	—
Cholesterol Side Chain Oxidation	100 ± 8[g]	—	68 ± 6[a]	—	—	94 ± 6	—	—	73 ± 5
Acetyl CoA Carboxylase	100 ± 6[h]	81 ± 9[b]	71 ± 7[a]	80 ± 7[a]	13 ± 2	9 ± 2	—	—	—
Fatty Acid Synthetase	100 ± 7[i]	122 ± 9	86 ± 5[b]	90 ± 6	82 ± 6	93 ± 5	—	—	—
Phosphatidate Phosphohydrolase	100 ± 7[j]	85 ± 7[b]	56 ± 5[a]	33 ± 3[a]	—	52 ± 5	32 ± 3	—	—
sn-Glycerol-3-phosphate Acyl Transferase	100 ± 8[k]	74 ± 6[a]	55 ± 4[a]	34 ± 5[a]	—	15 ± 5	43 ± 4	—	—

[a] $p \leq 0.001$; [b] $p \leq 0.010$; [c] 30.8 ± 3.1 mg% exchange of mitochondrial citrate; [d] 30.5 ± 2.74 mg of citrate lyased/gm wet tissue/30 min; [e] 28.5 ± 3.14 mg of acetyl CoA formed/gm wet tissue/30 min; [f] 384,900 ± 26,943 dpm cholesterol formed/gm wet tissue/60 min, [g] 6980 ± 558 dpm CO_2 formed/gm wet tissue/15 h; [h] 32,010 ± 1921 dpm/gm water tissue/30 min; [i] 37,656 ± 2635 dpm/gm wet tissue/30 min; [j] 16.70 ± 1.16 μg P_i/gm wet tissue/15 min; [k] 537800 ± 43024 dpm triglyceride formed/gm wet tissue/10 min.

also significantly reduced, 34% for serum cholesterol and 40% for serum triglyceride at 20 mg/kg/day (Table I). In hyperlipidemic mice (high lipid diet) serum cholesterol and triglyceride levels were elevated 176% and 120%, respectively, above normal lipid levels of 128 mg% an 138 mg/dl, respectively. After two weeks of drug administration with N-(p-Chlorobenzoyl)-sulfamate, serum cholesterol levels decreased to 71% above control values, and serum triglycerides were 32% above normal values.

In vitro mitochondrial citrate exchange, citrate lyase, HMG CoA reductase, and fatty acid synthetase activities were unaffected when drug was present (Table II). Acetyl CoA synthetase activity was reduced 26% at 200 μM, cholesterol side chain oxidation was reduced 32% at 100 μM, acetyl CoA carboxylase activity was reduced 29% at 100 μM. The inhibition of phosphatidate phosphohydrolase and acetyl transferase activities by N-(p-Chlorobenzoyl)-sulfamate followed a dose response curve with 200 μM resulting in 67% and 66% inhibition, respectively. *In vivo* enzymatic activities were also suppressed by the agent (Table III). Citrate lyase activity was inhibited 31 to 32% at 20 and 40 mg/kg/day. Acetyl CoA carboxylase activity was reduced 23% and 26% at 20 and 40 mg/kg/day. Acetyl transferase activity was inhibited 34 to 38% at 20 and 40 mg/kg/day. Phosphatidate phosphohydrolase activity was suppressed optimally at 40 mg/kg/day by 67%. Acetyl CoA synthetase, cholesterol biosynthesis and fatty acid synthetase activities were essentially unaffected by *in vivo* drug therapy.

A cholesterol distribution study in rats showed that N-(p-Chlorobenzoyl)-sulfamate administration resulted in a 47% increase in bile excretion of cholesterol (Table IV) with an increase in the radiolabeled cholesterol content of 44% in the small intestine and 14% in the fecal samples (Table V). There did not appear to be accumulation of cholesterol in the major organs after drug administration. The cholesterol and neutral lipid content was elevated in the bile, whereas bile triglyceride and phospholipids were not significantly elevated. Cholesterol, neutral lipid, and triglyceride content were

Table III. The In Vivo Effects of N-(p-Chlorobenzoyl)-sulfamate on Liver Enzyme Activities in CF_1 Male Mice after 15 Days Dosing at 10–60 mg/kg/Day.

Enzyme Assay (N = 6)	Citrate Lyase X ± S.D.	Acetyl CoA Synthetase X ± S.D.	Cholesterol Biosynthesis X ± S.D.	
1% Carboxymethylcellulose	100 ± 8*	100 ± 6*	100 ± 8*	
N-(p-Chlorobenzoyl)-sulfamate				
10 mg/kg	86 ± 6	108 ± 8	188 ± 9[b]	
20 mg/kg	69 ± 5[a]	100 ± 3	115 ± 7	
40 mg/kg	63 ± 4[a]	96 ± 5	117 ± 8[b]	
60 mg/kg	74 ± 7[a]	89 ± 9	126 ± 8[a]	
Saccharin 20 mg/kg/day	73 ± 6[a]	75 ± 6[a]	76 ± 7[a]	
	Acetyl CoA Carboxylase	**Fatty Acid Synthetase**	**sn-Glycerol-3-P Acyl Transferase**	**Phosphatidate Phospho-hydrolase**
1% Carboxymethylcellulose	100 ± 7	100 ± 8	100 ± 6	100 ± 7
N-(p-Chlorobenzoyl)-sulfamate				
10 mg/kg	61 ± 5[a]	108 ± 6	90 ± 7	70 ± 6[a]
20 mg/kg	77 ± 4[a]	113 ± 5	66 ± 5[a]	70 ± 6[a]
40 mg/kg	74 ± 6[a]	104 ± 7	62 ± 5[a]	33 ± 4[a]
60 mg/kg	60 ± 4[a]	144 ± 6[a]	83 ± 5[a]	12 ± 2[a]
Saccharin 20 mg/kg/day	44 ± 4[a]	85 ± 7	64 ± 5	45 ± 3

* See Table II for standard value of enzyme activities.
[a]$p \leq 0.001$; [b]$p \leq 0.010$.

Table IV. Effects of N-(p-Chlorobenzoyl)-sulfamate at 20 mg/kg/Day for 14 Days on Rat Lipid Distribution.

	mg Lipid X ± S.D.	Percent Control Cholesterol X ± S.D.	Neutral Lipid X ± S.D.	Triglyceride X ± S.D.	Phospholipid X ± S.D.	Protein X ± S.D.
Liver (N = 6)						
Control	100 ± 6	100 ± 7[c]	100 ± 5[d]	100 ± 5[e]	100 ± 8[f]	100 ± 6[g]
Treated	98 ± 6	89 ± 7	69 ± 4[a]	52 ± 5[a]	119 ± 12	97 ± 5
Small Intestine						
Control	100 ± 5	100 ± 7[h]	100 ± 5[i]	100 ± 6[j]	100 ± 8[k]	—
Treated	95 ± 5	95 ± 5	112 ± 6	110 ± 7	79 ± 5[a]	—
Feces						
Control	100 ± 7	100 ± 8[l]	100 ± 6[m]	100 ± 7[n]	100 ± 6[o]	—
Treated	119 ± 8	167 ± 14[a]	187 ± 12[a]	212 ± 9[a]	76 ± 6[a]	—
Bile						
Control		100 ± 8[p]	100 ± 7[q]	100 ± 6[r]	100 ± 8[s]	—
Treated		174 ± 9[a]	150 ± 5[a]	105 ± 7	107 ± 7	—
Lipoprotein						
Chylomicrons						
Control		100 ± 9[t]	100 ± 8[u]	100 ± 6[v]	100 ± 8[w]	100 ± 7[x]
Treated		84 ± 6[b]	56 ± 5[a]	53 ± 6[a]	234 ± 9[a]	115 ± 7
VLDL						
Control		100 ± 8[y]	100 ± 9[z]	100 ± 7[aa]	100 ± 8[bb]	100 ± 8[cc]
Treated		98 ± 3	77 ± 5[a]	61 ± 4[a]	57 ± 6[a]	106 ± 7
LDL						
Control		100 ± 9[dd]	100 ± 7[ee]	100 ± 8[ff]	100 ± 7[gg]	100 ± 8[hh]
Treated		98 ± 6	72 ± 7[a]	71 ± 4[a]	77 ± 8[a]	99 ± 7
HDL						
Control		100 ± 8[ii]	100 ± 9[jj]	100 ± 5[kk]	100 ± 6[ll]	100 ± 8[mm]
Treated		83 ± 7[b]	104 ± 6	126 ± 5[a]	86 ± 4[b]	103 ± 6

[a]$p \leq 0.001$; [b]$p \leq 0.010$; [c]24.03 mg cholesterol/gm tissue; [d]44.11 mg neutral lipid/gm tissue; [e]6.37 mg triglyceride/gm tissue; [f]7.19 mg phospholipid/gm tissue; [g]4.5 mg protein/gm tissue; [h]7.82 mg/gm; [i]6.98 mg/gm; [j]1.12 mg/gm; [k]2.06 mg/gm; [l]28.47 mg/gm; [m]33.94 mg/gm; [n]1.86 mg/gm; [o]1.39 kg/gm; [p]118 mg%; [q]5 mg/dl; [r]170 mg/ml; [s]1.75 mg/ml; [t]337 µg/ml; [u]67 µg/ml; [v]420 µg/ml; [w]149 µg/ml; [x]184 µg/ml; [y]190 µg/ml; [z]98 µg/ml; [aa]98 µg/ml; [bb]26 µ/ml; [cc]50 µg/ml; [dd]210 µg/ml; [ee]10 µg/ml; [ff]45 µg/ml; [gg]41 µg/ml; [hh]122 µg/ml; [ii]544 µg/ml; [jj]620 µg/ml; [kk]27 µg/ml; [ll]153 µg/ml; [mm]657 µg/ml.

Table V. Rat Organ Weights and Radioactive Distribution of 10 μCi ^{3}H-Cholesterol after 14 Days Dosing with N-(p-Chlorobenzoyl)-Sulfamate at 20 mg/kg/Day.

	Organ Weight (g)		DPM/Total Organ	
	Control	Treated	Control	Treated
Total body weight	355	338	—	—
Liver	13.35	10.47	46,578	19,684
Brain	1.75	1.66	8,383	7.769
Spleen	0.85	0.73	13,770	10,366
Lung	1.85	1.57	22,052	15,009
Kidney	2.75	2.33	18,920	13,631
Heart	1.35	1.06	7,344	6,402
Stomach	2.25	2.30	11,375	11,408
Small intestine	9.50	10.00	154,470	222,100
Large intestine	4.05	3.33	31,023	31,102
Chyme	2.56	3.53	18,375	19,203
Feces	2.65	3.06	54,388	62,118
Urine			1,764,266	1,481,972
Bile			364,182	534,141
Serum			3,754,890	3,457,892
			DPM/ml Sample	
Urine			281	236
Bile			1016	1490
Plasma			1043	960

increased in the feces after two weeks administration of drug, but phospholipid content was lower than those levels observed in control animals. Liver cholesterol, neutral lipid, and triglyceride levels were reduced, but phospholipids were increased 19 %. The liver weight of the treated animal was slightly reduced from the control value. Small intestine lipids showed essentially no change except a small reduction in phospholipids (21 %) (Table IV).

The lipoprotein fractions of the blood were also analyzed for drug induced alteration of lipid content. Chylomicrons showed a moderate reduction of cholesterol (16 %), whereas neutral lipid and triglyceride levels (44–47 %) were markedly lowered. Phospholipids were significantly elevated in the chylomicron fraction (134 %). The neutral lipid, triglyceride, and phospholipid levels were reduced (23–43 %) in the very low density and low density lipoprotein fractions. The cholesterol and phospholipid levels were marginally inhibited in the high density lipoprotein fraction, and triglyceride content was elevated. Protein content was not altered significantly in any of the four lipoprotein fractions after drug treatment. The LD_{50} in mice after a single intraperitoneal injection was 500 mg/kg.

Discussion

N-(p-Chlorobenzoyl)-sulfamate was an effective hypolipidemic agent in rodents when administered both orally and intraperitoneally. The effects on blood lipids were not dose dependent, and 20 mg/kg/day appeared to be the optimum dose for lowering serum lipid levels in mice. The reduction of serum cholesterol by N-(p-Chlorobenzoyl)-sulfamate is apparently caused by the accelerated excretion of cholesterol via the bile into the feces as measured by ^{3}H-cholesterol distribution and actual cholesterol content in the feces. N-(p-Chlorobenzoyl)-sulfamate had essentially no effect on cholesterol absorption from the intestine after oral administration, since only an 8 % reduction was observed in the absorption rate into the serum over a 24 h period.

The lowering of serum triglyceride levels can be accounted for by the observed inhibition of two regulatory enzymes of liver triglyceride synthesis, that is, *sn*-glycerol-3-phosphate acyl transferase and phosphatidate phosphohydrolase. Positive correlations between lowering of serum triglyceride levels and inhibition of these two enzyme activities have been demonstrated by Lamb *et al.* (14). Saccharin as well as (o-chlorobenzyl)sulfonamide inhibit these two enzyme activities (2). Lipid removed from the blood compartment did not appear to be deposited in other organs, such as liver and small intestine, as judged on the basis of ^{3}H-cholesterol distribution and organ weights; rather, cholesterol, neutral lipids, and triglycerides were excreted into the feces.

When the plasma lipoprotein fractions were examined, reduction of the levels of neutral lipids and triglycerides were observed with only marginal reduction of cholesterol levels. The reduction of these levels may reflect the effects of the drug on liver lipid synthesis, since triglyceride synthesis was inhibited but cholesterol synthesis was not. However, since the agent does have a safe therapeutic index, further investigation of the agent as a hypolipidemic drug is warranted.

References

(1) Wyrick, S. D., Hall, I. H., Dubey, A. S. (1983) J. Pharm. Sci. (in press).
(2) Hall, I. H., Voorstad, P. J., Chapman, J. M., Cocolas, G. H. (1983) J. Pharm. Sci. (in press).
(3) Ness, A. T., Pastewka, J. V., Peacock, A. C. (1964) Clin. Chim. Acta. 10, 229–237.
(4) Litchfield, Jr., J. T., Wilcoxon, F. (1949) J. Pharmacol. Exp. Ther. 96, 99–113.
(5) Goodridge, A. G. (1973) J. Biol. Chem. 248, 4318–4327.
(6) Hoffman, M., Weiss, L., Wieland, O. H. (1978) Anal. Biochem. 84, 441–448.
(7) Robinson, B. H., Williams, G. R., Halperin, M. L., Leznoff, C. C. (1970) Eur. J. Biochem. 15, 263–272.
(8) Robinson, B. H., Williams, G. R. (1970) Biochim. Biophys. Acta. 216, 63–70.
(9) Kritchevsky, D., Tepper, S. A. (1973) Atherosclerosis 18, 93–99.
(10) Haven, G. T., Krzemien, J. R., Nguyen, T. T. (1973) Res. Commun. Chem. Pathol. Pharmacol. 6, 253–261.
(11) Wada, F., Hirata, K., Sakameto, Y. (1969) J. Biochem. 65, 171–175.
(12) Greenspan, M. D., Lowenstein, J. M. (1968) J. Biol. Chem. 243, 6273–6280.
(13) Brady, R. O., Bradley, R. M., Trams, E. G. (1960) J. Biol. Chem. 235, 3093–3098.
(14) Lamb, R. G., Wyrick, S. D., Piantadosi, C. (1977) Atherosclerosis 27, 147–154.
(15) Mavis, R. D., Finkelstein, J. N., Hall, B. P. (1978) J. Lipid Res. 19, 467–477.
(16) Folch, J., Lees, M., Stanley, G. H. C. (1957) J. Biol. Chem. 226, 497–509.
(17) Bligh, E. G., Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
(18) Bragdon, J. H. (1951) J. Biol. Chem. 190, 513–517.
(19) Stewart, C. P., Henday, E. B. (1935) Biochem. J. 29, 1683–1689.
(20) Adam, A., van Cantfort, J., Gielen, J. (1976) Lipids 11, 610–615.
(21) Hatch, F. T., Lees, R. S. (1968) Adv. Lipid Res. 6, 1–68.
(22) Havel, R. J., Eder, H. A., Bragdon, J. H. (1955) J. Clin. Invest. 34, 1345–1353.
(23) Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.