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Hypolipidemic Activity of 4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone in Rodents

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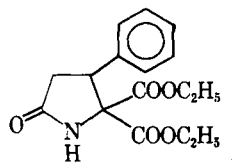
Abstract □ A series of pyrrolidinones have been reported to possess hypolipidemic activity in mice. The most active agent, 4-phenyl-5-5-dicarbethoxy-2-pyrrolidinone, effectively lowered both serum cholesterol and triglyceride levels at 20–30 mg/kg/d. The agent suppressed liver mitochondrial citrate exchange, phosphatidate phosphohydrolase, and *sn*-glycerol-3-phosphate acyl transferase activities. Lipid content of the liver, small intestine, and serum lipoprotein fractions was reduced by drug treatment, but lipid levels increased in the bile and fecal samples, suggesting the drug accelerated lipid excretion. The mode of action of the pyrrolidinone appears similar to that of the cyclic imides.

Keyphrases □ 4-Phenyl -5,5- dicarbethoxy -2- pyrrolidinone—hypolipidemic activity, rodents □ Hypolipidemic agents—potential, 4-phenyl-5,5-dicarbethoxy-2-pyrrolidinone, rodent screen

A series of substituted 2-pyrrolidinones have previously been examined for hypolipidemic activity in mice and have been observed to be active between 20–30 mg/kg/d. Similarly, types of moieties such as succinimide (1) also have hypolipidemic activity in the dosage range. One particular compound, 4-phenyl-5,5-dicarbethoxy-2-pyrrolidinone (2), demonstrated potent activity and lowered serum cholesterol and triglycerides >40% at 30 mg/kg/d after 16 d. The current study involves the mode of action of 4-phenyl-5,5-dicarbethoxy-2-pyrrolidinone in lowering lipid levels of the body.



4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone

EXPERIMENTAL

Antihyperlipidemic Screens in Normal Rodents—4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone was suspended in 1% aqueous carboxymethylcellulose and administered to male CF_1 mice (~25 g) intraperitoneally for 16 d or male Holtzman rats (~350 g) orally by an intubation needle for 14 d. On days 9 and 14 or 16, 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 16, and the triglyceride content was determined using 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 the test drug 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 animals' weights on day 0. After dosing for 14 d with the test drug, 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 the test drug 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 50–200 μmol of the test drug. *In vivo* enzymatic studies were determined using 10% homogenates of liver obtained from male CF_1 mice after administering the agents for 16 d at a dose ranging from 10 to 60 mg/kg/d ip. The liver homogenates for both *in vitro* and *in vivo* studies were prepared in 0.25 M sucrose plus 0.001 M EDTA.

Acetyl CoA synthetase (5) and ATP-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 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. The radioactivity was then 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 18 h of incubation at 37°C with the test drug, 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) activity 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 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 the test drug. 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

[1] Hycel Triglyceride Test Kit; Fisher.

[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, Boston, Mass.

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Table I—Effects of 4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone on Serum Lipid Levels in Rodents[a]

Dose, mg/kg/d	Mice			Rats		
	Serum Cholesterol		Serum Triglyceride,	Serum Cholesterol		Serum Triglyceride,
	Day 9	Day 16	Day 16	Day 9	Day 14	Day 14
Control						
(1% carboxymethylcellulose)	100 ± 5[c]	100 ± 6[d]	100 ± 6[e]	100 ± 9[f]	100 ± 7[g]	100 ± 8[h]
10	96 ± 6	50 ± 5[b]	63 ± 5[b]			
20	94 ± 5	58 ± 5[b]	58 ± 4[b]	90 ± 8	60 ± 6[b]	59 ± 5[b]
30	55 ± 6[b]	52 ± 5[b]	48 ± 5[b]			
40	65 ± 6[b]	62 ± 6[b]	65 ± 5[b]			
60	61 ± 4[b]	53 ± 4[b]	68 ± 6[b]			

[a] Expressed as percentage of control (mean ± *SD*); $n = 6$. [b] $p \leq 0.001$. [c] 118 mg%. [d] 122 mg%. [e] 137 mg%. [f] 73 mg%. [g] 78 mg%. [h] 110 mg%.

Table II—Effects of 4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone on CF_1 Mouse Liver Enzyme Activities *In Vitro*[a]

	Mitochondrial Citrate Exchange	ATP-Dependent Citrate Lyase	Acetyl CoA Synthetase	HMG CoA Reductase	Cholesterol Side-Chain Oxidation
Control					
(1% carboxymethylcellulose)	100 ± 7[c]	100 ± 5[d]	100 ± 7[e]	100 ± 7[f]	100 ± 9[g]
Treated					
0.050 mM	80 ± 6[b]	100 ± 6	95 ± 8	94 ± 7	—
0.100 mM	76 ± 7[b]	98 ± 5	96 ± 7	87 ± 6	56 ± 7[b]
0.200 mM	69 ± 7[b]	97 ± 6	98 ± 7	109 ± 8	—

	Acetyl CoA Carboxylase	Fatty Acid Synthetase	Phosphatidate Phosphohydrolase	*sn*-Glycerol-3-Phosphate Acyl Transferase
Control				
(1% carboxymethylcellulose)	100 ± 7[h]	100 ± 5[i]	100 ± 6[j]	100 ± 6[k]
Treated				
0.050 mM	107 ± 8	100 ± 6	45 ± 4[b]	100 ± 5
0.100 mM	91 ± 7	100 ± 7	43 ± 3[b]	91 ± 6
0.200 mM	111 ± 5	82 ± 5[b]	26 ± 3[b]	69 ± 6[b]

[a] Expressed as percentage of control (mean ± *SD*); $n = 6$. [b] $p \leq 0.001$. [c] 30.8% exchange of mitochondrial citrate. [d] 30.5 mg of citrate hydrolyzed/g wet tissue/20 min. [e] 28.5 mg of acetyl CoA formed/g wet tissue/20 min. [f] 384,900 dpm of cholesterol formed/g wet tissue/60 min. [g] 6080 dpm of CO_2 formed/g wet tissue/18 h. [h] 32,010 dpm/g wet tissue/30 min. [i] 37,656 dpm/g wet tissue/20 min. [j] 16.7 μg Pi/g wet tissue/15 min. [k] 537,800 dpm/g wet tissue/20 min.

with ether and counted[5]. *sn*-Glycerol-3-phosphate acyl transferase activity was determined with L-[2-^{3}H]glycerol-3-phosphate (7.1 Ci/mmol) and the microsomal fraction of the liver homogenates (14). The reaction was terminated after 20 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 15 min 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 the test drug for 16 d, the liver, small intestine, and fecal materials (24-h collection) were removed and a 10% homogenate in 0.25 M sucrose–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 number of milligrams of lipid was determined. The lipid was taken up in dichloromethane and cholesterol level (3), triglyceride levels[7], neutral lipid (18), and phospholipid content (19) were determined.

[^{3}H]Cholesterol Distribution in Rats—Male Holtzman rats (~350 g) were administered the test agent orally for 14 d. On day 13, 10 μCi of [^{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[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 the test drug intraperitoneally for 14 d at 20 mg/kg/d. On day 13, 10 μCi of [1,2-^{3}H]cholesterol (40.7 mCi/mmol) 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 counted[5].

Bile Cannulation Study—Male Holtzman rats (~400 g) were treated with the test drug at 20 mg/kg/d orally for 14 d. The rats were anesthetized with chlorpromazine[10] (25 mg/kg ip) followed after 30 min by pentobarbital[11] (22 mg/kg ip). The duodenal section of the small intestine was isolated, and ligatures were placed around the pyloric sphincter and distally to a site approximately one-third 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 the volume was measured. Aliquots were counted[5] as well as analyzed for cholesterol (3), neutral lipid (18), and phospholipid (19) content[13].

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

RESULTS

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

[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.
[13] In the first hour chlorpromazine decreases bile flow, while pentobarbital increases the flow. Neither agent interferes with lipid assay methods.

Table III—Effects of 4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone on CF_1 Mouse Liver Enzyme Activities *In Vivo*[a]

	ATP-Dependent Citrate Lyase	Acetyl CoA Synthetase	HMG CoA Reductase
Control (1% carboxymethylcellulose)	100 ± 8	100 ± 6	100 ± 7
Treated			
10 mg/kg/d	96 ± 7	78 ± 7	100 ± 8
20 mg/kg/d	94 ± 8	114 ± 8	94 ± 7
40 mg/kg/d	90 ± 7	94 ± 6	84 ± 6[c]
60 mg/kg/d	87 ± 7	68 ± 5[b]	83 ± 6[c]

	Acetyl CoA Carboxylase	Fatty Acid Synthetase	Phosphatidate Phosphohydrolase	*sn*-Glycerol-3-Phosphate Acyl Transferase
Control (1% carboxymethylcellulose)	100 ± 8	100 ± 6	100 ± 6	100 ± 7
Treated				
10 mg/kg/d	109 ± 7	105 ± 7	100 ± 6	81 ± 6[b]
20 mg/kg/d	76 ± 6[b]	115 ± 7[c]	98 ± 6	79 ± 5[b]
40 mg/kg/d	84 ± 6[c]	92 ± 6	43 ± 4[b]	77 ± 4[b]
60 mg/kg/d	93 ± 7	100 ± 6	59 ± 5[b]	52 ± 6[b]

[a] Expressed as percentage of control (mean ± *SD*); $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.010$.

Table IV—Effects of 4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone on Holtzman Rat Organ Lipid Levels[a]

	Lipid Extracted, mg	Cholesterol	Triglycerides	Neutral Lipids	Phospholipids	Protein
Liver						
Control	100 ± 7	100 ± 8[d]	100 ± 5[e]	100 ± 8[f]	100 ± 7[g]	100 ± 6[h]
Treated	76 ± 6[b]	73 ± 7[b]	71 ± 5[b]	45 ± 5[b]	76 ± 6[b]	97 ± 6
Small Intestine						
Control	100 ± 5	100 ± 7[i]	100 ± 6[j]	100 ± 7[k]	100 ± 8[l]	100 ± 8[m]
Treated	71 ± 6[b]	88 ± 7	20 ± 3[b]	63 ± 6[b]	98 ± 9	102 ± 7
Feces						
Control	100 ± 8	100 ± 6[n]	100 ± 7[o]	100 ± 6[p]	100 ± 7[q]	100 ± 5[r]
Treated	111 ± 7	117 ± 7[c]	137 ± 6[b]	109 ± 7	79 ± 6[b]	88 ± 6
Bile						
Control	—	100 ± 8[s]	100 ± 7[t]	100 ± 7[u]	100 ± 8[v]	—
Treated	—	112 ± 9	80 ± 6[b]	133 ± 8[b]	121 ± 5[b]	—
Lipoproteins						
Chylomicrons						
Control	—	100 ± 6[w]	100 ± 7[x]	100 ± 6[y]	100 ± 8[z]	100 ± 8[aa]
Treated	—	57 ± 4[b]	75 ± 5[b]	60 ± 5[b]	143 ± 5[b]	93 ± 7
Very-low density lipoproteins						
Control	—	100 ± 7[bb]	100 ± 6[cc]	100 ± 7[dd]	100 ± 8[ee]	100 ± 8[ff]
Treated	—	71 ± 6[b]	65 ± 5[b]	53 ± 4[b]	118 ± 7[c]	105 ± 6
Low-density lipoproteins						
Control	—	100 ± 8[gg]	100 ± 7[hh]	100 ± 7[ii]	100 ± 6[jj]	100 ± 9[kk]
Treated	—	63 ± 5[b]	86 ± 6[c]	41 ± 3[b]	128 ± 7	87 ± 7
High-density lipoproteins						
Control	—	100 ± 6[ll]	100 ± 7[mm]	100 ± 6[nn]	100 ± 8[oo]	100 ± 6[pp]
Treated	—	66 ± 7[b]	100 ± 8	93 ■ 7	103 ± 6	92 ± 7

[a] Expressed as percentage of control (mean ± *SD*); $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.010$. [d] 24.03 mg of cholesterol/g tissue. [e] 44.11 mg of neutral lipid/g tissue. [f] 6.37 mg of triglyceride/g tissue. [g] 7.19 mg. [h] 4.5 mg of protein/g wet tissue. [i] 7.82 mg/g. [j] 6.98 mg/g. [k] 1.12 mg/g. [l] 2.06 mg/g. [m] 42 mg/g. [n] 28.47 mg/g. [o] 33.94 mg/g. [p] 1.86 mg/g. [q] 1.39 kg/g. [r] 6.99 mg/g. [s] 118 mg%. [t] 5 mg/dL. [u] 170 mg/mL. [v] 1.75 mg/mL. [w] 337 μg/ml. [x] 67 mg/mL. [y] 420 μg/mL. [z] 149 μg/mL. [aa] 3 μg/mL. [bb] 190 μg/mL. [cc] 98 μg/mL. [dd] 22 μg/mL. [ee] 26 μg/mL. [ff] 50 μg/mL. [gg] 210 μg/mL. [hh] 10 μg/mL. [ii] 45 μg/mL. [jj] 41 μg/mL. [kk] 0.681 μg/mL. [ll] 544 μg/mL. [mm] 620 μg/mL. [nn] 27 μg/mL. [oo] 153 μg/mL. [pp] 5.677 μg/mL.

4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone proved to be a potent hypolipidemic agent in both mice and rats by the oral route as well as intraperitoneally (Table I). A dose-response curve showed that cholesterol levels were lowered approximately the same, 47–50%, at 10, 30, and 60 mg/kg/d. Serum triglyceride levels were reduced 52% maximally at 30 mg/kg/d in mice. In rats the serum cholesterol and triglyceride levels were approximately the same at 20 mg/kg/d as those observed in mice. After inducing a hyperlipidemic state in mice, in which case serum cholesterol levels were elevated 183% (354 mg%) above control values (125 mg%), drug treatment at 20 mg/kg reduced serum cholesterol to 184 mg%, 47% above normal. Serum triglyceride levels in the hyperlipidemic mice were elevated 168% (367 mg/dL) above control values. Drug administration lowered triglycerides to 176 mg/dL, which was 28% above normal triglyceride values (138 mg/dL).

In vitro enzyme assays (Table II) showed that the presence of the drug caused a decrease in mitochondrial citrate exchange to the cytoplasm, with 200 μM resulting in a 31% reduction. Cholesterol side-chain oxidation was reduced 44% at 100 μM. Phosphatidate phosphohydrolase activity was suppressed in a dose-dependent manner, with 200 μM resulting in 74% inhibition. *sn*-Glycerol-3-phosphate activity was lowered 31% at 200 μM.

In the *in vivo* enzymatic studies (Table III), marginal inhibition was observed in ATP-dependent citrate lyase at 60 mg/kg (13%) and in acetyl CoA synthetase at 10 (22%) and 60 mg/kg (32%). HMG CoA reductase activity was slightly inhibited at 40 and 60 mg/kg by 16–17%. Acetyl CoA carboxylase activity was suppressed 16% at 40 mg/kg/d. The maximum effect of the drug appeared to be on phosphatidate phosphohydrolase and *sn*-glycerol-3-phosphate acyl transferase activities. 4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone reduced phosphatidate phosphohydrolase activity 57% at 40 mg/kg/d and 41% at 60 mg/kg/d. *sn*-Glycerol-3-phosphate acyl transferase activity was reduced in a dose-dependent manner, with 60 mg/kg/d causing 48% reduction.

In an attempt to determine if the lipids were being removed from the blood compartment and being deposited in the tissues, the liver and small intestine were examined for lipid content after drug treatment (Table IV). In the liver, cholesterol was lowered 27%, triglycerides 29%, neutral lipids 45%, and phospholipids 24%. In the small intestine, cholesterol levels were reduced 12%; however, marked reduction of triglyceride levels was evident (80%). The drug treatment did not affect [^{3}H]cholesterol absorption from the intestine over a 24-h period after oral administration. Nevertheless, biliary excretion of lipids

Table V—Effects of 4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone on Rat [^{3}H]Cholesterol Distribution 24-h After Administration and Organ Weights After 14 d of Administration Orally

	Organ Weight, g		[^{3}H]Cholesterol/Total Organ, dpm	
	Control	Treated	Control	Treated
Brain	1.97	1.97	5877	1232
Lung	2.60	1.70	7069	3632
Heart	1.33	1.20	4004	1280
Liver	14.47	12.67	47429	25328
Kidney	3.37	3.13	8170	2592
Spleen	0.67	0.77	2315	2633
Adrenal	0.0504	0.050	—	—
Stomach	2.97	2.43	11375	1440
Small intestine	10.17	6.33	41656	73152
Large intestine	4.10	3.57	14104	10832
Chyme	2.57	7.58	9226	30048
Feces	2.63	5.03	8349	9664

was altered by drug treatment. Cholesterol excretion was elevated 12%, neutral lipids 37%, and phospholipids 21%. The fecal sample also demonstrated increases in lipid content, *i.e.*, 17% and 37% increases in cholesterol and triglycerides, respectively, and a 21% reduction in phospholipids.

Examination of the lipoprotein fractions of the blood showed reduction of cholesterol content in all four fractions. Triglycerides were lowered in chylomicrons and in the very low-density fraction, which contain most of the triglyceride content of the lipoprotein fractions. Neutral lipids were also lowered in the chylomicron, very low-, and low-density lipoprotein fractions. Phospholipid content was elevated in these three fractions. Examination of the effects of drug treatment on [^{3}H]cholesterol distribution in the body shows that there is a reduction of cholesterol in the brain, lung, heart, liver, kidney, and stomach (Table V). However, high concentrations of labeled material were found in the small intestine (76%), chyme (226%), and feces (16%). The organ weights of the treated animals were lower than those of the control animals. The LD_{50} in mice was >2 g/kg ip.

DISCUSSION

4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone proved to be a potent hypolipidemic agent, lowering serum cholesterol and triglyceride levels >45%. The agent was more active than clofibrate, which is inactive at 10–60 mg/kg/d. Clofibrate requires dosages of 150–200 mg/kg/d to reduce cholesterol ~15% and triglyceride levels 25%. The pyrrolidinone was active in the dosage range where succinimide (1) had proven to be active as well as the cyclic imide, phthalimide, saccharin, and 1,8-naphthalimide (24). In fact, its mode of action of lowering serum lipids seems to be similar to the cyclic imides. For example, inhibition of mitochondrial citrate exchange would lower the cytoplasmic levels of acetyl CoA, a key intermediate precursor in the *de novo* synthesis of cholesterol and fatty acids. Two regulatory enzymes of the triglyceride pathway, phosphatidate phosphohydrolase and *sn*-glycerol-3-phosphate acyl transferase activities, were inhibited by the pyrrolidinones as well as the cyclic imides. Positive correlations between the inhibition of the activities of these enzymes with the lowering of serum triglyceride levels have been made previously (14).

Removal of lipids from the blood was not related to an increase in organ lipids. Three observations support this thesis: (*a*) the lipid content of liver and small intestine was actually reduced; (*b*) there was no increase in organ weights over the control values; (*c*) the [^{3}H]cholesterol content was lower in the major organs of the treated animals compared with the control animals.

The lipids appeared to be excreted in the bile at an accelerated rate after drug treatment. This excretion of [^{3}H]cholesterol was also reflected in the higher radioactive content observed in the small intestine, chyme, and feces. Similar observations have been made with phthalimide and saccharin (25).

Cholesterol, triglycerides, and neutral lipid content of rat serum lipoprotein fractions were also lowered by treatment with the pyrrolidinones. Cholesterol content probably did not change when examining the high-density to low-density ratio. High cholesterol in the high-density lipoprotein fraction and low content in the low-density lipoprotein supposedly protects against myocardial infarction. Nevertheless, lowering the cholesterol content of the low-density lipoprotein fraction is probably beneficial since this is the lipoprotein fraction which deposits lipids in the atherosclerotic plaque. These preliminary studies do suggest that pyrrolidinones may have potential as hypolipidemic agents and are worthy of further study.

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