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## RESEARCH ARTICLES

# Antihyperlipidemic Activity of Phthalimide Analogues in Rodents

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**Abstract** □ Phthalimide analogues have been shown to effectively lower serum cholesterol and triglyceride levels in rats and mice. The mode of action of these agents was not to suppress the appetite of animals, but rather to reduce the activities of key enzymes in the early synthesis of liver cholesterol and fatty acids in the triglyceride pathway. Phthalimide analogues were effective in accelerating biliary excretion of cholesterol and blocking its absorption from the gut. After 16 days dosing, it was evident that higher levels of lipids were being excreted than in control mice. The major serum lipoprotein fractions were reduced in cholesterol, triglyceride, and neutral lipid content, but not phospholipid content in rats after 14 days of administration.

**Keyphrases** □ Phthalimide—analogues, antihyperlipidemic activity, cholesterol and triglyceride reduction □ Antihyperlipidemic agents—phthalimide analogues, cholesterol and triglyceride reduction □ Cholesterol—antihyperlipidemic effect of phthalimide analogues □ Triglyceride—antihyperlipidemic effect of phthalimide analogues

Continued investigation for new hypolipidemic agents has revealed that phthalimide analogues are potent in lowering serum lipids in rodents (1). In mice phthalimide lowers serum cholesterol 43% after 16 days of dosing at 20 mg/kg ip. Serum triglyceride levels were reduced 56% after 14 days of drug administration. Although phthalimide was the most active member of the series, other analogues demonstrate similar magnitudes of hypolipidemic activity (2). Preliminary *in vitro* studies indicate that phthalimide analogues suppressed the activity of liver enzymes involved in the early *de novo* synthesis of cholesterol and fatty acids. A more detailed study of the effects of these agents on lipid distribution and metabolism was undertaken.

## EXPERIMENTAL

**Source of Compounds**—Phthalimide was purchased commercially[1]. The synthesis and chemical characterization of 1-*N*-phthalimidobutan-3-one and 3-*N*-phthalimidopropionic acid has been reported previously (1).

**Antihyperlipidemic Screens in Normogenic Rodents**—Compounds to be tested were suspended in 1% carboxymethylcellulose in water and administered to male $CF_1$ (~25 g) mice intraperitoneally or male Holtzman rats (~200 g) orally by an intubation needle for 16 days. On days 9 and 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, and the triglyceride content was determined using a commercial kit[2] (4). Animals were maintained on standard rodent food[3] throughout the experiment.

**Hyperlipidemic-Induced Mice**—Male $CF_1$ mice (~25 g) were placed on a commercial diet[4] which contained butter fat (400 g), cellulose[5] (60 g), cholesterol (53 g), choline dihydrogen citrate (4 g), salt mixture oil[6] (40 g), sodium cholate (40 g), sucrose (223 g), vitamin-free casein (200 g), and total vitamin supplement for a 2-week period. After the cholesterol and triglyceride levels were assayed and observed to be elevated, the mice were administered test drugs at 20 mg/kg/day ip for an additional 2-week period. Serum cholesterol and triglyceride levels were measured after 14 days 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 16 days with test drugs, a number of organs were excised, trimmed of fat, and weighed. The organ weights were expressed as a percentage of the total body weight of the animal. The average food intake[3] in g/rat/day was determined over the 16-day period of dosing.

**Toxicity Studies**—The acute toxicity ($LD_{50}$ value) (5) 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 in the group was determined for each dosage.

**Enzymatic Studies**—*In vitro* enzymatic studies used 10% homogenates of male $CF_1$ mouse liver with 2.5 μmoles of the test drugs and male Holtzman rat liver with 0.100–10 m*M* concentration of the test drugs. *In vivo* enzymatic studies used 10% homogenates of livers from male $CF_1$ mice obtained after administering the agents intraperitoneally for 16 days at 10–60 mg/kg/day. The liver homogenates for both *in vitro* and *in vivo*

[1] Ruger Chemical Co., Inc.

[2] Fisher, Hycel Triglyceride Test Kit.
[3] Wayne Blox Rodent Chow.
[4] U.S. Biochemical Corporation Basal Atherogenic Test Diet.
[5] Celufil.
[6] Wesson Oil.

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Table I—Effects of Phthalimide Analogues on Serum Cholesterol and Triglyceride Levels of Male Holtzman Rats and Male $CF_1$ Mice [a]

| Compound | Rats: Dose, mg/kg/day | Rats: Serum Cholesterol Day 9 | Rats: Serum Cholesterol Day 16 | Rats: Triglyceride Day 14 | Mice: Dose, mg/kg/day | Mice: Serum Cholesterol Day 9 | Mice: Serum Cholesterol Day 14 | Mice: Triglyceride Day 14 |
|---|---|---|---|---|---|---|---|---|
| Control (1% carboxymethylcellulose) | | 100 ± 9[d] | 100 ± 7[e] | 100 ± 8[f] | | 100 ± 5[g] | 100 ± 6[h] | 100 ± 6[i] |
| Phthalimide | 5 | 91 ± 8 | 79 ± 5[b] | 66 ± 5[b] | 10 | 67 ± 7[b] | 43 ± 4[b] | 46 ± 4[b] |
| | 10 | 81 ± 9[c] | 66 ± 7[b] | 56 ± 5[b] | 20 | 62 ± 5[b] | 59 ± 5[b] | 44 ± 3[b] |
| | 20 | 72 ± 6[b] | 57 ± 4[b] | 58 ± 4[b] | 40 | 49 ± 6[b] | 45 ± 3[b] | 52 ± 6[b] |
| | 50 | 70 ± 4[b] | 62 ± 5[b] | 62 ± 6[b] | 60 | 51 ± 4[b] | 40 ± 5[b] | 56 ± 7[b] |
| 1-*N*-Phthalimidobutan-3-one | 10 | 70 ± 8[b] | 59 ± 8[b] | 59 ± 13[b] | 10 | 73 ± 9[b] | 66 ± 5[b] | — |
| | 20 | 67 ± 5[b] | 51 ± 5[b] | 68 ± 3[b] | 20 | 67 ± 9[b] | 63 ± 9[b] | 58 ± 7[b] |
| | — | — | — | — | 30 | 66 ± 7[b] | 54 ± 6[b] | — |
| 3-*N*-Phthalimidopropionic acid | — | — | — | — | 10 | 84 ± 6[b] | 80 ± 6[b] | 52 ± 4[b] |
| | 20 | 67 ± 5[b] | 51 ± 5[b] | 68 ± 3[b] | 20 | 74 ± 8[b] | 55 ± 4[b] | 53 ± 5[b] |
| | — | — | — | — | 40 | 84 ± 7[c] | 74 ± 5[b] | 58 ± 7[b] |
| | — | — | — | — | 60 | 87 ± 6[b] | 76 ± 6[b] | 56 ± 6[b] |

[a] Percent of control, expressed as mean ± *SD*; $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.010$. [d] 73 mg %. [e] 78 mg %. [f] 110 mg %. [g] 118 mg %. [h] 122 mg %. [i] 137 mg %.

Table II—Effects of Phthalimide Analogues on Serum Cholesterol and Triglyceride Levels in Hyperlipidemic-Induced Mice [a]

| Compound | Serum Cholesterol: 2-week Diet | Serum Cholesterol: 14 Days of Dosing | Serum Triglyceride: 2-week Diet | Serum Triglyceride: 14 Days of Dosing |
|---|---|---|---|---|
| Control (1% carboxymethylcellulose) | 100 ± 6[c] | 100 ± 7[d] | 100 ± 5[e] | 100 ± 4[f] |
| Control (hyperlipidemic diet) | 289 ± 9[b] | 290 ± 9[b] | 131 ± 5[b] | 131 ± 5[b] |
| Phthalimide | 289 ± 8[b] | 137 ± 7[b] | 132 ± 6[b] | 57 ± 5[b] |
| 1-*N*-Phthalimidobutan-3-one | 288 ± 9[b] | 130 ± 6[b] | 135 ± 6[b] | 86 ± 5[b] |
| 3-*N*-Phthalimidopropionic acid | 288 ± 10[b] | 126 ± 5[b] | 130 ± 5[b] | 75 ± 5[b] |

[a] Percent of control, expressed as mean ± *SD*; $n = 6$. [b] $p \leq 0.001$. [c] 74 ± 4 mg %. [d] 76 ± 5 mg %. [e] 136 ± 7 mg %. [f] 139 ± 5 mg %.

studies were prepared in 0.25 *M* sucrose and 0.001 *M* EDTA [(ethylenedinitrilo)tetraacetic acid].

Acetyl coenzyme A synthetase (6) and adenosine triphosphate-dependent citrate lyase (7) activities were determined spectrophotometrically at 540 nm as the hydroxamate of acetyl coenzyme A formed after 30 min at 37°. Mitochondrial citrate exchange was determined by the procedure of Robinson *et al.* (8, 9) using sodium [$^{14}$C]bicarbonate (41 mCi/mmole) which was 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, with the test drugs for 10 min. Then the radioactivity was measured in the mitochondrial and supernatant fraction in scintillation fluid[7] and expressed as a percentage. Cholesterol side-chain oxidation was determined by the method of Kritchevsky *et al.* (10) using [26-$^{14}$C]cholesterol (50 mCi/mmole) and mitochondria isolated from rat liver homogenates. After an 18-hr incubation at 37° 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[8] and counted. 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) was measured using [1-$^{14}$C]acetate (56 Ci/mmole) using a postmitochondrial supernatant (9000×*g* for 20 min) for 60 min at 37° (11). The digitonide derivative of cholesterol was isolated and counted (12). Acetyl coenzyme A carboxylase activity was measured by the method of Greenspan and Lowenstein (13). Initially, the enzyme had to be polymerized for 30 min at 37°, and then the assay mixture containing sodium [$^{14}$C]bicarbonate (41.0 mCi/mmole) was added and incubated for 30 min at 37° with the test drugs. Fatty acid synthetase activity was determined by the method of Brady *et al.* (14) using [2-$^{14}$C]malonyl-coenzyme A (37.5 mCi/mmole) incorporated into newly synthesized fatty acids, which were extracted with ether and counted. Acyl transferase activity was determined with L-[2-$^{3}$H]glycerol-3-phosphate (7.1 Ci/mmole) and the microsomal fraction of the liver homogenates (15). The reaction was terminated after 10 min, and the lipids were extracted with chloroform–methanol (1:2) containing 1% 1 *N* HCl and counted.

[7] Fisher, SOX-1 Scintiverse; Packard Scintillation Counter.
[8] Hyamine hydroxide; New England Nuclear.

Table III—Effects of Phthalimide Analogues on Body Weight and Food Consumption of Rats after 14 Days of Dosing [a]

| | Body Weight Increase Over 14 Days, % | Food Consumed Per Day, g |
|---|---|---|
| Control | 138 ± 3 | 22.5 |
| Phthalimide | 142 ± 4 | 22.3 |
| 1-*N*-Phthalimidobutan-3-one | 136 ± 3 | 21.5 |
| 3-*N*-Phthalimidopropionic acid | 137 ± 3 | 20.0 |

[a] $n = 6$.

Phosphatidate phosphohydrolase activity was measured as the inorganic phosphate released after 30 min from phosphatidic acid by the method of Mavis *et al.* (16). The released inorganic phosphate after development with ascorbic acid and ammonium molybdate was determined at 820 nm.

The *in vitro* oxidative phosphorylation process in male $CF_1$ mouse liver was also examined with an oxygen electrode[9] connected to an oxygraph[10] at 37°. The reaction vessel contained 55 μmoles of sucrose, 22 μmoles of monobasic potassium phosphate, 22 μmoles of potassium chloride, 90 μmoles of succinate or 60 μmoles of α-ketoglutarate as substrate, 2 μmoles of adenosine triphosphate, and 2.5 μmoles of the test compounds in a total volume of 1.8 ml. After the basal metabolic (state 4) rate was obtained, 0.257 μmole of adenosine diphosphate was added to obtain the adenosine diphosphate stimulated respiration (state 3) rate (17). The rates were calculated as μl of oxygen consumed/mg of liver/hr.

**Liver, Small Intestine, and Fecal Lipid Extraction**—In male $CF_1$ mice that had been administered test drugs for 16 days, the liver, small intestine, and fecal materials (24-hr collection) were removed and a 10% homogenate in 0.25 *M* sucrose and 0.001 *M* EDTA was prepared. An aliquot (2 ml) of the homogenate was extracted by the Folch *et al.* (18) and Bligh and Dyer (19) methods and the number of milligrams of lipid weighed. The lipid was dissolved in methylene chloride, and the cholesterol level (3), triglyceride levels[11], neutral lipid content (20), and phospholipid content (21) were determined.

**[$^{14}$C]Cholesterol Distribution in Mice and Rats**—Male $CF_1$ mice (~25 g) were administered test agents intraperitoneally for 14 days and rats were administered test agents orally. On day 13, 10 μCi of [4-$^{14}$C]-cholesterol (52.5 mCi/mmole) was administered, and feces were collected for 0–6, 6–12, and 12–24 hr postadministration. Twenty-four hours after cholesterol administration, the major organs were excised and samples of blood, chyme, and urine were collected. Homogenates (10%) were prepared of the tissues which were combusted[12] and counted. Some tissue samples were plated on filter paper[13], dried, and digested for 24 hr in base[8] at 40° and counted. Results were expressed as dpm/mg of wet tissue and dpm/mg of total organ.

**Cholesterol Absorption Study**—Male Holtzman rats (~400 g) were administered test drugs intraperitoneally for 14 days at 20 mg/kg/day.

[9] Clark electrode.
[10] Gilson Instruments.
[11] Bio-Dynamics/bmc Triglyceride Kit.
[12] Packard Tissue Oxidizer.
[13] Whatman No. 3.

Table IV—Effects of Phthalimide Analogues on Rat Organ Weights after 14 Days of Administration [a]

| | Liver | Lung | Heart | Kidney | Spleen |
|---|---|---|---|---|---|
| Control | 100 ± 5 | 100 ± 4 | 100 ± 3 | 100 ± 4 | 100 ± 6 |
| Phthalimide | 85 ± 6 [c] | 100 ± 5 | 93 ± 3 [c] | 89 ± 5 [c] | 73 ± 5 [b] |
| 3-*N*-Phthalimidopropionic acid | 84 ± 7 [c] | 91 ± 4 [c] | 87 ± 2 [b] | 88 ± 5 [c] | 60 ± 5 [b] |
| | Brain | Adrenals | Stomach | Small Intestine | Large Intestine |
| Control | 100 ± 5 | 100 ± 8 | 100 ± 12 | 100 ± 10 | 100 ± 8 |
| Phthalimide | 100 ± 6 | 95 ± 7 | 98 ± 9 | 92 ± 11 | 108 ± 10 |
| 3-*N*-Phthalimidopropionic acid | 110 ± 7 | 106 ± 8 | 97 ± 13 | 101 ± 9 | 109 ± 5 |

[a] Percent of control, expressed as mean ± *SD*; $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.010$.

Table V—*In Vitro* Effects of Phthalimide Analogues (2.5 μmoles) on Mouse Liver Enzyme Activities after 16 Days of Dosing [a]

| Compound | Mitochondrial Citrate Exchange | Acetyl Coenzyme A Synthetase | Citrate Lyase | HMG CoA Reductase | Cholesterol Side Chain |
|---|---|---|---|---|---|
| 1% Carboxymethylcellulose | 100 ± 10 [d] | 100 ± 11 [e] | 100 ± 9 [f] | 100 ± 7 [g] | 100 ± 8 [h] |
| Phthalimide | 19 ± 7 [b] | 70 ± 8 [b] | 42 ± 6 [b] | 85 ± 7 | 111 ± 9 |
| 1-*N*-Phthalimidobutan-3-one | 10 ± 4 [b] | 53 ± 7 [b] | 34 ± 4 [b] | 72 ± 7 [b] | 90 ± 7 |
| 3-*N*-Phthalimidopropionic acid | 6 ± 3 [b] | 57 ± 7 [b] | 87 ± 6 [b] | 86 ± 4 [b] | 75 ± 5 [b] |

| Compound | Acetyl Coenzyme A Carboxylase | Fatty Acid Synthetase | Phosphatidate Phosphohydrolase | *sn*-Glycerol-3-*P*-acyl Transferase |
|---|---|---|---|---|
| 1% Carboxymethylcellulose | 100 ± 6 [i] | 100 ± 7 [j] | 100 ± 7 [k] | 100 ± 8 [l] |
| Phthalimide | 8 ± 4 [b] | 105 ± 8 | 55 ± 5 [b] | 28 ± 8 [b] |
| 1-*N*-Phthalimidobutan-3-one | 17 ± 3 [b] | 109 ± 7 | 65 ± 3 [b] | 46 ± 5 [b] |
| 3-*N*-Phthalimidopropionic acid | 18 ± 4 [b] | 107 ± 7 | 59 ± 4 [b] | 11 ± 5 [b] |

| Compound | Oxidative Phosphorylation: α Ketoglutarate State 4 | α Ketoglutarate State 3 | Succinate State 4 | Succinate State 3 |
|---|---|---|---|---|
| 1% Carboxymethylcellulose | 100 ± 6 [m] | 100 ± 7 [n] | 100 ± 5 [o] | 100 ± 6 [p] |
| Phthalimide | 64 ± 4 [b] | 65 ± 6 [b] | 64 ± 7 [b] | 65 ± 5 [b] |
| 1-*N*-Phthalimidobutan-3-one | 79 ± 6 [b] | 76 ± 7 [b] | 83 ± 5 [b] | 58 ± 4 [b] |
| 3-*N*-Phthalimidopropionic acid | 69 ± 5 [b] | 64 ± 5 [b] | 68 ± 6 [b] | 59 ± 3 [b] |

[a] Percent of control, expressed as mean ± *SD*; $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.010$. [d] 30.8 ± 3.1 mg % exchange of mitochondrial citrate. [e] 28.5 ± 3.14 mg of acetyl coenzyme A formed/g wet tissue/30 min. [f] 30.5 ± 2.74 mg of citrate hydrolyzed/g wet tissue/30 min. [g] 384,900 ± 26,943 dpm of cholesterol formed/g wet tissue/60 min. [h] 6080 ± 5.58 dpm of $CO_2$ formed/g wet tissue/18 hr. [i] 32,010 ± 1921 dpm/g wet tissue/30 min. [j] 37,656 ± 2635 dpm/g wet tissue/30 min. [k] 16.70 ± 1.16 μg Pi/g wet tissue/15 min. [l] 537,800 ± 43,024 dpm of triglyceride formed/g wet tissue/10 min. [m] 3.51 ± 0.21 μl of oxygen consumed/hr/mg of tissue. [n] 5.21 ± 0.36 μl of oxygen consumed/hr/mg of tissue. [o] 5.92 ± 0.30 μl of oxygen consumed/hr/mg of tissue. [p] 11.31 ± 0.67 μl of oxygen consumed/hr/mg of tissue.

On day 13, 10 μCi of 1,2-[$^3$H]-cholesterol (40.7 Ci/mmole) was administered to the rat orally. Twenty-four hours later, the blood was collected, and the serum was separated by centrifugation (22). Both the serum and the precipitate were counted.

**Bile Cannulation Study**—Male Holtzman rats (~400 g) were treated with test drugs at 20 mg/kg/day orally for 14 days; the rats were anesthetized with chlorpromazine (25 mg/kg) followed after 30 min by pentobarbital (22 mg/kg ip). The duodenum section of the small intestine was isolated and ligatures were placed around the pyloric sphincter and at a site distally approximately one-third of the way down the duodenum. Sterile isotonic saline was injected into the sectioned off duodenum segment. The saline expanded the duodenum and the common bile duct. Once the bile duct was identified, a loose ligature was placed around the bile duct, and a nich was introduced into the duct immediately before it enters the duodenum. Plastic tubing[14] was introduced into the duct, passing the ligature, and tied in place. The ligatures around the duodenum were then removed. Once bile was freely moving down the cannulated tube, [1,2-$^3$H]cholesterol (40.7 Ci/mmole) was injected intravenously into the rats. The bile was collected over the next 6 hr and measured (in ml). Aliquots were counted as well as analyzed for cholesterol content (3).

**Plasma Lipoprotein Fractions**—Male Holtzman rats (~400 g) were administered test drugs at 20 mg/kg/day 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 (23) and Havel *et al.* (24) into the chylomicrons, very low-density lipoproteins, high-density lipoproteins, and low-density lipoproteins. Each of the fractions were analyzed for cholesterol (3), triglyceride[11], neutral lipids (20), phospholipids (21), and protein levels (25).

[14] PE-10 Intramedic Polyethylene tubing.

## RESULTS

Clearly, phthalimide, 1-*N*-phthalimidobutan-3-one, and 3-*N*-phthalimidopropionic acid were effective in lowering serum cholesterol and triglycerides in rats and mice. The 20-mg/kg dose appeared to be the most effective dose in rodents (Table I). The phthalimide analogues were not only effective in normogenic mice, but also in hyperlipidemic-induced mice where serum cholesterol was reduced ~150% and triglyceride levels were reduced to below normal levels (Table II). The phthalimide analogues essentially had no effect on body weight increase in rats over the period of drug administration or the consumption of food compared with the control (Table III). Organ weights of the treated rats after 14 days of dosing were suppressed slightly in the case of the liver and moderately in the case of the spleen (Table IV). All other organs appeared to be within the normal range.

Examination of mouse liver enzymatic activities (Table V) showed that phthalimide analogues present at 2.5 μmoles suppressed mitochondrial exchange of citrate to the cytoplasm and cytoplasmic acetyl coenzyme

Table VI—*In Vitro* Inhibition of Rat Liver Lipid Enzymes

| Enzyme | $ID_{50}$ Values ($10^{-3}$ *M*): Phthalimide | 1-*N*-Phthalimidobutan-3-one | 3-*N*-Phthalimidopropionic Acid |
|---|---|---|---|
| Acetyl Coenzyme A Synthetase | 1.285 | 1.333 | 1.361 |
| Acetyl Coenzyme A Carboxylase | 1.221 | 2.100 | 2.227 |
| Phosphatidate Phosphohydrolase | 0.168 | 7.440 | 2.800 |
| *sn*-Glycerol-3-*P*-acyl Transferase | 5.174 | 7.616 | 7.168 |

**Table VII—*In Vivo* Effects of Phthalimide Analogues on Male $CF_1$ Mouse Liver Enzymes [a]**

| Compound | Dose mg/kg/day | Acetyl Coenzyme A Synthetase | HMG CoA Reductase | Acetyl Coenzyme A Carboxylase | Phosphatidate Phosphohydrolase | Fatty Acid Synthetase | Liver Lipids | Small Intestine Lipids |
|---|---|---|---|---|---|---|---|---|
| Control (1% carboxymethylcellulose) | — | 100 ± 7[d] | 100 ± 6[e] | 100 ± 5[f] | 100 ± 8[g] | 100 ± 6[h] | 100 ± 9[i] | 100 ± 7 |
| Phthalimide | 10 | 76 ± 5[b] | 89 ± 9 | 60 ± 5[b] | 91 ± 9 | 79 ± 8[b] | 70 ± 8[b] | 106 ± 8 |
| | 20 | 76 ± 6[b] | 89 ± 10 | 62 ± 6[b] | 67 ± 8[b] | 78 ± 7[b] | 70 ± 7[b] | 58 ± 5 |
| | 40 | 75 ± 4[b] | 88 ± 8 | 56 ± 5[b] | 29 ± 5[b] | 82 ± 4[b] | 68 ± 8[b] | 77 ± 6 |
| | 60 | 84 ± 5[b] | 105 ± 10 | 57 ± 4[b] | 3 ± 2[b] | 76 ± 6[b] | 64 ± 6[b] | 88 ± 7 |
| 3-*N*-Phthalimidopropionic acid | 10 | 76 ± 6[b] | 68 ± 6[b] | 79 ± 6[b] | 69 ± 6[b] | 91 ± 5 | 73 ± 5[b] | 96 ± 7 |
| | 20 | 79 ± 7[b] | 59 ± 5[b] | 66 ± 5[b] | 76 ± 7[b] | 96 ± 6 | 67 ± 4[b] | 76 ± 6 |
| | 40 | 82 ± 8[c] | 79 ± 4[b] | 58 ± 4[b] | 88 ± 8 | 107 ± 7 | 66 ± 5[b] | 77 ± 6 |
| | 60 | 82 ± 6[c] | 84 ± 6[c] | 47 ± 4[b] | 83 ± 8[c] | 109 ± 5 | 62 ± 6[b] | 92 ± 8 |

[a] Percent of control, expressed as mean ± *SD*; $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.010$. [d] 28.5 ± 3.14 mg of acetyl coenzyme A formed/g wet tissue/30 min. [e] 384,900 ± 26,943 dpm of cholesterol formed/g wet tissue/60 min. [f] 32,010 ± 1921 dpm/g wet tissue/30 min. [g] 37,656 ± 2635 dpm/g wet tissue/30 min. [h] 16.70 ± 1.16 μg Pi released/g wet tissue/15 min. [i] 79.5 ± 5.56 mg/g wet tissue.

A synthetase and adenosine triphosphate-dependent citrate lyase activities significantly. 3-Hydroxy-3-methylglutaryl coenzyme A reductase activity and side-chain oxidation of cholesterol were unaffected by the agents. Acetyl coenzyme A carboxylase, phosphatidate phosphohydrolase, and *sn*-glycerol-3-phosphate acyl transferase activities were suppressed by the phthalimide analogues, whereas fatty acid synthetase activity was unaffected. Both the basal and adenosine diphosphate-stimulated respiration states of oxidative phosphorylation of liver were reduced in the presence of the analogues. $ID_{50}$ values obtained from rat liver assays are noted in Table VI for acetyl coenzyme A synthetase, acetyl coenzyme A carboxylase, phosphatidate phosphohydrase, and *sn*-glycerol-3-phosphate acyl transferase. $ID_{50}$ values for 3-hydroxy-3-methylglutaryl coenzyme A reductase and fatty acid synthetase could not be obtained in the concentration range employed for these studies. When the same enzyme activities were tested *in vivo* in mice, it can readily be seen that the same enzyme activities were inhibited significantly (Table VII) after 16 days of dosing. In addition, suppression of 3-hydroxy-3-methyl glutaryl coenzyme A reductase activity was observed; in particular 3-*N*-phthalimidopropionic acid produced significant reduction of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Dose-related suppression of acetyl coenzyme A carboxylase and phosphatidate phosphohydrolase activities and lipid content was observed for phthalimide, and acetyl coenzyme A carboxylase activity and lipid content suppression was observed for the 3-*N*-phthalimidopropionic acid.

Analysis of the lipid content (Table VIII) revealed that cholesterol,

**Table VIII—Effects of Phthalimide Analogues on Lipid Content of Mouse Liver and Small Intestine after 16 Days of Dosing [a]**

| | Liver | | Small Intestine | |
|---|---|---|---|---|
| Dosage | Phthalimide | 3-*N*-Phthalimidopropionic Acid | Phthalimide | 3-*N*-Phthalimidopropionic Acid |
| | | Total Cholesterol | | |
| Control | 100 ± 7[d] | 100 ± 7[d] | 100 ± 7[h] | 100 ± 7[h] |
| 10 mg/kg | 74 ± 6[b] | 63 ± 6[b] | 113 ± 6 | 108 ± 6 |
| 20 mg/kg | 34 ± 4[b] | 48 ± 5[b] | 194 ± 8[b] | 242 ± 8[b] |
| 40 mg/kg | 56 ± 5[b] | 76 ± 7[b] | 169 ± 9[b] | 204 ± 7[b] |
| 60 mg/kg | 63 ± 5[b] | 58 ± 6[b] | 169 ± 8[b] | 95 ± 6 |
| | | Neutral Lipids | | |
| Control | 100 ± 4[e] | 100 ± 4[e] | 100 ± 5[i] | 100 ± 5[i] |
| 10 mg/kg | 51 ± 5[b] | 25 ± 3[b] | 113 ± 6[c] | 78 ± 6[b] |
| 20 mg/kg | 36 ± 5[b] | 22 ± 2[b] | 45 ± 5[b] | 48 ± 5[b] |
| 40 mg/kg | 18 ± 4[b] | 22 ± 3[b] | 45 ± 4[b] | 55 ± 6[b] |
| 60 mg/kg | 15 ± 5[b] | 32 ± 2[b] | 105 ± 7 | 70 ± 5[b] |
| | | Triglycerides | | |
| Control | 100 ± 5[f] | 100 ± 5[f] | 100 ± 6[j] | 100 ± 6[j] |
| 10 mg/kg | 37 ± 7[b] | 54 ± 4[b] | 70 ± 5[b] | 109 ± 6 |
| 20 mg/kg | 31 ± 6[b] | 39 ± 3[b] | 47 ± 4[b] | 62 ± 5[b] |
| 40 mg/kg | 38 ± 6[b] | 40 ± 4[b] | 48 ± 3[b] | 70 ± 5[b] |
| 60 mg/kg | 30 ± 7[b] | 44 ± 4[b] | 70 ± 5[b] | 93 ± 6 |
| | | Phospholipids | | |
| Control | 100 ± 8[g] | 100 ± 8[g] | 100 ± 8[k] | 100 ± 8[k] |
| 10 mg/kg | 74 ± 7 | 128 ± 7[b] | 88 ± 7 | 101 ± 7 |
| 20 mg/kg | 144 ± 8 | 121 ± 4[b] | 62 ± 6[b] | 48 ± 5[b] |
| 40 mg/kg | 139 ± 8 | 128 ± 9[b] | 84 ± 7[c] | 44 ± 4[b] |
| 60 mg/kg | 137 ± 9 | 127 ± 7[b] | 85 ± 8 | 74 ± 6[b] |

[a] Percent of control, expressed as mean ± *SD*; $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.010$. [d] 12.24 mg/g tissue. [e] 28.35 mg/g tissue. [f] 4.77 mg/g tissue. [g] 4.39 mg/g tissue. [h] 7.81 mg/g tissue. [i] 7.18 mg/g tissue. [j] 1.06 mg/g tissue. [k] 2.02 mg/g tissue.

neutral lipids, and triglyceride content were reduced in liver homogenate with an elevation in phospholipid content. In the small intestine, cholesterol content was elevated and neutral lipid, triglyceride, and phospholipid content was reduced at the optimum dose, 20 mg/kg.

Radiolabeled cholesterol studies (Tables IX and X) demonstrate that lipids removed from the blood compartment were not being deposited in major organs: *i.e.*, liver, heart, brain, kidney, and lung in rats (where the cholesterol was administered orally) and mice (where the cholesterol was administered intraperitoneally). The labeled cholesterol in the treated animals was found in higher concentrations in the intestine, chyme, and feces. Cholesterol absorption (Table XI) from the gut over a 24-hr period was reduced by phthalimide and the propionic analogue. In conjunction with this observation, it can also be seen that the agents accelerated bile flow and biliary secretion of cholesterol (Table XII). The increase in cholesterol secretion is also reflected in the lipid extractions from feces of mice after treatment with phthalimide (Table XIII). Elevations in neutral lipids and phospholipids were also noted in the fecal excretion at all doses employed. Significant reduction of the lipid and protein content of serum lipoprotein fractions (Table XIV) were observed in rats after treatment with all three agents. Cholesterol, neutral lipids, and triglycerides were reduced in the chylomicron, very low-, low- and high-density lipoproteins. Phospholipid content was elevated in the chylomicron, very low-, and high-density lipoprotein fractions. Protein content was reduced in the chylomicron and very low-density fraction for all three agents. However, only phthalimide treatment resulted in a reduction of the protein of the low- and high-density lipoprotein fractions.

Data are expressed in Tables I–VIII, XIII, and XIV as percent of control (mean ± the standard deviation). The probable significant level ($p$) between each test group and the control group was determined by the Student's $t$ test.

## DISCUSSION

A positive correlation between elevated serum cholesterol and triglycerides and the subsequent development of atherosclerosis has been made (26). Over the years, diet and chemotherapy have been the treatments of choice. However, the number of drugs distributed commercially for the treatment of hyperlipidemic states has been decreasing due to incurred hepatotoxicity, cardiovascular changes, and GI disturbances or due to the fact that the agents did not effectively reduce both serum cholesterol and triglycerides in humans.

The required doses to elicit hypolipidemic activity for phthalimide analogues are in a safe therapeutic range compared with the $LD_{50}$ of >2 g/kg (2). The agents were more active than clofibrate in reducing both serum cholesterol and triglyceride levels in rodents (27). Clofibrate reduced total lipid and cholesterol by 15–20% of the control values at doses of 100–200 mg/kg daily by oral or subcutaneous routes of administration (27).

The suppression of cholesterol synthesis by the phthalimide analogues was not exclusively at the regulatory site of the cholesterol pathway, *e.g.*, clofibrate blocks 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Rather, earlier steps in the *de novo* synthesis of acetyl coenzyme A appeared to be blocked by phthalimide analogues. Reduction of available citrate from the mitochondria, where it is generated from glycolysis, may be a critical factor in the synthesis of cytoplasmic acetyl coenzyme A. Reduction of mitochondrial citrate exchange in the rat liver has been shown with other agents, *e.g.*, 1,2,3-benzenetricarboxylate and 2-*p*-iodobenzylmalonate (8). The inhibition of acetate incorporation into

**Table IX—Effects of Phthalimide Analogues after 14 Days of Dosing on [$^{3}$H]Cholesterol Distribution 24 hr after Administration [a]**

| Organ | Control | | Phthalimide | | 3-*N*-Phthalimidopropionic Acid | |
|---|---|---|---|---|---|---|
| | Total Organ dpm | Recovery, % | Total Organ dpm | Recovery, % | Total Organ dpm | Recovery, % |
| Brain | 42,412 | 1.21 | 23,394 | 0.64 | 21,694 | 0.62 |
| Heart | 37,638 | 1.07 | 26,593 | 0.76 | 25,893 | 0.74 |
| Lung | 100,584 | 2.87 | 80,827 | 2.31 | 75,229 | 2.15 |
| Liver | 901,785 | 23.77 | 627,374 | 17.93 | 534,300 | 15.27 |
| Spleen | 67,760 | 1.93 | 56,684 | 1.62 | 41,638 | 1.19 |
| Kidney | 69,192 | 1.97 | 47,237 | 1.35 | 58,783 | 1.68 |
| Stomach | 127,446 | 3.64 | 80,477 | 2.30 | 52,835 | 1.51 |
| Small Intestine | 851,406 | 24.33 | 862,507 | 24.65 | 1,067,550 | 30.51 |
| Large Intestine | 246,924 | 7.05 | 500,709 | 14.31 | 244,581 | 6.99 |
| Chyme | 163,977 | 4.68 | 220,088 | 6.29 | 269,074 | 7.69 |
| Feces | 889,892 | 25.43 | 974,126 | 27.84 | 1,106,389 | 31.62 |

[a] $n = 6$.

fatty acids in neonatal chicks has also been shown for 1,2,3-benzenetricarboxylate. Reduction of cytoplasmic levels of acetyl coenzyme A would markedly affect both cholesterol and fatty acid synthesis. Hepatic triglyceride synthesis appeared to be blocked at the regulatory sites, *i.e.*, *sn*-glycerol-3-phosphate acyl transferase and phosphatidate phosphohydrase. The inhibition of phosphatidate phosphohydrolase activity by the phthalimide analogues may explain the elevation in levels of phospholipids observed in the liver and lipoprotein fractions. A number of agents have been identified that inhibit these two enzymes [*e.g.*, 1,3-bis(substituted phenoxy)-2-propanone, 1-methyl-4-piperidyl bis(*p*-chlorophenoxy)acetate, and clofibrate (28)] with a concomitant reduction of serum triglycerides (15). $ID_{50}$ values obtained for inhibition of these enzyme activities in the rat liver seem to be realistic considering the *in vivo* dose required for potent hypolipidemic activity of the phthalimide analogues.

The phthalimide analogues did not uncouple liver oxidative phosphorylation processes, as has been observed for clofibrate (29) and which is probably due to the detergent action of clofibrate causing disruption of the mitochondrial membranes. The phthalimide did suppress both basal and adenosine diphosphate-stimulated respiration, which may reduce available energy for synthetic processes. The phthalimide analogues, like clofibrate (30), accelerated the excretion of cholesterol and probably cholesterol esters through the biliary route; these probably are not reabsorbed from the gut *via* the extrahepatic circulation process. Data from the biliary study suggest that this mode of action of the phthalimide analogues may be of sufficient importance to account for some of the observed reduction of serum lipids.

The lipids removed from the blood compartment are not being deposited in the major organs of the body, which should be an ideal characteristic of a hypolipidemic agent. Other studies have indicated that the reduction of serum cholesterol and triglycerides after 16 and 14 days of dosing, respectively, were returned to predrug administration levels (31), indicating that the pharmacological effects of the phthalimide analogues are freely reversible after 2 weeks. Low-density lipoprotein, derived from intravascular very low-density lipoprotein, is the major lipoprotein that transports cholesterol (60–70%) to the peripheral tissue and in most species back to the liver (32, 33). Low-density lipoproteins are deposited in the atherosclerotic plaque shedding their cholesterol into the form cells (34–36). An increase in dietary cholesterol is reflected as an increase in cholesterol levels of low-density and, to a less extent, high-density and intermediate-density lipoproteins (37). High-density lipoproteins, which originate from the liver and intestine, play an important role in the interconversion of lipid components to various lipoprotein fractions and in the return of lipids from the peripheral tissue to the liver. Both the low- and high-density lipoproteins are actively taken up by the liver low-density lipoprotein receptors (33, 38). High-density lipoproteins may participate in the transport of cholesterol out of the atherosclerotic plaque or may slow down the atherogenic process (39). Studies have suggested that high-density lipoproteins are inversely related to the incidence of coronary heart disease (*e.g.*, Troms study) (40–43). However, other studies in humans dispute these findings (44–46). Other workers have proposed that the ratio of low-density to high-density lipoprotein is more important in predicting the incidence of atherosclerosis (46, 47). The cholesterol content of each fraction or the ratio of high-density lipoprotein cholesterol to total cholesterol has also been discussed as possible parameters for predicting coronary heart disease (46, 47).

**Table X—[$^{3}$H]Cholesterol Content 24 hr after Injection of 10 μCi in $CF_1$ Mice Administered Drug (20 mg/kg/day) for 14 Days [a]**

| Organ | Control | | Phthalimide | |
|---|---|---|---|---|
| | Total dpm | Tritium Recovered, % | Total dpm | Tritium Recovered, % |
| Brain | 8305 | 0.124 | 7042 | 0.105 |
| Lung | 33,949 | 0.506 | 20,589 | 0.307 |
| Heart | 23,774 | 0.354 | 7041 | 0.105 |
| Liver | 436,066 | 6.502 | 302,133 | 4.505 |
| Spleen | 54,303 | 0.810 | 55,329 | 0.825 |
| Kidney | 82,954 | 1.237 | 45,672 | 0.681 |
| Stomach | 266,464 | 3.973 | 146,941 | 2.191 |
| Small Intestine | 607,318 | 9.056 | 737,660 | 10.999 |
| Large Intestine | 791,297 | 11.799 | 542,437 | 8.0881 |
| Subtotal | | 34.361 | | 27.806 |
| Feces | | | | |
| 0–6 hr | 374,855 | 5.589 | 1,013,973 | 15.119 |
| 6–12 hr | 2,126,220 | 31.703 | 2,333,767 | 34.798 |
| 12–24 hr | 1,901,108 | 28.333 | 1,494,109 | 22.277 |
| Total excreted in 24 hr | | 65.625 | | 72.194 |
| Plasma/ml | 256,730 | | 197,092 | |

[a] $n = 6$.

**Table XI—Effects of Phthalimide Analogues on 24-hr [$^{3}$H]-Cholesterol Absorption in Rats After 14 Days of Dosing**

| Compound | Plasma [a] | Percent of Control |
|---|---|---|
| Control | 14280 | |
| Phthalimide | 6283 | 44 [b] |
| 3-*N*-Phthalimidopropionic acid | 5569 | 39 [b] |

[a] dpm assuming 17 ml total volume/rat; $n = 6$. [b] $p \leq 0.001$.

Treatment with clofibrate does not significantly alter the high-density lipoprotein fraction in humans, but lowers the cholesterol of this fraction slightly; however, clofibrate does not protect against coronary heart disease (46). Treatment with probucol for short periods of time reduces the high-density lipoproteins as well as the ratio of cholesterol content of the high-density lipoproteins to total cholesterol content with no increase in the incidence of heart disease (44). A number of preliminary studies have alluded to the fact that high-density lipoproteins are taken up by the liver where the cholesterol is released from the apoprotein and acts as a precursor for bile acids and biliary cholesterol (33, 48, 49).

Treatment with phthalimide derivatives for a 2-week period lowers cholesterol and triglyceride levels of chylomicrons, very low-, low-, and high-density lipoprotein fractions of the rat. Although the lipid distribution among the lipoproteins fractions in the rat is different from the human, some analogies may be drawn. The reduction of cholesterol and

**Table XII—Effects of Phthalimide Analogues on Bile Flow and Biliary [$^{3}$H]Cholesterol Excretion in Rats After 14 Days of Dosing [a]**

| | 6-hr Bile Flow | Total cpm | Cholesterol, mg% |
|---|---|---|---|
| Control | 3.36 ± 0.16 | 1016 ± 81 | 111 ± 8 |
| Phthalimide | 7.60 ± 0.40 [b] | 1686 ± 144 [b] | 201 ± 12 [b] |
| 3-*N*-Phthalimido-propionic acid | 5.23 ± 0.57 [b] | 1554 ± 103 [b] | 171 ± 10 [b] |

[a] $n = 6$. [b] $p \leq 0.001$.

**Table XIII—Effects of Phthalimide on Lipid Content of Fecal Material Collected after 16 Days of Dosing [a]**

| Dosage | Total Cholesterol | Neutral Lipids | Triglycerides | Phospholipids |
|---|---|---|---|---|
| | | 0–6-hr Fecal Collection | | |
| Control | $100 \pm 7^{d}$ | $100 \pm 8^{e}$ | $100 \pm 6^{f}$ | $100 \pm 5^{g}$ |
| 10 mg/kg | $139 \pm 8^{b}$ | $121 \pm 8^{c}$ | $96 \pm 5$ | $166 \pm 6^{b}$ |
| 20 mg/kg | $134 \pm 7^{b}$ | $151 \pm 6^{b}$ | $90 \pm 4$ | $192 \pm 8^{b}$ |
| 40 mg/kg | $188 \pm 9^{b}$ | $156 \pm 7^{b}$ | $91 \pm 6$ | $177 \pm 7^{b}$ |
| 60 mg/kg | $115 \pm 5^{c}$ | $128 \pm 6$ | $99 \pm 8$ | $116 \pm 6^{c}$ |
| | | 6–12-hr Fecal Collection | | |
| Control | $100 \pm 7^{h}$ | $100 \pm 8^{i}$ | $100 \pm 6^{j}$ | $100 \pm 6^{k}$ |
| 10 mg/kg | $150 \pm 7^{b}$ | $137 \pm 7^{b}$ | $100 \pm 6$ | $112 \pm 6$ |
| 20 mg/kg | $157 \pm 8^{b}$ | $204 \pm 8^{b}$ | $102 \pm 5$ | $145 \pm 7^{b}$ |
| 40 mg/kg | $140 \pm 6^{b}$ | $162 \pm 7^{b}$ | $106 \pm 6$ | $182 \pm 7^{b}$ |
| 60 mg/kg | $144 \pm 6^{b}$ | $161 \pm 8^{b}$ | $112 \pm 7$ | $125 \pm 6^{b}$ |
| | | 12–24-hr Fecal Collection | | |
| Control | $100 \pm 8^{l}$ | $100 \pm 6^{m}$ | $100 \pm 7^{n}$ | $100 \pm 6^{o}$ |
| 10 mg/kg | $148 \pm 6^{b}$ | $133 \pm 5^{b}$ | $97 \pm 6$ | $148 \pm 8^{b}$ |
| 20 mg/kg | $146 \pm 7^{b}$ | $230 \pm 8^{b}$ | $98 \pm 7$ | $190 \pm 7^{b}$ |
| 40 mg/kg | $133 \pm 6^{b}$ | $216 \pm 7^{b}$ | $98 \pm 7$ | $150 \pm 6^{b}$ |
| 60 mg/kg | $99 \pm 7$ | $154 \pm 6^{b}$ | $97 \pm 7$ | $129 \pm 5^{b}$ |

[a] Percent of control, expressed as mean ± $SD$; $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.010$. [d] 19.77 mg/g tissue. [e] 17.62 mg/g tissue. [f] 1.74 mg/g tissue. [g] 1.85 mg/g tissue. [h] 29.47 mg/g tissue. [i] 33.94 mg/g tissue. [j] 1.86 mg/g tissue. [k] 1.61 mg/g tissue. [l] 28.47 mg/g tissue. [m] 33.94 mg/g tissue. [n] 1.86 mg/g tissue. [o] 1.39 mg/g tissue.

**Table XIV—Effects of Phthalimide Analogues on Holtzman Lipoprotein Fraction after 14 Days of Dosing [a]**

| Compound | Cholesterol | Neutral Lipids | Triglycerides | Phospholipids | Protein |
|---|---|---|---|---|---|
| | | | Chylomicrons | | |
| 1% Carboxymethylcellulose | $100 \pm 9^{b}$ | $100 \pm 8^{c}$ | $100 \pm 6^{d}$ | $100 \pm 10^{e}$ | $100 \pm 7^{f}$ |
| Phthalimide | $42 \pm 4^{v}$ | $57 \pm 6^{v}$ | $49 \pm 4^{v}$ | $374 \pm 13^{v}$ | $61 \pm 5^{v}$ |
| 1-*N*-Phthalimidobutan-3-one | $53 \pm 4^{v}$ | $73 \pm 6^{v}$ | $52 \pm 5^{v}$ | $99 \pm 11$ | $61 \pm 6^{v}$ |
| 3-*N*-Phthalimidopropionic acid | $66 \pm 5^{v}$ | $17 \pm 3^{v}$ | $51 \pm 6^{v}$ | $197 \pm 15^{v}$ | $57 \pm 6^{v}$ |
| | | | Very Low-Density Lipoprotein | | |
| 1% Carboxymethylcellulose | $100 \pm 8^{g}$ | $100 \pm 9^{h}$ | $100 \pm 7^{i}$ | $100 \pm 7^{j}$ | $100 \pm 8^{k}$ |
| Phthalimide | $47 \pm 5^{v}$ | $75 \pm 7^{v}$ | $33 \pm 4^{v}$ | $205 \pm 10^{v}$ | $43 \pm 3^{v}$ |
| 1-*N*-Phthalimidobutan-3-one | $75 \pm 6^{v}$ | $78 \pm 6^{w}$ | $78 \pm 7^{v}$ | $213 \pm 9^{v}$ | $86 \pm 7$ |
| 3-*N*-Phthalimidopropionic acid | $85 \pm 8$ | $85 \pm 6$ | $40 \pm 4^{v}$ | $359 \pm 5^{v}$ | $43 \pm 5^{v}$ |
| | | | Low-Density Lipoprotein | | |
| 1% Carboxymethylcellulose | $100 \pm 9^{l}$ | $100 \pm 7^{m}$ | $100 \pm 8^{n}$ | $100 \pm 7^{o}$ | $100 \pm 8^{p}$ |
| Phthalimide | $42 \pm 3^{v}$ | $68 \pm 6^{v}$ | $44 \pm 4^{v}$ | $81 \pm 6^{v}$ | $67 \pm 7^{v}$ |
| 1-*N*-Phthalimidobutan-3-one | $55 \pm 5^{v}$ | $77 \pm 5^{v}$ | $49 \pm 4^{v}$ | $42 \pm 4^{v}$ | $101 \pm 9$ |
| 3-*N*-Phthalimidopropionic acid | $57 \pm 7^{v}$ | $73 \pm 5^{v}$ | $50 \pm 4^{v}$ | $71 \pm 6^{v}$ | $98 \pm 8$ |
| | | | High-Density Lipoprotein | | |
| 1% Carboxymethylcellulose | $100 \pm 8^{q}$ | $100 \pm 9^{r}$ | $100 \pm 4^{s}$ | $100 \pm 6^{t}$ | $100 \pm 8^{u}$ |
| Phthalimide | $44 \pm 5^{v}$ | $48 \pm 4^{v}$ | $51 \pm 3^{v}$ | $212 \pm 7^{v}$ | $66 \pm 6^{v}$ |
| 1-*N*-Phthalimidobutan-3-one | $56 \pm 5^{v}$ | $52 \pm 3^{v}$ | $65 \pm 5^{v}$ | $324 \pm 12^{v}$ | $101 \pm 7$ |
| 3-*N*-Phthalimidopropionic acid | $52 \pm 8^{v}$ | $64 \pm 5^{v}$ | $53 \pm 4^{v}$ | $225 \pm 8^{v}$ | $104 \pm 8$ |

[a] Percent of control, expressed as mean ± $SD$; $n = 6$. [b] 337 μg/ml. [c] 67 μg/ml. [d] 420 μg/ml. [e] 149 μg/ml. [f] 221 μg/ml. [g] 190 μg/ml. [h] 98 μg/ml. [i] 221 μg/ml. [j] 26 μg/ml. [k] 50 μg/ml. [l] 210 μg/ml. [m] 10 μg/ml. [n] 45 μg/ml. [o] 41 μg/ml. [p] 122 μg/ml. [q] 544 μg/ml. [r] 620 μg/ml. [s] 27 μg/ml. [t] 153 μg/ml. [u] 657 μg/ml. [v] $p \leq 0.001$. [w] $p \leq 0.010$.

triglyceride content of low- and high-density lipoproteins was approximately of the same magnitude after drug treatment which probably did not change the low-density to high-density lipoprotein ratio during drug treatment. The reduction of cholesterol in both lipoprotein fractions may reflect the increase in cholesterol excretion in the bile after phthalimide treatment. Supposedly, the cholesterol esters are exchanged between high- and low-density lipoproteins (50), so a reduction of one by drug therapy should reflect in the other fraction ultimately.

After phthalimide treatment it was noted that the phospholipid content of the very low-density and chylomicron fractions were elevated. Clofibrate is also known to increase the phospholipid content of liver and serum (51). The very low-density and chylomicron remnants have their phospholipid content enzymatically removed before they can be taken up by the liver. If the phospholipid, *e.g.*, phosphatidyl choline, remains on these remnants then the remnants are not taken up by the liver (52). The inability to take up these fractions may be critical to the regulation of liver cholesterol synthesis, since in hyperlipidemic patients and rats the cholesterol released from very low-density lipoproteins inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (53). However, the cholesterol release from very low-density lipoproteins of normal rats did not inhibit the enzyme, suggesting that the actual content of cholesterol in the very low-density lipoprotein fraction may be the one decisive factor in the regulation of liver cholesterol synthesis (54).

Mixed results have been observed regarding the cholesterol content of high- and low-density lipoprotein fractions. In the fibroblast system, they inhibit the regulatory enzyme of cholesterol synthesis. However, in the hepatocyte tissue culture system, the high cholesterol content of high- and low-density lipoprotein did not inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (54). Further studies are being conducted with the relationship of lipoprotein fractions of rats after phthalimide treatment.

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