Effect of Sulfadimethoxine on Thiopental Distribution and Elimination in Rats

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Abstract □ The effect of sulfadimethoxine on the distribution and elimination of thiopental was examined by comparing the change in the steady-state volume of distribution (V_{ss}) determined from both *in vivo* plasma elimination and *in vitro* serum and tissue binding studies in rats. The plasma disappearance of thiopental after a 12-mg/kg iv dose followed a biexponential decline in both the control and sulfadimethoxine-treated rats. The plasma thiopental concentrations under the steady-state plasma sulfadimethoxine concentration (500 μg/ml) were significantly lower than those of the control rats. In the sulfadimethoxine-treated rats, the pharmacokinetic parameter β significantly decreased while V_{ss} significantly increased to 3.6-fold that of the control rats. With sulfadimethoxine, a significant increase was observed in the apparent dissociation constant (K_d) of thiopental to serum protein by equilibrium dialysis, but the total number of binding sites was not altered. The *in vitro* serum free fraction of thiopental was increased to about 2.6-fold in the presence of sulfadimethoxine. The free fraction of thiopental in the main distribution tissues (liver, muscle, and adipose) was determined by equilibrium dialysis with and without sulfadimethoxine. No significant changes were observed in the presence of sulfadimethoxine. The calculated V_{ss}, determined by the free fractions from *in vitro* binding experiments, also showed a significant increase. The ratio of V_{ss} with sulfadimethoxine to that of the control rats was 2.8. The total clearance did not change, but the intrinsic clearance decreased to one-half of that of the control rats due to the increase of the serum free fraction by sulfadimethoxine. It was concluded that sulfadimethoxine caused a displacement of thiopental in plasma protein binding, which significantly increased the free fraction of thiopental, and this result may explain the significant increase of V_{ss} and the decrease of both β and intrinsic clearance. Tissue binding of thiopental, however, was unaffected by sulfadimethoxine.

Keyphrases □ Sulfadimethoxine—effect on elimination and distribution of thiopental in rats, *in vitro* and *in vivo* studies □ Thiopental—elimination and distribution in rats, effect of sulfadimethoxine on thiopental metabolism, *in vitro* and *in vivo* studies □ Metabolism—effect of sulfadimethoxine on thiopental metabolism in rats, *in vitro* and *in vivo* studies

The apparent volume of distribution and total body clearance are influenced by age, disease, and drug–drug interaction. These changes are based on alterations in plasma and/or tissue binding, metabolism, and hepatic blood flow (1–5). The volume of distribution at steady state (V_{ss}) can be expressed by:

$$V_{ss} = V_p + (f_p/f_t)V_t \quad \text{(Eq. 1)}$$

where V_p is the plasma volume, V_t is the volume of the other body tissues, and f_p and f_t are the fractions of the drug present in unbound form in the plasma and tissue, respectively (6, 7). Equation 1 shows that V_{ss} is influenced by alterations in f_p or f_t due to the displacement of protein-bound drug. When metabolism is the rate-determining step of drug elimination, total body clearance (Cl_{tot}) can be expressed by:

$$Cl_{tot} \simeq f_p Cl_{int} \quad \text{(Eq. 2)}$$

where Cl_{int} is the intrinsic clearance of unbound drug (7). Equation 2 shows that both the increase in f_p, due to displacement by the second drug, and the change in Cl_{int}, due to metabolic inhibition or induction, may affect Cl_{tot}.

The present study determined the effect of sulfadimethoxide on the distribution and elimination of thiopental by comparing the changes of V_{ss}, which were determined from both *in vivo* plasma elimination and *in vitro* serum and tissue binding studies. The change of Cl_{int} also is discussed.

EXPERIMENTAL

Adult male Wistar rats[1], 245–280 g, were used. Under light ether anesthesia, the femoral vein and artery were cannulated with polyethylene tubing[2]. Cannulated rats were kept in restraining cages under normal housing conditions for 1 day prior to the experiments. All animals were fasted overnight (~15 hr) but had water *ad libitum* before the experiments. After a loading dose of 200 mg of sulfadimethoxine[3]/kg, 41.3 mg/kg/hr was infused through the femoral vein cannula for 3 hr with a constant-rate infusion pump[4]; with this dosage, steady-state concentrations of sulfadimethoxine (500 μg/ml) were obtained within 20–35 min after the beginning of the infusion.

At 50 min after the initiation of infusion, the rats were given 12 mg of thiopental[5]/kg in saline through the other femoral vein cannula over a 5-sec interval with a 500-μl syringe. Blood samples (0.25 ml) then were obtained at 1, 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 min in heparinized polyethylene centrifuge tubes[6]. The body temperature was kept at 37° by a heat lamp. Plasma was separated by centrifugation for 20 sec in a tabletop microfuge[6] and assayed for thiopental by the method of Brodie *et al.* (8). Sulfadimethoxine in plasma did not interfere with the assay of thiopental. The method of Tsuda and Matsunaga (9) was employed for the assay of sulfadimethoxine in plasma. The thiopental concentration data for individual animals were fitted to the equation $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ for the plasma concentration C_t at time t by nonlinear least-squares regression (10). Pharmacokinetic constants (Table I) were determined from the biexponential equation constants, *i.e.*, A, α, B, and β, using conventional equations (11).

Serum was separated from the blood, obtained through the carotid artery, by centrifugation for 10 min at 3000 rpm after standing for 60 min at room temperature. The serum free thiopental fraction was determined by equilibrium dialysis at 37° for 16 hr using semimicrocells[7] and a semipermeable membrane[8] against 0.05 *M* isotonic tromethamine–hydrochloric acid buffer (pH 7.4), containing 0.05–0.5 m*M* thiopental and 2.4 m*M* sulfadimethoxine. The protein binding of thiopental to serum and tissues was unchanged between 16 and 20 hr of dialysis at 37°.

Previous studies in this laboratory compared binding data of thiopental determined from equilibrium dialysis at 37° for 20 hr and from flow dialysis, but the difference between the two methods was insignificant (12). The effect of dialysis on the serum binding of thiopental also was examined at 37° for 24 hr with polarization analysis (13) using 1-anilino-8-naphthalenesulfonate and compared with the dialysis at 4° for 24 hr, but no denaturation of proteins after dialysis was observed. The sulfadimethoxine concentration in the protein chamber after dialysis was in the

[1] Nihon Seibutsu Zairyo, Tokyo, Japan.
[2] Type PE-10 (for femoral vein) and PE-50 (for femoral artery), Clay Adams, Becton–Dickinson Co., Parsippany, N.J.
[3] Daiichi Pharmaceutical Industries Co., Tokyo, Japan.
[4] Natsume Seisakusho Co., Tokyo, Japan.
[5] Tanabe Pharmaceutical Industries, Osaka, Japan.
[6] Beckman Instruments, Fullerton, Calif.
[7] Kokugo-gomu Co., Tokyo, Japan.
[8] Type 36/32, Visking Co., Chicago, Ill.

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Table I—Thiopental Pharmacokinetics in Rats[a]

Rat	Body Weight, g	A, µg/ml	α, min^{-1}	B, µg/ml	β, min^{-1}	k_{12}, min^{-1}	k_{21}, min^{-1}	k_{el}, min^{-1}	V_1, ml/kg	V_{ss}, ml/kg	$V_{d\beta}$, ml/kg	AUC, (µg min)/ml	Cl_{tot}[b], ml/(min kg)	Cl_{int}[c], ml/(min kg)
						Without Sulfadimethoxine								
1	250	269.7	21.03	15.2	0.007	19.774	1.126	0.138	42.1	782.3	787.8	2060.0	5.83	
2	270	24.7	0.27	14.5	0.007	0.155	0.104	0.017	306.3	762.7	793.9	2291.3	5.24	
3	245	49.7	3.00	22.6	0.009	2.063	0.944	0.030	166.0	524.1	528.0	2445.6	4.91	
Mean	255	114.7	8.10	17.4	0.008	7.330	0.724	0.062	171.5	689.7	703.2	2265.6	5.33	38.4
±*SE*	7.6	77.8	6.51	2.6	0.001	6.253	0.315	0.038	76.3	83.0	87.6	112.1	0.27	
						With Sulfadimethoxine								
11	250	6.0	0.26	6.1	0.003	0.125	0.132	0.006	989.3	1926.2	1946.4	2201.8	5.45	
12	280	6.6	0.08	5.4	0.002	0.038	0.036	0.005	1003.3	2071.6	2143.5	2433.0	4.93	
13	245	133.9	2.32	6.1	0.005	2.101	0.105	0.119	85.7	1794.1	1885.2	1178.1	10.18	
14	265	12.1	0.27	2.8	0.001	0.212	0.052	0.007	808.1	4129.1	4221.4	2030.4	5.91	
Mean	260	39.7	0.73	5.1[d]	0.003[d]	0.619	0.081	0.034	721.6	2480.2[d]	2549.1[d]	1960.8	6.62	17.9
±*SE*	9.1	31.4	0.53	0.8	0.001	0.495	0.023	0.028	216.6	552.5	560.1	273.6	1.20	

[a] Pharmacokinetic parameters were calculated by conventional equations (11) using biexponential equation constants from plasma disappearance curves. [b] Total clearance (Cl_{tot}) was calculated using Cl_{tot} = dose/AUC. [c] Intrinsic clearance (Cl_{int}) was calculated using $Cl_{tot} = (Q_h s f_p Cl_{int})/(Q_h s + f_p Cl_{int})$, where Q_h is the hepatic blood flow of 59.1 ml/min/kg (15), f_p is the plasma free fraction, s is the blood-to-plasma distribution ratio, and Cl_{tot} is the total clearance. The hematocrit value was 0.45. [d] Significantly different from the control rats ($p < 0.05$).

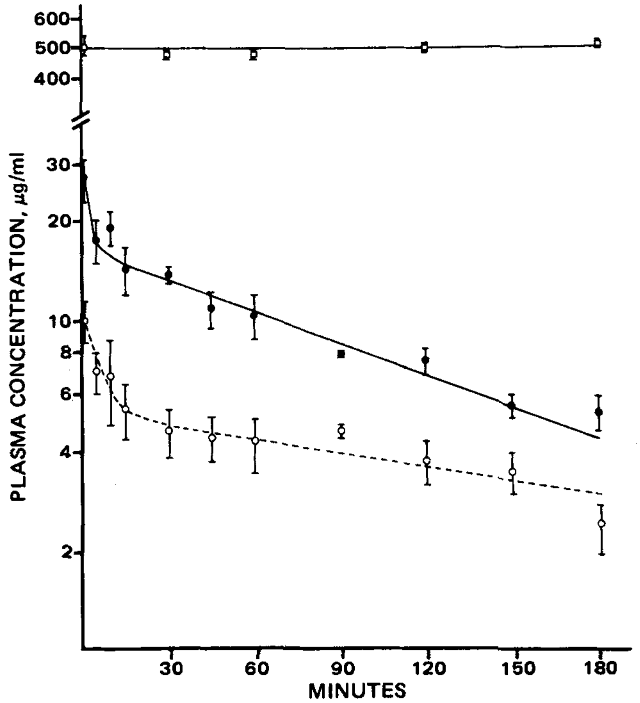

Figure 1—*Plasma disappearance curves of thiopental after 12 mg/kg iv. Each point and vertical bar represent the mean and standard error of three or four rats. Curves were calculated by the SALS method (10) using a digital computer. Key:* ●, *plasma concentration of control rats;* O, *plasma concentration of sulfadimethoxine-treated rats, which were infused with sulfadimethoxine (41.3 mg/kg/hr) after a loading dose of 200 mg/kg; and* □, *concentration of sulfadimethoxine.*

same range as that of the *in vivo* steady-state concentration of sulfadimethoxine (500 µg/ml) in plasma.

Liver, abdominal muscle, and adipose tissues were excised after the carotid artery bleeding. A 50% liver homogenate and a 25% muscle homogenate were prepared in 0.05 *M* tromethamine–hydrochloric acid buffer (pH 7.4) using a homogenizer[9]. The adipose homogenate was used without dilution and predialysis, but the liver and muscle homogenates were predialyzed against 0.05 *M* tromethamine–hydrochloric acid buffer (pH 7.4) at 4° for 24 hr. Equilibrium dialysis was performed on these three tissue homogenates at 37° for 16 hr, using 1 ml of buffer and 1 ml of each homogenate. The initial concentrations of thiopental in the buffer solution were 0.1 m*M* for the liver and muscle homogenates and 0.5 m*M* for adipose homogenate, and the initial concentration of sulfadimethoxine was 2 m*M* for all homogenates. After dialysis, the concentrations of both unbound thiopental and sulfadimethoxine in the buffer solution were determined as already described.

The unbound (free) fraction (f) in serum or tissue homogenate was calculated by:

$$C_{in} - 2C_f = C_b \quad \text{(Eq. 3)}$$

$$\frac{C_f}{C_f + pC_b} = f \quad \text{(Eq. 4)}$$

where C_{in} is the initial concentration in the buffer solution, C_f is the concentration of unbound drug in the buffer solution after dialysis, C_b is the concentration of bound drug in serum or tissue after dialysis, and p is the dilution factor for the tissue homogenate. In this study, $p = 1$ for the serum and adipose homogenates, $p = 2$ for the liver homogenate, and $p = 4$ for the muscle homogenate.

The blood-to-plasma distribution ratio (s) of thiopental was determined to calculate the intrinsic clearance (Cl_{int}). The blood was incubated with 5 µg/ml of thiopental at 37° for 20 min with and without sulfadimethoxine. The blood sulfadimethoxine concentration was 454 µg/ml (corresponding to the *in vivo* plasma concentration of 500 µg/ml). After centrifugation, an aliquot of the plasma was removed and the concentration of thiopental was determined as already described. An analytical blank without substrate was determined in the same manner. The hemolysis during incubation was negligible.

[9] Silverson Co., Bucks, United Kingdom.

Table II—*In Vitro* Thiopental Free Fraction in Rat Tissues[a]

Tissue	Control Rats	Sulfadimethoxine-Treated Rats[b] 1 m*M*	2 m*M*
Liver[b]	0.076 ± 0.004[c]	0.064 ± 0.009[c]	0.075 ± 0.004[c]
Muscle[d]	0.101 ± 0.014	0.105 ± 0.016	0.114 ± 0.017
Adipose[e]	0.011 ± 0.003	0.008 ± 0.001	0.008 ± 0.001

[a] The free fraction was determined by equilibrium dialysis. [b] n = 3. [c] Mean ± *SE*. [d] n = 6–12. [e] n = 4 or 5.

All means are presented with their standard error (mean ± *SE*). The Student t test was utilized to determine a significant difference between control and sulfadimethoxine-treated rats with $p = 0.05$ as the minimal level of significance.

RESULTS

The plasma disappearance of thiopental after intravenous administration of 12 mg/kg is shown in Fig. 1. The disappearance of thiopental followed biexponential curves in both the control and sulfadimethoxine-treated rats. The plasma sulfadimethoxine concentration was kept at a constant level of 500 μg/ml during the 3-hr sampling period. The plasma thiopental concentrations in rats under constant infusion of sulfadimethoxine were significantly lower than those in controls without sulfadimethoxine. The pharmacokinetic constants were computed by a nonlinear iterative least-squares method (10) and are listed in Table I. In the sulfadimethoxine-treated rats, a significant decrease was observed in β while a significant increase (3.6-fold of the control rats) was observed in V_{ss}. The total (body) clearance, however, did not change significantly.

Scatchard plots of thiopental binding to serum protein obtained from equilibrium dialysis are shown in Fig. 2. In both experiments, *i.e.*, with and without sulfadimethoxine, there was no evidence for the existence of more than one class of binding site in the concentration range tested, which corresponds to the *in vivo* plasma concentration range of thiopental after intravenous administration of 12 mg/kg. The apparent dissociation constant (K_d) of thiopental was 0.1 m*M*, and the total number of binding sites (binding capacity) was 0.6 m*M*. In the presence of 500 μg/ml of sulfadimethoxine, a typical competitive inhibition was observed, and K_d was increased to 0.4 m*M*, but the binding capacity was not altered.

The serum free fraction (f_p) of thiopental showed nonlinearity in the concentration range studied with and without sulfadimethoxine; therefore, the mean values of f_p obtained from the *in vivo* mean plasma concentration of thiopental for 3 hr, *i.e.*, 0.035 m*M* for the control without sulfadimethoxine and 0.014 m*M* in the presence of sulfadimethoxine, were used to calculate the intrinsic clearance of unbound drug (Cl_{int}) using the *in vivo* total body clearance. The f_p of thiopental increased from 0.153 to 0.402 with sulfadimethoxine. The blood-to-plasma distribution ratios (s) were 0.922 for the control and 1.382 for the sulfadimethoxine-treated rats. Using these ratios and the hepatic blood flow obtained from the literature (14), the *in vivo* intrinsic clearance (Cl_{int}) of thiopental was calculated (Table I). With sulfadimethoxine, the Cl_{int} was decreased to about one-half that of the control rats. The *in vivo* free fractions of thiopental at 30 min (β-phase) after intravenous administration of 12 mg of thiopental/kg determined by ultrafiltration were 0.131 for the control rats and 0.269 for the sulfadimethoxine-treated rats. A remarkable increase in the free fraction was observed in the sulfadimethoxine-treated rats.

The free fraction of thiopental to the mainly distributed tissue homogenates (*i.e.*, liver, muscle, and adipose tissues) (8) was determined by equilibrium dialysis, and the *in vitro* apparent volume of distribution [$V_{ss(in\ vitro)}$] was calculated by:

$$V_{ss(in\ vitro)} = V_p + \frac{f_p}{f_l} V_l + \frac{f_p}{f_m} V_m + \frac{f_p}{f_a} V_a \quad \text{(Eq. 5)}$$

where f is the free fraction of thiopental in each tissue, V is the anatomical plasma and tissue volumes, and the subscripts p, l, m, and a denote plasma, liver, muscle, and adipose, respectively. In this study, reported values (14) of 44 ml/kg for both V_p and V_l and of 500 ml/kg for V_m were used, but the value of 40 ml/kg for V_a was experimentally determined. In spite of the presence of sulfadimethoxine, alterations of the free fraction of thiopental binding to liver, muscle, and adipose homogenates were insignificant, suggesting that sulfadimethoxine does not alter the binding of thiopental to these tissues (Table II).

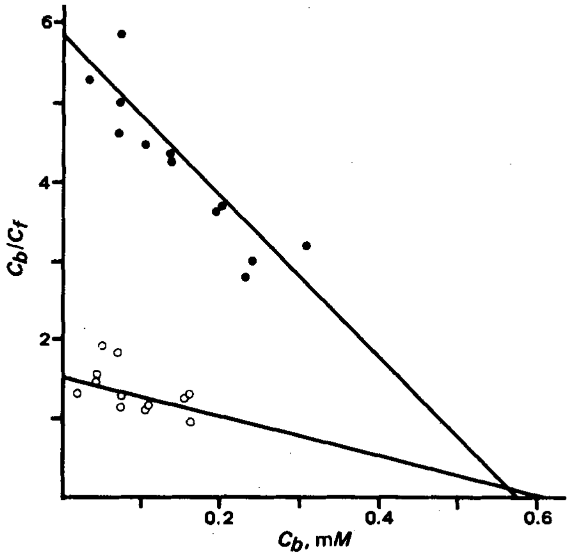

Figure 2—*Scatchard plot of data for the binding of thiopental to rat serum with (○) and without (●) sulfadimethoxine. Equilibrium dialysis was performed at 37° for 16 hr against 0.05* M *isotonic tromethamine–hydrochloric acid buffer (pH 7.4) containing 0.05–0.5* mM *thiopental. The concentration of sulfadimethoxine was 2.4* mM. *Lines were fitted by a linear least-squares regression.*

The mean apparent volumes of distribution (V_{ss}) calculated from each plasma disappearance curve were 689.7 ± 83.0 ml/kg for the control rats and 2480.2 ± 552.5 ml/kg for the sulfadimethoxine-treated rats (Table I); $V_{ss(in\ vitro)}$ values, calculated by Eq. 5 from the *in vitro* binding studies for the main distribution tissues (*i.e.*, liver, muscle, and adipose), were 1442 ml/kg for the control rats and 4083 ml/kg for the sulfadimethoxine-treated rats. The ratio of $V_{ss(in\ vitro)}$ with sulfadimethoxine to that of the control was 2.8, which seems to be comparable to the ratio of 3.6 from the *in vivo* experiments.

DISCUSSION

The importance of tissue binding on drug distribution and elimination as well as plasma binding has been emphasized recently (6, 15, 16). In this study, the effect of sulfadimethoxine on the elimination and distribution of thiopental was examined in both plasma disappearance and *in vitro* serum and tissue binding studies in an attempt to predict the drug interaction from *in vitro* binding data.

In plasma protein binding, a typical displacement of thiopental by sulfadimethoxine was observed (Fig. 2); but in tissue binding, no significant difference was observed in the free fraction of thiopental with or without sulfadimethoxine (Table II). This finding suggests that sulfadimethoxine does not alter the binding of thiopental to these tissues. The possible reasons for this discrepancy would be that thiopental is bound mainly to albumin in plasma but is bound in part to albumin in tissues but mainly to lipid or other macromolecules, which have nonspecific binding activities, due to the high lipophilicity of thiopental.

The overestimation of $V_{ss(in\ vitro)}$ compared to values from the plasma disappearance curves (Table I) might be due to underestimation of the free fraction of thiopental in tissue binding (Table II). In this study, diluted (25–50%) homogenates were used for liver and muscle binding studies, and this approach may cause the underestimation of the free fraction of thiopental due to the protein concentration dependency in the thiopental binding. Recently, Fichtl *et al.* (17) reported that the displacement of thiopental by phenylbutazone was not revealed in tissue binding using rabbit muscle homogenate. With respect to the displacement of thiopental in plasma binding by sulfadimethoxine, little has been reported, and only the displacement of tolbutamide binding to the plasma protein by sulfadimethoxine in sheep was reported (18). Thus, the increase in V_{ss} in the presence of sulfadimethoxine might be explained by the alteration of plasma binding of thiopental. Furthermore, a significant difference also was shown in V_{d_β} between the control and sulfadimethoxine-treated rats (Table I), which also can be explained by the alteration of the plasma binding of thiopental.

The possibility of inhibition in the metabolism of thiopental by sulfadimethoxine also should be considered. Previously, the inhibition in the microsomal oxidation of tolbutamide by sulfaphenazole was reported

(19). In this study, in the presence of sulfadimethoxine, the Cl_{int} of thiopental also decreased to one-half that of the control rats (Table I). These findings suggest that the inhibition in thiopental metabolism might be affected by sulfadimethoxine.

The values for the tissue binding reported in this paper are only relative; they are not absolute. Further elaborate studies are necessary for the precise evaluation of tissue binding.

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NOTES

Effects of Imide Analogs on Enzymes Required for Cholesterol and Fatty Acid Synthesis

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Received March 20, 1980, from the *Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514.* Accepted for publication August 21, 1980.

Abstract □ Twelve imide analogs were examined for their ability to lower serum cholesterol and triglyceride levels in mice. Potent activity was observed for compounds containing a phthalimide or saccharin ring structure. The ability to lower serum cholesterol appears to be related to the ability to suppress acetyl-CoA synthetase activity. The availability of acetyl-CoA in the cytoplasm is a key regulatory component for cholesterol and fatty acid synthesis. The capacity to reduce serum triglycerides was related directly to the ability of the compound to inhibit acetyl-CoA carboxylase activity, the regulatory enzyme of fatty acid synthesis.

Keyphrases □ Imide analogs—effects on enzymes required for cholesterol and fatty acid synthesis, serum cholesterol and triglyceride levels, mice □ Cholesterol synthesis—effects of 12 imide analogs on related enzymes □ Fatty acid synthesis—effects of 12 imide analogs on related enzymes □ Triglyceride levels—effects of imide analogs on enzymes required for cholesterol and fatty acid synthesis

The antihyperlipidemic effects of potassium phthalimide and *N*-substituted phthalimides at 20 mg/kg/day in rodents were reported previously (1). Side-chain lengths of four carbon atoms or their equivalent for the *N*-substituted acids, esters, and ketones resulted in the greatest inhibition. β-Hydroxy-β-methylglutaryl-CoA reductase activity was not affected by these agents significantly, but inhibition of acetyl-CoA synthetase activity was related directly to the ability to lower serum lipids. Furthermore, the agents appeared to accelerate cholesterol excretion in the feces. No toxic or teratogenic effects were noted for these compounds, *i.e.*, $LD_{50} \geq 2$ g/kg.

The present study involves variation of the type of nucleus and the side chain to improve antihyperlipidemic activity and examination of the enzymes involved early in cholesterol and triglyceride synthesis for inhibition by these agents.

EXPERIMENTAL

Twelve compounds were selected for this study (Table I). Phthalimide[1] (I), succinimide[1] (III), 1,8-naphthalimide[2] (V), saccharin[3] (VII), dibutyl phthalate[4] (X), and the standard, acetazolamide[5], were purchased commercially.

[1] Kodak Co.
[2] Aldrich Chemical Co.
[3] Ruger Chemical Co.
[4] Matheson, Coleman and Bell.
[5] Lederle Laboratories.

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