GLOSSARY OF SYMBOLS

D_σ = diffusion constant of species σ in membrane phase
C_σ = concentration of species σ
ΔC_σ = difference in concentration across the barrier of species σ
ΔJ_R = difference in reaction rate at barrier boundaries
η = reciprocal of relaxation length characteristic of system
k_1 = rate constant for association reaction
k_2 = rate constant for dissociation reaction
K = equilibrium constant of the reaction
J_σ = matter flux of species σ expressed in moles cm.$^{-2}$ sec.$^{-1}$

P, Q, R, A, B, and C are constants computed and utilized for calculation of θ.

a_1, b_1, and c_1 are concentration gradients at $x = 0$, respectively, of species α, β, and γ.

All other parameters are combinations of these quantities, whose algebraic relations are defined by equations in the paper.

REFERENCES

(1) L. S. Schanker, *Ann. Rev. Pharmacol.*, **1**, 29(1969).
(2) M. Mayersohn and M. Gibaldi, *Biochim. Biophys. Acta*, **196**, 296(1970).
(3) M. Mayersohn and M. Gibaldi, *J. Pharm. Sci.*, **60**, 225(1971).
(4) G. Szabo, G. Eisenman, and S. M. Ciani, in "Coral Gables Conference on Physical Principles of Biological Membranes," F. Snell, Ed., Gordon and Breach, New York, N. Y., 1970, p. 71.
(5) G. Levy and E. J. Mroszczak, *J. Pharm. Sci.*, **57**, 235(1968).
(6) R. H. Reuning and G. Levy, *ibid.*, **58**, 79(1969).
(7) V. S. Vaidhyanathan, *ibid.*, **60**, 886(1971).
(8) J. F. Danielli, *Symp. Soc. Exp. Biol.*, **8**, 502(1954).
(9) R. Blumenthal and A. Katchalsky, *Biochim. Biophys. Acta*, **173**, 357(1969).
(10) P. F. Scholander, *Science*, **131**, 585(1960).
(11) V. S. Vaidhyanathan, *J. Theoret. Biol.*, **33**, 1(1971).
(12) W. D. Stein, in "The Movement of Molecules Across Cell Membranes," Academic, New York, N. Y., 1967.
(13) V. S. Vaidhyanathan, *J. Theoret. Biol.*, **31**, 53(1971).
(14) D. R. Olander, *AIChE J.*, **6**, 233(1960).

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Blood Levels of Sulfamethizole in Dogs following Administration of Timed-Release Tablets Employing Lipase–Lipid–Drug Systems

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Abstract □ Blood levels of sulfamethizole in dogs following the administration of timed-release tablets are reported. Tablet formulations containing 5% glyceryl monostearate were employed for *in vivo* studies because the *in vitro* release from this formulation extended more than 12 hr. The formulations employed a pancreatic lipase-glyceryl trilaurate and glyceryl tristearate system, with enzyme-substrate combinations serving as a release-controlling vehicle to produce a timed-release effect. The main portion of the drug was released through the lipolytic digestion of the substrate by the lipase in addition to some release due to leaching and surface dissolution. A timed-release effect and uniform blood levels were observed over 12 hr. from tablets made from lipase–lipid–drug granules. Blood levels from tablets containing lipase were significantly higher and more consistent than blood levels obtained from tablets without lipase. The variations in blood levels observed in dogs receiving tablets with lipase were much less than variations observed in dogs receiving tablets without lipase.

Keyphrases □ Timed-release lipase–lipid–sulfamethizole tablets—effect of lipase, blood levels, dogs □ Lipase effect—blood levels of dogs following administration of timed-release lipase–lipid–sulfamethizole tablets □ Drug-release rates—effect of lipase on timed-release lipase–lipid–sulfamethizole tablets, blood levels, dogs

Enzymes play an important role in the breakdown and digestion of nutrient materials in the GI tract. This concept was utilized to control the drug release from a substrate system containing sulfamethizole incorporated in a lipase–lipid matrix. Lipase causes a controlled digestion of the substrate through the hydrolysis of the ester substrate controlling the release of the drug to produce a timed-release effect.

The concentration of lipase in the intestinal tract varies considerably with time (1) and from person to person. The range of lipase activity of 169 persons with normal pancreatic functions, in terms of the amount of acid liberated by the action of lipase on olive oil, was 110–1360 μeq. acid/min./ml. of duodenal fluid (2). The incorporation of lipase in the system, in addition to its release-controlling mechanism, would help minimize the wide range of variations in the concentration of lipase in the intestinal tract.

The purpose of this investigation was to study the blood levels of sulfamethizole following the oral administration of timed-release tablets during *in vivo* studies in dogs after *in vitro* dissolution patterns were established. The results of *in vitro* dissolution studies conducted on timed-release tablets employing lipase–lipid–sulfamethizole systems were reported previously (3).

EXPERIMENTAL

Composition of Spray-Congealed Granules—The manufacture of spray-congealed granules of lipase-lipid-sulfamethizole systems and the composition of initial-release granules were described pre-

Table I—Sulfamethizole Blood Levels (mg. %) in Dogs after Receiving Double-Layered Tablets Containing 25% Drug in Initial-Release and 75% Drug in Drug–Lipid Granules with 5% Glyceryl Monostearate

Hours	Tablets with Lipase[a]			Tablets without Lipase[b]	
	Dog 1	Dog 2	Dog 3	Dog 1	Dog 2
0.5	—	2.520	1.470	2.205	0.735
1.5	1.155	—	1.680	1.600	1.050
3.0	1.050	1.470	1.470	0.840	1.600
6.0	0.840	1.260	1.050	0.735	0.840
10.0	0.840	0.945	0.945	—	0.630
12.0	0.945	0.945	0.945	0.735	0.525

[a] The weight of each dog employed was 10.1 kg. [b] The weight of each dog employed was 9.0 kg.

viously (3). The composition of granules used in the preparation of tablets for *in vivo* studies was as follows:

glyceryl trilaurate[1]	16.00 g.
glyceryl tristearate[2]	34.00 g.
sulfamethizole NF[3]	25.00 g.
crude lipase[4] (5% w/w of substrate and drug)	
glyceryl monostearate[1] (5% w/w of substrate and drug)	

Preparation of Tablets—The preparation of timed-release tablets employing spray-congealed lipase–lipid–sulfamethizole granules and initial-release granules was described in an earlier report (3). Based on blood level results, appropriate changes in tablet formulation were made to achieve timed-release uniform blood levels over 12 hr. The weight of individual tablets was checked, and tablets were checked with a Stokes hardness tester to ensure that the hardness was between 6 and 7 kg.

Dog Studies—The weights of the dogs[5] employed in each group are given in Tables I–III. The dogs were fed[6] at 8:00 p.m. the evening before the experiment, and no food was given during the night and the 12-hr. period of the experiment. Water was permitted *ad libitum*. The dose of the drug administered was calculated on the basis of 43 mg./kg. of body weight. Dogs were used once and were selected so that their body weight was such that it was not necessary to use a fraction of a tablet. Seven tablets with lipase or six tablets without lipase, each weighing 200 mg., were administered orally to dogs ranging in age from 6.5 to 8 months.

In the first study, double-layered tablets containing 25% drug in initial-release and 75% drug in drug–lipid granules with lipase were given to three dogs; two dogs received similar tablets without lipase. In the second study, double-layered tablets containing 20% drug in initial-release and 80% drug in drug–lipid granules with lipase were given to three dogs; similar tablets without lipase were administered to two dogs. In the third study, tablets containing 100% drug in drug–lipid granules with lipase were given to seven dogs; six dogs received similar tablets without lipase. Control blood samples drawn from either the cephalic vein of the front leg or the saphenous vein of the hind leg of each dog prior to the administration of tablets were treated just like blood samples and were used as blanks. Blood samples were drawn at appropriate time intervals after the administration of tablets, and 1-ml. portions were assayed for total sulfamethizole using a modified Bratton–Marshall (4) assay procedure for sulfonamides. The percent transmittance of the resulting solutions was measured on a colorimeter[7] at 545 nm., and the concentration of sulfamethizole was calculated by comparison with the standard reference curve.

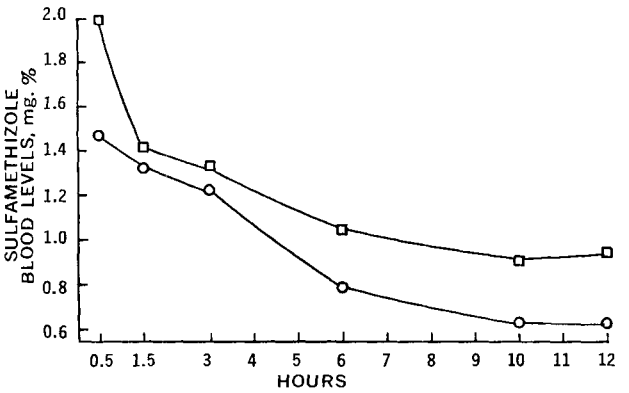

Figure 1—*Average sulfamethizole blood levels (mg. %) in dogs after receiving double-layered tablets containing 25% drug in initial-release granules and 75% drug in drug–lipid granules with 5% glyceryl monostearate. Key: □, tablets with lipase; and ○, tablets without lipase.*

[1] C. P. Hall Co.
[2] Sterotex HM, The Capital City Products Co.
[3] C. M. Bundy Co.
[4] Reheis Chemical Co.
[5] Registered beagles, supplied by Martin Animal Clinic, Ripley, Miss.
[6] Purina Dog Chow.

Table II—Sulfamethizole Blood Levels (mg. %) in Dogs after Receiving Double-Layered Tablets Containing 20% Drug in Initial-Release and 80% Drug in Drug–Lipid Granules with 5% Glyceryl Monostearate

Hours	Tablets with Lipase[a]			Tablets without Lipase[b]	
	Dog 1	Dog 2	Dog 3	Dog 1	Dog 2
0.5	1.188	2.450	1.200	1.785	0.840
1.5	2.160	1.575	1.900	2.205	1.365
3.0	1.296	1.365	2.400	2.100	0.945
6.0	1.188	1.365	1.200	1.050	0.630
10.0	1.188	1.155	1.100	0.840	0.630
12.0	1.188	1.260	1.100	0.630	0.735

[a] The weight of each dog employed was 10.0 kg. [b] The weight of each dog employed was 9.0 kg.

RESULTS AND DISCUSSION

In the present studies, blood levels of sulfamethizole in dogs were measured following the administration of various timed-release tablets made from spray-congealed granules of lipase–lipid–sulfamethizole. Sulfamethizole was used as a model drug in the formulation of timed-release tablets to test the efficacy of the timed-release system because of its short biological half-life of 2.1 hr. (5). The release of the active ingredient is brought about by the fact that the lipase causes the controlled digestion of glyceryl trilaurate and glyceryl tristearate and subsequent release of the embedded drug particles. The integrity of the timed-release system in rendering the drug available for *in vivo* absorption was already shown during *in vitro* studies (3). Once the drug is in solution, it will be absorbed depending upon the rate of release from the dosage form.

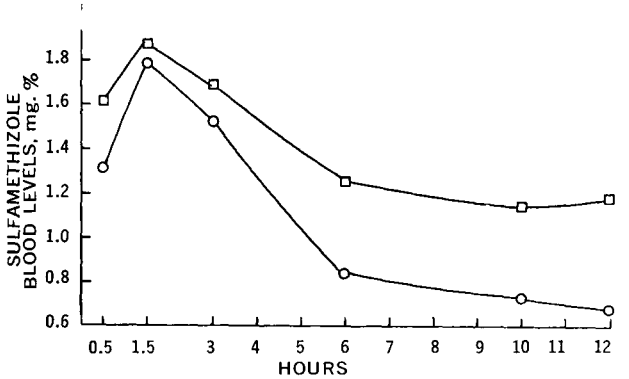

Figure 2—*Average sulfamethizole blood levels (mg. %) in dogs after receiving double-layered tablets containing 20% drug in initial-release granules and 80% drug in drug–lipid granules with 5% glyceryl monostearate. Key: □, tablets with lipase; and ○, tablets without lipase.*

[7] Spectronic 20, Bausch & Lomb.

Table III—Sulfamethizole Blood Levels (mg. %) in Dogs after Receiving Tablets Containing 100% Drug in Drug–Lipid Granules with 5% Glyceryl Monostearate

Hours	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	Dog 7	Average
				Tablets with Lipase[a]				
0.5	0.945	1.050	0.945	0.945	0.945	1.155	0.945	0.990
1.5	0.840	1.050	0.945	0.945	0.840	1.050	0.945	0.945
3.0	0.840	1.155	1.155	1.050	1.050	1.155	0.840	1.035
6.0	0.945	1.050	1.155	1.260	0.945	1.050	0.945	1.050
10.0	0.840	0.840	1.050	1.050	0.840	1.050	0.840	0.930
12.0	0.840	0.840	1.050	1.050	0.840	0.945	0.945	0.930
				Tablets without Lipase[b]				
0.5	1.260	0.630	0.630	0.630	0.840	0.630	—	0.770
1.5	0.630	0.630	1.155	0.735	0.630	0.840	—	0.770
3.0	0.735	0.735	0.735	0.840	0.735	1.365	—	0.857
6.0	0.630	0.840	0.840	0.735	0.420	1.260	—	0.787
10.0	0.630	0.630	1.155	0.735	0.630	0.630	—	0.735
12.0	0.630	0.525	0.630	0.840	0.630	0.735	—	0.665

[a] The weight of each dog employed was 9.9 kg. [b] The weight of each dog employed was 8.9 kg.

The individual blood levels observed after the administration of tablets containing 25% drug in initial-release and 75% drug in drug–lipid granules with 5% glyceryl monostearate are listed in Table I; average blood levels are plotted in Fig. 1. Three dogs received tablets with lipase and two dogs received tablets without lipase. As seen in Fig. 1, the rates of decrease in blood levels were approximately the same and the blood levels occurring from tablets with lipase were considerably higher than those from tablets without lipase. The lipid substrate demonstrated a timed-release effect on the release of drug, and sustained blood levels were observed after 6 hr. in dogs receiving tablets with or without lipase. Since the blood levels at the end of 12 hr. decreased considerably and showed a partial sustaining effect in the first 6 hr., the amount of drug in initial-release granules in tablets was decreased to 20%. The individual blood levels obtained after the administration of tablets containing 20% drug in initial-release and 80% drug in drug–lipid granules with 5% glyceryl monostearate are listed in Table II; average blood levels are plotted in Fig. 2. Tablets with lipase were administered to three dogs, and tablets without lipase were given to two dogs. The blood levels from tablets containing lipase were sustained better than were blood levels from the tablets containing 25% drug in initial-release granules (Fig. 1). The decrease in blood levels occurring from tablets without lipase was steady, with very little timed-release effect. Lower blood levels were observed at 0.5 hr. from tablets with and without lipase because of a decrease of 5% drug in initial-release granules. However, the blood levels continued to increase, reaching the peak at approximately 1.5 hr. and decreasing slowly in tablets with lipase and faster and to a greater extent in tablets without lipase.

Since the initial blood levels were higher than the levels at the end of 12 hr., tablets made only with drug–lipid granules were used to see if constant blood levels could be maintained. Individual blood levels occurring from tablets containing 100% drug in drug–lipid granules with 5% glyceryl monostearate are listed in Table III, and average blood levels are plotted in Fig. 3. A timed-release effect was observed, and initial blood levels were maintained over 12 hr. when seven dogs received tablets with lipase and six dogs received tablets without lipase. Significantly higher blood levels were obtained following the administration of tablets with lipase compared to tablets without lipase. A t test of analysis was performed on the data obtained in the last study to show the difference in the average sulfamethizole blood levels in dogs receiving tablets with and without lipase; 85–99% confidence limits (Fig. 3) were obtained. It is obvious from the standard errors plotted around the averages in Fig. 3 that the variations in blood levels in dogs receiving tablets with lipase were much less than the variations in dogs receiving tablets without lipase. Based on this observation, it is possible that the lipase included in the formulation might have helped to minimize the variability of lipase activity in the intestinal tract of the test animals.

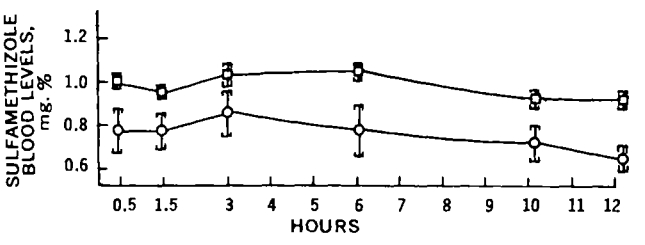

Figure 3—*Average sulfamethizole blood levels (mg. %) in dogs after receiving tablets containing 100% drug in drug–lipid granules with 5% glyceryl monstearate. Key: □, tablets with lipase; and ○, tablets without lipase. Standard errors are plotted around the averages.*

The presence of the drug in blood after the administration of tablets with and without lipase is the result of the absorption of the drug released by surface dissolution, leaching, attrition as a result of digestive fluids, and physical forces to which the tablets were exposed in the GI tract. In addition to all these factors, the more consistent and significantly higher blood levels resulting from tablets with lipase can probably be attributed to the control of drug release by the lipase–lipid–drug system.

REFERENCES

(1) M. J. Schiffrin and E. S. Nasset, in "Medical Physiology," 11th ed., P. Bard, Ed., C. V. Mosby, St. Louis, Mo., 1961, p. 426.

(2) W. Rick, *Aerztl. Lab.*, **7**, 290(1961).

(3) K. A. Javaid, J. H. Fincher, and C. W. Hartman, *J. Pharm. Sci.*, **60**, 1709(1971).

(4) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).

(5) M. J. Robinson, A. Bondi, Jr., and J. V. Swintosky, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 874(1958).

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