60S(1969).
(10) L. Ravin, E. G. Shami, and E. Rattie, *J. Pharm. Sci.*, **59**, 1290(1970).
(11) I. C. Edmundson and K. A. Lees, *J. Pharm. Pharmacol.*, **17**, 193(1965).
(12) A. R. Ebian, M. A. Moustafa, S. A. Khalil, and M. M. Motawi, *ibid.*, **25**, 13(1973).
(13) W. I. Higuchi, E. L. Rowe, and E. N. Hiestand, *J. Pharm. Sci.*, **52**, 162(1963).
(14) D. E. Wurster and P. W. Taylor, *ibid.*, **54**, 169(1965).
(15) J. E. Carless and A. A. Foster, *J. Pharm. Pharmacol.*, **18**, 697(1966).
(16) E. Shefter and T. Higuchi, *J. Pharm. Sci.*, **52**, 781(1963).

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Blood Concentration Profiles of Acetaminophen following Oral Administration of Fatty Acid Esters of Acetaminophen with Pancreatic Lipase to Dogs

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Abstract □ Fatty acid esters of acetaminophen were administered orally to dogs, and blood concentrations of acetaminophen were determined at various time intervals. Blood concentrations of acetaminophen following oral administration of a short chain ester, *p*-acetamidophenyl acetate, were not significantly different from those found using acetaminophen. Blood concentrations of acetaminophen following oral administration of intermediate hydrocarbon chain-length compounds were less than those of the control at 1 and 3 hr postdosing. There appears to be a direct relationship between the *in vitro* hydrolysis rates and the blood concentration *in vivo*. Concomitant oral administration of acetaminophen derivatives, pancreatic lipase, and calcium salts resulted in an increase in the blood levels of acetaminophen as compared to administration of the esters alone. Calcium carbonate was included as a source of calcium ion to activate the lipase involved in the hydrolysis of the fatty acid esters. A combination of *p*-acetamidophenyl acetate, *p*-acetamidophenyl dodecanoate, pancreatic lipase, and calcium carbonate was shown to achieve a prolonged release of acetaminophen. *p*-Acetamidophenyl acetate was thought to provide the initial release of acetaminophen; *p*-acetamidophenyl dodecanoate, being hydrolyzed more slowly, provided the prolonged release, which maintained therapeutic blood concentrations for 13 hr following a single dose of the combination in dogs.

Keyphrases □ Acetaminophen—blood concentration profiles after oral administration of *p*-acetamidophenyl acetate and dodecanoate with pancreatic lipase, dogs □ Fatty acid esters of acetaminophen—acetaminophen blood concentration after oral administration with pancreatic lipase, dogs □ *p*-Acetamidophenyl acetate and dodecanoate—acetaminophen blood concentration following oral administration with pancreatic lipase, dogs

Timed release, sustained release, and prolonged action are popular terms for describing oral dosage forms that first release an initial dose and then gradually release a remaining quantity of drug sufficient to maintain a uniform therapeutic effect over an extended period (1).

The unique physiological and morphological features in the GI tract present both a challenge and an opportunity in the design of oral dosage forms. Although the physiological environment is an important factor in designing all dosage forms, many dosage forms are designed to operate in a particular set of biochemical conditions. For example, amide and ester derivatives are prepared frequently to alter the physical and chemical properties of a drug molecule to obtain a more acceptable dosage form. These chemical modifications often lead to pharmacologically inactive compounds; however, the presence of autogenous enzyme systems and/or other suitable conditions found in the various segments of the GI tract facilitate the hydrolysis and release of the active form.

Table I—Blood Concentration (Micrograms per Milliliter) of Acetaminophen following an Oral Dose of 20 mg/kg in Five Dogs

	Minutes				
Subject	30	90	150	240	330
D1	10.27	10.88	22.10	15.03	8.91
D2	28.16	20.74	18.84	14.15	10.68
D3	18.57	22.44	18.16	10.75	11.22
D4	16.46	22.44	24.01	21.56	12.51
D5	13.67	20.40	21.08	15.44	12.58
Average	17.43	19.38	20.84	15.39	11.18
±*SE*	3.02	2.17	1.07	1.75	0.66

Dittert *et al.* (2) found that esters of acetaminophen were substrates for serum lipase. Work in this laboratory indicates that, by controlling the chain length of the esters, it would be theoretically possible to impose a rate-limiting step prior to absorption of the drug from the GI tract, thereby achieving an extended duration of action from acetaminophen (3). Because of the insoluble nature of fatty acid esters of acetaminophen, it was not thought that any appreciable quantity of intact ester would be absorbed.

The objectives of this work were to investigate the feasibility of employing fatty acid ester derivatives of

Table II—Blood Concentration (Micrograms per Milliliter) for Acetaminophen following an Oral Dose of 40 mg/kg in Six Dogs

Subject	Hours					
	1	3	5	7	10	13
D6	20.13	33.33	17.41	11.83	5.71	4.76
D7	27.21	17.00	6.39	2.99	0.68	0.54
D8	55.36	27.48	9.66	4.08	0.00	0.00
D9	55.90	45.30	20.13	7.21	3.81	0.68
D10	19.32	40.40	17.00	3.67	3.13	2.86
D11	58.22	39.45	22.44	9.39	2.18	0.95
Average	39.36	33.83	15.51	6.53	2.59	1.63
±*SE*	7.75	4.20	2.53	1.45	0.86	0.74

a drug in an enzyme substrate system to control the rate of absorption following oral administration and to establish a possible role for the concomitant administration of pancreatic lipase to provide a more uniform enzyme activity in the GI tract.

EXPERIMENTAL

Acetaminophen Assay in Blood—A 15.1-mg sample of acetaminophen[1], weighed on an analytical balance, was transferred to a 100-ml volumetric flask, and the flask was filled to the mark with distilled water. Aliquots of 0.5, 0.4, 0.3, 0.2, 0.1, 0.050, and 0.025 ml were diluted to 0.5 ml with distilled water and mixed with 0.5 ml of whole blood from pooled samples of blood drawn before the administration of acetaminophen. The removal of serum proteins and red blood cells was accomplished by a method used in the determination of blood sugar (4).

A 2.0-ml portion of 0.12 *N* barium hydroxide solution[2] was added, and the mixture was stirred[3]. After standing for about 2 min, a deep reddish-brown color developed. A 2.0-ml portion of 2% zinc sulfate solution[2] was added with mixing, resulting in the precipitation of red blood cells and serum protein. The samples were centrifuged at about 4000 rpm for 5 min. After filtration of the aqueous portion through a 0.8-μm filter[4], the absorbance of the clear aqueous filtrate of each sample was determined[5] against a reagent blood blank at 245 nm.

Protocol for *In Vivo* Studies—Groups of six to eight healthy, treated line-bred beagle dogs[6] were selected for the blood concentration studies. All food was withheld from the dogs for 24 hr prior to the beginning and during the experiment. The dogs were about 1 year in age and weighed between 9.1 and 13.6 kg (20 and 30 lb). All doses were calculated on a weight basis equivalent to 40 mg of acetaminophen/kg. This amount is comparable to a dose of 2.8 g for a human weighing 70 kg.

Control blood samples for all experiments were drawn from the cephalic vein of either front leg just prior to the administration of the dose. All doses were administered in hard gelatin capsules followed by 10 ml of water to force dogs to swallow the capsules. Water was available to the animals during the experiment. At 1-, 3-, 5-, 7-, 10-, and 13-hr intervals after drug administration, blood samples of about 2 ml were drawn from the cephalic vein(s) with a 2.5-ml disposable syringe, previously flushed with a heparin solution (10,000 USP units/ml)[7], using a 2.54-cm (1-in.), 22-gauge needle.

Immediately following the withdrawal of each blood sample, the needle was removed from the barrel of the syringe and the contents were delivered to clean glass vials. All blood samples were sealed and stored over ice until analyzed. No samples were stored longer than 24 hr, and most samples were prepared for analysis immediately.

Control studies for acetaminophen absorption were performed at two different dose levels to establish blood concentration–time profiles for the model drug compound. Five dogs were administered a dose of 20 mg of acetaminophen/kg. In this control study, blood samples were drawn at 30-, 90-, 150-, 240-, and 330-min intervals after drug administration. Six dogs were administered 40 mg of acetaminophen/kg, and blood samples were taken at 1-, 3-, 5-, 7-, 10-, and 13-hr intervals.

Table III—Blood Concentration (Micrograms per Milliliter) of Acetaminophen following an Oral Dose of *p*-Acetamidophenyl Acetate Equivalent to 40 mg of Acetaminophen/kg in Three Dogs

Subject	Hours					
	1	3	5	7	10	13
D12	36.59	41.76	13.31	5.03	2.45	0.41
D13	22.17	41.22	16.60	2.99	0.68	0.00
D14	36.59	27.21	13.60	4.90	2.31	1.36
Average	31.78	36.73	14.50	4.31	1.81	0.59

Table IV—Blood Concentration (Micrograms per Milliliter) of *p*-Acetamidophenyl Decanoate Equivalent to 40 mg of Acetaminophen/kg in Six Dogs

Subject	Hours					
	1	3	5	7	10	13
D15	2.85	11.56	9.79	4.90	4.35	4.08
D16	0.68	5.44	7.75	7.35	5.85	3.40
D17	1.22	9.52	11.15	5.44	2.45	1.63
D18	0.68	2.58	4.63	5.03	4.76	0.00
D19	5.03	9.66	9.93	6.39	6.94	2.45
D20	0.00	2.72	1.36	0.00	0.68	0.00
Average	1.75	6.91	7.44	4.85	4.17	1.93
±*SE*	0.77	1.58	1.53	1.04	0.93	0.70

The short chain ester derivatives of acetaminophen through the octanoate were administered without pancreatic lipase[8] or other additives. The intermediate length and long chain esters were studied both with and without lipase and other additives. When studied with lipase, these esters were mixed with the enzyme preparation and encapsulated in two or three No. 00 hard gelatin capsules. Lipase preparations were assayed prior to each experiment by the method of Lazo-Wasem (5). Capsules containing the long chain esters and lipase were stored in a refrigerator and/or over ice until administered.

Blood samples were analyzed by the procedure previously described. To 0.5 ml of blood, 0.5 ml of distilled water was added. A 2.0-ml portion of 0.12 *N* barium hydroxide solution was added and

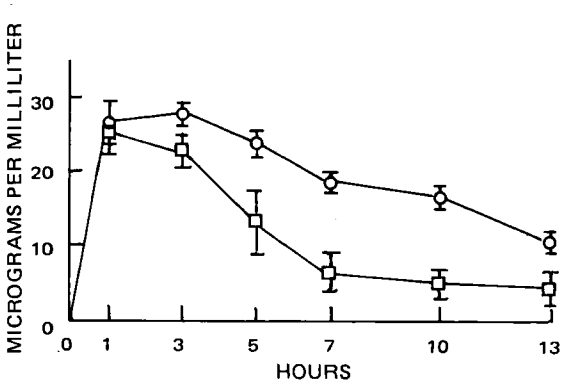

Figure 1—*Blood concentrations of acetaminophen after an oral dose of* p-*acetamidophenyl acetate, equivalent to 20 mg of acetaminophen, or* p-*acetamidophenyl dodecanoate, equivalent to 40 mg of acetaminophen, and 58.9 mg of lipase and 10 mg of calcium carbonate/kg in six dogs. Key:* ○, *average with standard error; and* □, *control average with standard error.*

[1] Eastman Organic Chemicals.
[2] Fisher Scientific Co.
[3] Vortex Jr. mixer, Scientific Products.
[4] Millipore, Millipore Corp.
[5] Beckman DU spectrophotometer, Beckman Instruments.
[6] Supplied by Dr. Joseph Martin, Ripley, Miss.
[7] Hyrex Co.
[8] Supplied by Rheis Chemical Co.

Table V—Blood Concentration (Micrograms per Milliliter) of Acetaminophen following an Oral Dose of *p*-Acetamidophenyl Decanoate Equivalent to 40 mg of Acetaminophen, 54 mg of Lipase, and 10 mg of Calcium Chloride/kg in Seven Dogs

Subject	Hours					
	1	3	5	7	10	13
D26	9.39	20.68	12.65	9.93	9.79	6.80
D27	8.84	22.17	12.24	11.83	5.03	4.35
D28	11.83	12.38	7.21	6.39	11.02	6.94
D29	7.07	20.68	16.05	13.06	8.84	5.17
D30	5.58	18.64	12.79	10.34	5.44	4.90
D31	8.16	19.59	10.07	8.16	7.89	4.63
D32	8.71	13.33	8.57	6.39	5.44	1.36
Average	8.51	18.21	11.37	9.44	7.64	4.88
±*SE*	0.74	1.45	1.11	0.98	0.90	0.70
Level of significance to control (Table IV)	$p < 0.01$	$p < 0.01$	$p < 0.1$	$p < 0.05$	$p < 0.05$	$p < 0.05$

mixed with the blood sample. After standing for 2 min, a 2.0-ml portion of a 2% zinc sulfate solution was added and mixed. The samples were centrifuged and filtered. The absorbance of the filtrates was determined against a blood reagent blank.

RESULTS AND DISCUSSION

Control studies for acetaminophen absorption were performed *in vivo* at two dose levels to establish blood concentration–time profiles. The doses of all animal studies were calculated on a weight basis. Five dogs were given an oral dose of 20 mg/kg as noted in Table I. This dose level produced an average blood concentration peak of 20.84 μg/ml after 2.5 hr. However, some individual animals exhibited a peak concentration level after 1 hr, and it was difficult to assess what happened between samples. After 5.5 hr, the concentration decreased to about half that of the peak concentration.

Since prolonged-release medication required a larger dose, a second control study was conducted at 40 mg of free acetaminophen/kg in six dogs; the results are given in Table II. In this study, a maximum concentration was observed after 1 hr and an exponential decay began at 3 hr and continued through a 10-hr period. A plot of the data on a semilogarithmic graph gave a linear decay, beginning at the 3-hr interval and continuing through the 10-hr interval. A half-life for blood concentration was determined graphically from the data in Table II and was found to be in the order of 1 hr and 40 min.

The availability of acetaminophen after oral administration and administration of its ester derivatives was determined by the following equation (6, 7):

$$\frac{A_T}{V_d} = C_T + K_e\,(AUC) \qquad \begin{matrix} t = T \\ t = 0 \end{matrix} \qquad \text{(Eq. 1)}$$

where A_T is the amount of drug absorbed up to time T, V_d is the distribution volume, C_T is the concentration at time T, K_e is the elimination rate constant, and AUC is the area under the curve. When the data in Table II were treated by this method, the half-life for absorption was found to be 40 min, and acetaminophen was more available when administered as the free drug.

Three dogs were given oral doses of *p*-acetamidophenyl acetate equivalent to 40 mg of acetaminophen/kg. A comparison of blood levels of acetaminophen shown in Table III with the control study presented in Table II indicates a slight increase in the half-life for absorption from 40 to 60 min, although there was only a slight change in the availability of acetaminophen with equivalent doses. Treatment of the data in Table II gave an $(A_T/V_d)_{max}$ of 75.3 μg/ml as compared to 71.5 μg/ml for the data in Table III. These estimates of relative availability most likely underestimate the true value.

From observations on the *in vitro* hydrolysis of acetaminophen derivatives, it was anticipated that intermediate and long chain esters would reduce the availability of acetaminophen (3). The study recorded in Table IV illustrates a reduced availability of aceta-

Table VI—Blood Concentration (Micrograms per Milliliter) of Acetaminophen following an Oral Dose of *p*-Acetamidophenyl Acetate, Equivalent to 20 mg of Acetaminophen, or *p*-Acetamidophenyl Dodecanoate, Equivalent to 40 mg of Acetaminophen, and 58.9 mg of Lipase and 10 mg of Calcium Carbonate/kg in Six Dogs

Subject	Hours					
	1	3	5	7	10	13
D85	19.04	33.33	27.34	17.00	17.00	13.33
D86	23.13	29.92	24.08	15.24	12.79	11.02
D87	28.02	27.75	20.13	16.60	16.46	13.33
D88	38.09	27.75	21.08	19.59	21.36	6.53
D89	27.89	25.17	28.56	17.68	16.60	8.84
D90	29.64	24.21	26.12	25.17	18.23	13.19
Average	27.64	28.02	24.55	18.55	17.07	11.04
±*SE*	2.62	1.35	1.39	1.44	1.18	1.14
	Controls[a]					
D91	23.13	23.81	18.36	8.71	10.07	12.24
D92	23.26	30.47	21.76	8.30	5.85	0.00
D93	30.06	29.79	19.04	15.04	11.97	11.29
D94	34.14	24.08	8.30	7.21	3.81	2.86
D95	21.90	15.37	6.39	0.27	0.00	1.36
D96	23.67	19.86	6.26	1.22	0.54	0.54
Average	26.03	23.90	13.35	6.79	5.37	4.72
±*SE*	2.00	2.36	4.38	2.26	2.00	2.30
Level of significance	$p < 0.70$	$p < 0.20$	$p < 0.01$	$p < 0.005$	$p < 0.001$	$p < 0.05$

[a] Inactive lipase.

minophen following oral administration of equivalent doses of *p*-acetaminophenyl decanoate. The availability of drug from this ester form was 34% of that produced by acetaminophen. Concomitant administration of *p*-acetamidophenyl decanoate, pancreatic lipase (3 Wilson units/mg), and calcium chloride increased the availability from 34 to 73% as calculated from the data reported in Table V.

To investigate the feasibility of using a combination of ester derivatives with pancreatic lipase and calcium ions to achieve a prolonged release of acetaminophen, *p*-acetamidophenyl acetate and *p*-acetamidophenyl dodecanoate, equivalent to 20 and 40 mg/kg of acetaminophen, respectively, were administered with 59 mg/kg of pancreatic lipase and 10 mg/kg of calcium carbonate. The acetate ester was included to provide the initial release, and the dodecanoate ester was selected because of its slower hydrolysis rate. The amounts of pancreatic lipase and calcium carbonate were determined to be adequate from preliminary studies.

The results of this combination using a short chain ester and an intermediate ester are shown in Table VI and in Fig. 1. At the 1-, 3-, and 5-hr intervals, the blood concentrations were somewhat higher than an average of 21 μg/ml; at 7 and 10 hr, they were slightly below. The control used in this study was identical to the experiment, except that the pancreatic lipase used in the dose was inactivated by moisture and heat. No statistical difference at a 90% confidence level was noted at the 1- and 3-hr intervals; however, at 5-, 7-, 10-, and 13-hr intervals, a significant difference was observed. The hydrolysis and subsequent absorption from *p*-acetamidophenyl dodecanoate were facilitated by the inclusion of lipase in the dose. The availability of acetaminophen was increased by a factor of 1.7 when the combination included the active enzyme preparation.

REFERENCES

(1) R. H. Blythe, *Drug Stand.*, **26**, 1(1958).

(2) L. W. Dittert, H. C. Caldwell, H. J. Adams, G. M. Irvin, and J. V. Swintosky, *J. Pharm. Sci.*, **57**, 774(1968).

(3) C. T. Bauguess, F. Sadik, J. H. Fincher, and C. W. Hartman, *ibid.*, **64**, 117(1975).

(4) M. Somogyi, *J. Biol. Chem.*, **160**, 69(1945).

(5) E. A. Lazo-Wasem, *J. Pharm. Sci.*, **50**, 999(1961).

(6) R. Dominguez and E. Pomerene, *Proc. Soc. Exp. Biol. Med.*, **60**, 173(1945).

(7) M. Gibaldi, "Introduction to Biopharmaceutics," Lea and Febiger, Philadelphia, Pa., 1971, p. 65.

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Correlation of Kinetic Parameters and Thermal Behavior of Segmented Polyurethane Elastomers with Biological Responses

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Abstract □ Kinetic studies of thermal degradation of 16 segmented polyether polyurethane samples, containing various amounts of 3,4-diaminotoluene and dibutyltin diacetate as additives, were carried out by thermogravimetry. From a single dynamic thermogravimetric experiment, the temperatures of initiation of degradation, 10, 25, and 50% (w/w) of degradation, as well as the activation energies for degradation, were determined. The activation energies were computed from the thermogravimetric curves using Broido's graphical approximation method, which applies to first-order decomposition kinetics. The results of stepwise multiple linear regression analysis indicate that the biological responses to elastomer samples, such as tissue culture, hemolysis, intramuscular implant, intradermal irritation, systemic toxicity, and histopathological rating, and the cumulative biological response index are highly correlated with thermal stability and kinetic measurements of the materials.

Keyphrases □ Segmented polyurethane elastomers—correlation of kinetic parameters and thermal behavior with biological responses □ Polyurethane elastomers, segmented—correlation of kinetic parameters and thermal behavior with biological responses □ Biomaterials, segmented polyurethane elastomers—correlation of kinetic parameters and thermal behavior with biological responses

Segmented polyether polyurethane elastomers demonstrate superior properties of strength and flex-life (1) and are excellent candidates for biomedical applications (2–9). These polymers consist of at least two major segments in the repeating chain structural unit: a crystalline *hard* segment (urea), melting above 200°, and a *soft* segment (polyether glycol), melting below 50° (10–12). Both segments are polymeric and connected by urethane linkages. The final product is termed a polyether/urethane/urea or a segmented polyurethane.

The chemistry and techniques involved in the production of these polymer materials are well known (13, 14). In addition, several investigations considered the chemical, physical, mechanical, and biological properties (5), the dependence of morphological structure on the chain characteristics and cast conditions (15), the hemolytic activity (16, 17), and the toxicity (18, 19) of the polymeric material relevant to biological applications. However, no systematic studies have been reported that compare and correlate physical properties with biological responses for segmented polyurethane systems containing different kinds and amounts of additives.

The purposes of this paper, therefore, are to consider one system of segmented polyurethanes containing various quantities of additives and to illustrate the correlation between the measured thermal