Succinylsulfathiazole Crystal Forms III: Crystal Growth Studies

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Abstract □ Crystal growth accompanying the transformation of succinylsulfathiazole crystal forms in aqueous suspensions was studied using a projecting microscope. The effects of increase of temperature, agitation, inclusion of seeds of Form II (the water-stable dihydrate), sulfathiazole, methylcellulose, and polysorbate 80 on the particle-size distribution of anhydrous succinylsulfathiazole Form I were examined. Rates of crystal growth, calculated as increase of diameter per unit time, were given under different experimental conditions. Increase of temperature, agitation, and seeding with nuclei of Form II had significant growth-accelerating effects. Sulfathiazole and polysorbate 80 had growth-retarding effects. Methylcellulose inhibited the crystal growth of Form I for over a year. Aqueous suspensions of Form II did not show any change in particle-size distribution. The crystal growth was shown to be a direct consequence of the transformation of the crystal form. Physical conditions and additives which had accelerating or retarding effects on the rate of transformation of Form I had similar effects on the crystal growth rate of succinylsulfathiazole in aqueous suspensions.

Keyphrases □ Succinylsulfathiazole—crystal growth, effect of temperature, agitation, seeding, methylcellulose, and polysorbate 80, particle-size distributions □ Crystals—succinylsulfathiazole, factors affecting growth, particle-size distributions □ Transformations, crystal—succinylsulfathiazole, various factors affecting growth, particle-size distributions

Succinylsulfathiazole crystal forms, their preparation, characterization, and kinetics of interconversions in the presence and absence of some additives, were described previously (1, 2). These reports noted that crystal growth, caking, and the formation of cement-like precipitates in aqueous suspensions of succinylsulfathiazole were consequences of the transformation of metastable crystal forms to a water-stable form.

Changes in particle-size distribution accompanying transformation of crystal forms of various drugs have been described. Studies on methylprednisolone (3), cholesterol (4, 5), cortisone acetate (6, 7), an experimental antihypertensive compound (8), oxyclozanide (9), theophylline (9), and the antiviral compound SK & F-30097 (10) are some examples.

The present work is concerned with a quantitative study of the changes in particle-size distribution accompanying the transformation of anhydrous succinylsulfathiazole Form I to the dihydrate water-stable Form II in aqueous suspension. The effects of some physical factors, *e.g.*, temperature and agitation, on the crystal growth rate are examined. The effects of including various additives, *e.g.*, seeds of Form II, sulfathiazole, methylcellulose, and polysorbate 80, are also investigated.

A correlation of the results of this study with those of the kinetic studies previously reported (1, 2) would help to evaluate the possible use of metastable crystal forms in aqueous pharmaceutical suspensions and its consequences on the physical stability of the suspensions produced.

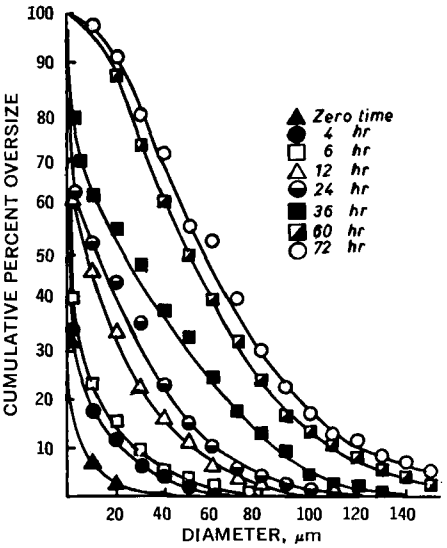

Figure 1—*Change in particle-size distribution with time of aqueous suspension of succinylsulfathiazole Form I at 22°.*

EXPERIMENTAL

Materials and Apparatus—Succinylsulfathiazole Forms I and II were obtained as micronized commercial samples[1], and their identity was checked by IR spectrophotometry (1). Sulfathiazole BPC, methylcellulose, and polysorbate 80 (USP grade) were used.

A projecting microscope[2] was used for particle-size analyses. IR spectra were determined with a double-beam grating spectrophotometer[3].

Methods—Micronized succinylsulfathiazole Form I (0.3 g) was thoroughly mixed with a few drops of distilled water. The smooth paste produced was then gradually diluted with water to produce a 1% (w/v) suspension. The bottle containing the suspension was kept undisturbed in a thermostated water bath maintained at 22 ± 0.1°.

At various time intervals, the suspension was briefly shaken, a drop was transferred to a microscope slide, and the projected diameter was measured for about 100 particles. The particle diameters were classified into 5-μm size range intervals. The procedure was repeated with 5–10 drops of the suspension, taken at the same time. The total counting time varied from 30 to 40 min.

The effect of temperature was examined by repeating this procedure, with the suspension immersed in a thermostated water bath at 37 ± 0.1°.

To investigate the effect of agitation, bottles containing suspensions, prepared as previously described, were rotated at 100 rpm in thermostated water baths at 22 ± 0.1 and 37 ± 0.1° in separate experiments. Sampling was carried out as before.

An experiment was carried out to investigate the effect of seeding aqueous suspensions of succinylsulfathiazole Form I with (10% by weight of the solid phase) Form II on the rate of crystal growth at 22 ± 0.1°.

[1] Courtesy of Chemical Industries Development, Guiza, Egypt.
[2] Reichert, Austria.
[3] Perkin-Elmer model 237-B.

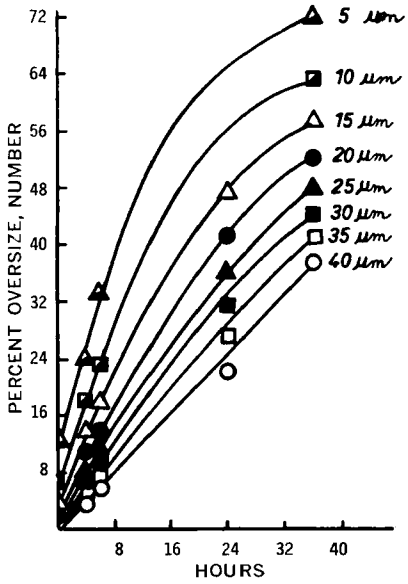

Figure 2—*Change in cumulative count with time of succinylsulfathiazole suspension at 22°.*

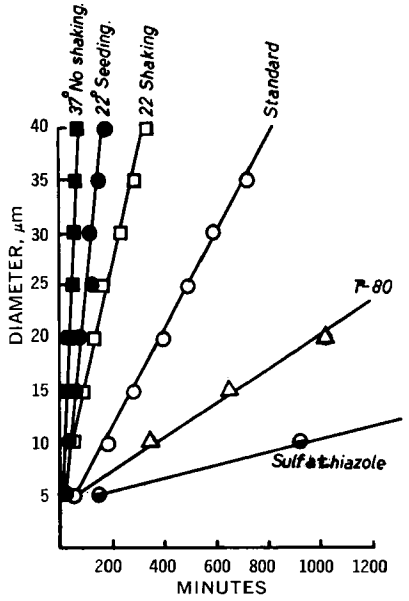

Figure 4—*Comparison of rates of growth above 15% cumulative count.*

The effect of additives, namely sulfathiazole (10% by weight of the solid phase), methylcellulose (0.5% w/v), and polysorbate 80 (1% w/v), on the rate of crystal growth of succinylsulfathiazole Form I in aqueous suspensions at 22 ± 0.1° was examined in separate experiments.

Succinylsulfathiazole Form II was suspended, in place of Form I, in water, and the particle-size distribution was determined as previously described at various time intervals.

IR spectrophotometry (1, 2) was used to follow various stages in the transformation of Form I to Form II in aqueous suspensions under the different experimental conditions adopted.

RESULTS AND DISCUSSION

Particle-size distribution data of anhydrous succinylsulfathiazole Form I in aqueous suspension at 22°, plotted as cumulative oversize frequency percentage *versus* diameter at various time intervals, are shown in Fig. 1. A marked horizontal displacement of the curves to the right with time, indicating crystal growth, was observed. The rate of crystal growth of Form I was derived by following the principles of Edmundson and Lees (11) as applied by Carless *et al.* (6, 7) and Ravin *et al.* (10) in their crystal growth studies.

For this purpose, a plot of the cumulative oversize frequency percentage *versus* time (Fig. 2) was established, from the data in Fig. 1, for various particle diameters. The increase in diameter with time at various cumulative oversize frequency percentages (obtained by following horizontal lines cutting the curves in Fig. 2 at the specified oversize frequency percentages) was then plotted (Fig. 3). The slopes of the lines in Fig. 3 represent the crystal growth rate as the increase of diameter per unit time—*viz.*, in micrometers per hour.

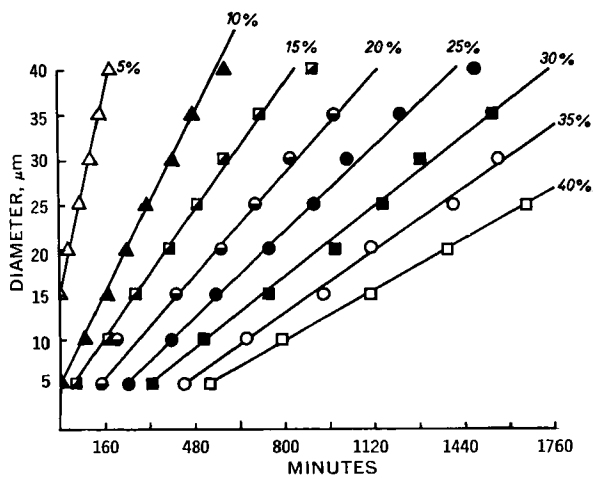

Figure 3—*Rates of growth of aqueous suspension of succinylsulfathiazole Form I at 22°.*

Values of the crystal growth rates at various cumulative oversize frequency percentages are shown in Table I. The rate of crystal growth decreased 5-7 times when the cumulative oversize frequency percentage level at which measurements were made was varied from 5 to 35. IR examination of the solid phase with time showed that a simultaneous transformation of anhydrous succinylsulfathiazole Form I to the dihydrate Form II in aqueous suspension accompanied crystal growth.

Increasing the temperature (37°) was found to increase the rate of crystal growth of succinylsulfathiazole Form I in aqueous suspension about 10 times (Table I). It was previously shown (1, 2) that an increase in temperature increased the rate of transformation of Form I to Form II in aqueous suspension. This would, in turn, lead to the production of the required number of nuclei of Form II necessary for a steady-state transformation (12) in a shorter period.

Since crystal growth was also observed (1) to be a direct consequence of the transformation of crystal form, it is not surprising to find that the rate of crystal growth was increased by an increase of temperature. Furthermore, increased temperature accelerates the diffusion-controlled processes of solution and deposition on the nuclei of the water-stable Form II (13, 14), thus increasing the proportion of large size particles.

Agitation at 100 rpm increased the rate of crystal growth of succinylsulfathiazole Form I in aqueous suspension (Table I). Both nuclei formation and deposition on the nuclei of the water-stable Form II formed involve diffusion-controlled processes (13, 14). Acceleration of the latter processes with agitation is responsible for the crystal growth observed. The effect of agitation becomes negligible at higher temperatures (37°) as the increase in the rate of crystal growth by an increase in temperature largely outweighs the effect of agitation (Table I).

Seeding an aqueous suspension of succinylsulfathiazole Form I with nuclei of the water-stable Form II increased the crystal growth rate (Table I). By increasing the number of nuclei of the water-stable Form II, seeding produced an approximately fourfold increase in the rate of crystal growth.

The presence of sulfathiazole in an aqueous suspension of succinylsulfathiazole Form I decreased the rate of crystal growth approximately 10 times (Table I). This might be explained by the possible fit of sulfathiazole molecules to specific sites in the developing nuclei of succinylsulfathiazole Form II, which results in a re-

Table I—Rate of Crystal Growth as Increase of Diameter per Unit Time at Various Cumulative Oversize Frequency Percentages of Aqueous Suspensions of Succinylsulfathiazole Form I under Different Conditions

	Rate of Growth, μm/hr						
	5%	10%	15%	20%	25%	30%	35%
Standard suspension (22°)	7.89	3.75	2.62	2.16	1.74	1.50	1.27
Standard suspension (37°)	70.80	48.00	31.74	25.09	20.88	16.92	14.46
Seeding (10% Form II, 22°)	30.00	17.50	13.35	11.75	9.85	7.40	6.23
Agitation (100 rpm, 22°)	16.99	9.00	6.76	5.33	4.50	3.67	2.85
Agitation (100 rpm, 37°)	70.80	42.90	27.30	22.20	18.40	15.42	12.49
Polysorbate 80 (1% w/v, 22°)	3.17	1.60	0.80	—	—	—	—
Sulfathiazole (10%, 22°)	0.78	0.45	0.30	—	—	—	—

duction of their number in addition to a decrease in their growth rate.

Polysorbate 80, by imposing an interfacial barrier (3, 15), reduced the rate of transformation of Form I to Form II in aqueous suspension and, consequently, decreased the crystal growth rate (Table I).

Methylcellulose inhibited the transformation of succinylsulfathiazole Form I to Form II in aqueous suspension for over a year (2). Consequently, little change in particle-size distribution was observed in the presence of methylcellulose.

Succinylsulfathiazole Form II, being stable in aqueous suspension (1), did not exhibit any measurable changes of particle-size distribution with time.

A comparison of the effect of various experimental conditions on the rate of crystal growth of succinylsulfathiazole Form I in aqueous suspension is shown in Fig. 4. An increase in temperature had the greatest growth-accelerating effect. Seeding and agitation also had marked, but weaker, growth-accelerating effects. Greater growth-retarding effects could be observed in the presence of sulfathiazole as compared to polysorbate 80. However, methylcellulose was the most effective growth retardant. In all cases, the rate of growth, calculated as an increase in diameter per unit time, was found to be greater the smaller the cumulative oversize frequency percentage level at which measurement was made.

An attempt was made to correlate the rate of crystal growth to the particle diameter of the growing crystals. The plot in Fig. 2 shows that smaller particles grew to bigger size particles at lower cumulative oversize frequency percentage levels than they did at higher levels; *i.e.*, there would be a greater proportion of large size particles at the lower frequency levels. Since rates of growth were found to be greater at lower frequency levels (where larger particles predominate), it follows that the rate of crystal growth is directly proportional to the particle diameter. This result is in agreement with the findings (6, 7) for cortisone acetate and represents an example of the third case of the crystal growth patterns described by Higuchi and Lau (3), for which they found no simple physical model.

The significance of the change in count of various particle diameters was evaluated by plotting absolute frequency percentage *versus* time curves. This type of plot (Fig. 5) was derived from the cumulative oversize frequency percentage *versus* diameter curves (Fig. 1) following the method of Carless *et al.* (6, 7). Figure 5 shows that there were a decrease in count of smaller particles (3.5 μm) with time, an increase followed by a decrease in count of intermediate-size particles (*e.g.*, 7.5 and 12.5 μm), and a progressive increase in count of larger particles (*e.g.*, 22.5 and 72.5 μm).

The decrease in count with time could be attributed to a predominant dissolution of smaller particles (15), whereas the increase in count represents predominant crystal growth. Thus, small particles are the first to go into solution, and larger particles grow at the expense of the dissolving small ones. Particles of intermediate size grow at first, as a result of the dissolution of smaller particles, until they become relatively the smaller particles in the distribution. Then they start to dissolve to give way for the growth of still larger particles. The point of inflection observed in the curves representing intermediate-size particles (*e.g.*, 12.5 μm) might be taken to mean that the two processes of dissolution and crystal growth reach a state of equilibrium.

A comparison of the curves representing the change in frequency percentage of the 12.5-μm diameter particles under various experimental conditions is shown in Fig. 6. The inflection in these curves took place at different time values. In cases where no growth retardants were present, the point of inflection corresponded to about one-third the transformation of Form I to Form II [as determined by IR analysis (2)]. Since the transformation of a crystal form is known to be a prerequisite for crystal growth, it might be assumed that a transformation of about one-third of Form I to Form II would provide the necessary driving force for the predominant crystal growth in aqueous suspensions of Form I.

The transformation-retarding additives, sulfathiazole and polysorbate 80, by interfering at the interface of dissolving and growing sites of both crystal forms, make it necessary for at least a two-thirds transformation of Form I to take place (as determined by IR analysis) before the inflection indicating predominant crystal growth may be observed (Fig. 6).

Another interesting result of the crystal growth studies of succinylsulfathiazole Form I in aqueous suspension was that the physical conditions and additives employed not only affected the crystal growth rate but also the time at which a fixed proportion of the crystal form had been changed, the time at which equilibrium particle size distribution was attained, and the maximum particle size

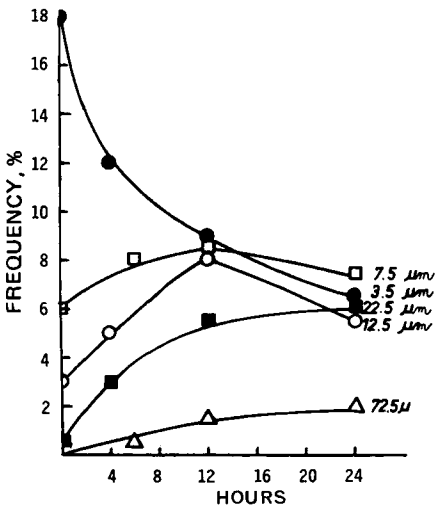

Figure 5—*Change in frequency percentage with time of aqueous suspension of succinylsulfathiazole Form I at 22°.*

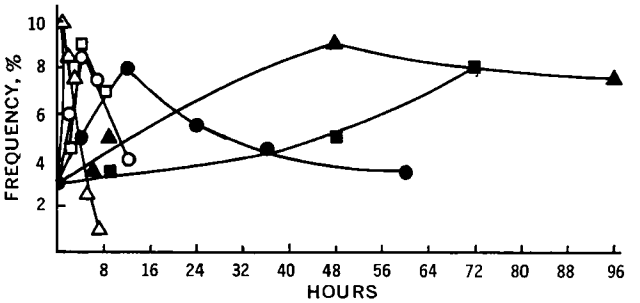

Figure 6—*Change in frequency percentage of 12.5-μm particles with time of aqueous suspensions of succinylsulfathiazole Form I at 22° under different conditions. Key: △, 37°; ▲, polysorbate 80; □, seeding at 22°; ■, sulfathiazole (10% w/w) at 22°; ○, 22° (shaking); and ●, standard at 22°.*

Table II—Effect of Various Experimental Conditions on Transformation of Crystal Form and the Time and Extent of Crystal Growth in Aqueous Suspension of Succinylsulfathiazole Form I

Condition	Time at Inflection in Count of 12.5-μm Particles	Time Required to Attain Equilibrium Particle-Size Distribution	Cumulative Oversize Frequency Percentage (100-μm Diameter)
Standard suspension of Form I (22°)	12	72	17
Standard suspension (37°)	1	7	25
Seeding (10% Form II)	4	10	3.5
Agitation (100 rpm)	4	12	2.5
Polysorbate 80 (1% w/v)	48	72	5
Sulfathiazole (10%)	72	96	1

reached. Table II shows that when the additive had an accelerating effect on the transformation of crystal form, a reduction in the time necessary to achieve the equilibrium particle-size distribution and an increase in the number of relatively larger size particles (100 μm) were also observed and vice versa.

Unexpectedly, seeding and agitation, although increasing the crystal growth rate markedly, did not lead to the production of a high proportion of large particles (100 μm). This finding might be attributed to an increase in the number of nuclei of the water-stable Form II under these conditions. Since the amount of solid material in suspension is roughly constant throughout the experiments, distribution of the solid material on the large number of nuclei present results in the production of only fewer numbers of large particles.

An increase in temperature, on the other hand, accelerated the transformation of crystal form and crystal growth and also produced a relatively high proportion of the 100-μm particles (Table II). It could be assumed that, although an increase in temperature accelerated the transformation of crystal form, the number of nuclei required for a steady-state transformation (2, 12) was not increased under these conditions. These nuclei grew, therefore, to larger size particles.

As a result of the foregoing discussion, the mechanism of crystal growth is thought to consist of two stages. The first involves transformation of the metastable crystal form to the water-stable form. The second stage involves growth of formed nuclei of the latter form. Evidence has been accumulating that the rate-controlling step in the crystal growth process is the change of crystal form. When this was inoperative, *e.g.*, when the water-stable form was suspended in water, no growth was observed. When the transformation of crystal form was retarded, crystal growth was also inhibited (*e.g.*, in the presence of methylcellulose, sulfathiazole, and polysorbate 80).

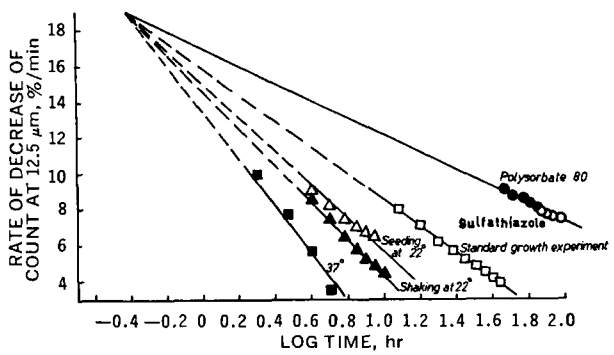

Figure 7—*Comparison of rates of dissolution of succinylsulfathiazole under different conditions.*

Table III—Effect of Experimental Conditions on the Rate of Transformation of Crystal Form and Crystal Growth in Aqueous Suspension of Succinylsulfathiazole Form I

Condition	K[a] Transformation × 10^4, min^{-1}	K Growth (10% Cumulative Oversize Frequency)
Standard suspension (22°)	6.33	3.75
Standard suspension (37°)	33.60	48.00
Seeding (10% Form II)	27.59	17.50
Polysorbate 80 (1% w/v)	4.99	1.60

[a] Data from Ref. 2.

On the other hand, when the transformation of the crystal form was fast or was increased by altering the prevailing physical conditions (*e.g.*, by increasing temperature or agitation), the crystal growth that followed was also quite marked. Seeding effects are also further evidence that the rate-controlling step is the transformation of crystal form. Thus, the inclusion of seeds of the water-stable Form II in aqueous suspensions of Form I increased not only the rate of transformation of crystal form (2) but also the crystal growth rate.

An attempt was made to find out which stage of the growth mechanism is affected by the various experimental conditions and additives employed. For this purpose, the rate of decrease in the count of the 12.5-μm particles, indicating predominant dissolution, *versus* time on a logarithmic scale was plotted (Fig. 7). Figure 7 shows that the rate of decrease in the count (a measure of the dissolution rate) at a very short time after the start of the experiments (about 0.4 hr), obtained by backward extrapolation, was practically the same under various experimental conditions. This finding might be interpreted by assuming that all experimental variables affected the transformation of crystal form and, consequently, the crystal growth of Form II. Form I, existing alone for a very short time, had a high dissolution rate which was not yet affected by the experimental variables. As time went on, transformation of Form I to Form II took place with variable velocities because of the experimental conditions prevailing. Since the developing water-stable Form II is about five times less soluble than Form I (16), the curves in Fig. 7 could be seen to deviate from each other as time progressed.

Table III shows that the rate of crystal growth under different conditions was increased or decreased in the same way as the rate of transformation of crystal form was affected. These arguments are, therefore, supporting evidence of previous conclusions and findings (1, 2) that experimental conditions affect the rate of transformation of Form I to Form II in aqueous suspension and, consequently, the crystal growth rate.

It might be concluded that, in agreement with previous recommendations (1, 2), the formulation of physically stable suspensions of succinylsulfathiazole would best be achieved by using water-stable Form II or, alternatively, including an efficient transformation retardant, *e.g.*, methylcellulose, with Form I. Attention must also be drawn to the incidence of polymorphism among pharmaceuticals as one important factor that could alter the physical stability of pharmaceutical preparations.

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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