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Kinetics of Drug–Drug Interactions in Sheep: Tolbutamide and Sulfadimethoxine

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Abstract □ The interaction between sulfadimethoxine and tolbutamide in sheep involving displacement from protein binding sites was investigated quantitatively. A 52% increase in the unbound plasma concentration of tolbutamide was observed *in vitro* at 37° after the addition of sulfadimethoxine (100 μg/ml) to sheep plasma containing tolbutamide (50 μg/ml). Transient changes in tolbutamide's unbound and total plasma concentrations were noted after acute intravenous administration of sulfadimethoxine to sheep receiving a constant intravenous infusion of tolbutamide. These observations were consistent with displacement of tolbutamide from plasma and tissue binding sites and redistribution of the displaced tolbutamide into body water spaces. The steady state of both agents featured little change in the total plasma tolbutamide concentration, a 150% increase in the unbound plasma tolbutamide concentration, and an inhibition of tolbutamide oxidation by sulfadimethoxine. A model is presented and mathematical relationships are derived that permit a quantitation of the interaction and that indicate that sulfadimethoxine's constant of metabolic inhibition (K_I) for tolbutamide metabolism is 65 μg/ml.

Keyphrases □ Tolbutamide—interaction with sulfadimethoxine, kinetic analysis, sheep □ Sulfadimethoxine—interaction with tolbutamide, kinetic analysis, sheep □ Drug interactions—tolbutamide and sulfadimethoxine, kinetic analysis, sheep □ Interactions, drug—tolbutamide and sulfadimethoxine, kinetic analysis, sheep □ Kinetics—analysis of tolbutamide–sulfadimethoxine interaction, sheep □ Antidiabetic agents—tolbutamide, interaction with sulfadimethoxine, kinetic analysis, sheep □ Antibacterials—sulfadimethoxine, interaction with tolbutamide, kinetic analysis, sheep

The coadministration of two or more drugs may produce ineffective therapy, exaggerated therapeutic responses, or even toxic responses. While numerous reports on such drug interactions have appeared in review articles (1–7) and books (8–10), they generally provide little more than a compilation of observed events. In one publication (11), attempts were made to discuss and relate the molecular events underlying various drug interactions.

The application of pharmacokinetic principles to the quantitation of drug–drug interactions has been limited but successful in the interpretation of the data. Kinetic analysis of the metabolic interaction of tolbutamide and sulfaphenazole explained observed clinical adverse effects and permitted a prediction of plasma levels of both agents when coadministered (12). Kinetic evaluation of the interaction between warfarin and phenylbutazone demonstrated the complexities that may be involved in metabolic interactions (13, 14). By using a perfusion-limited pharmacokinetic model (15), it was possible to assess contributions of enzyme induction and increased hepatic blood flow in mixed enzymatic–hemodynamic drug interactions (16).

Displacement of one drug by another from protein binding sites has frequently been suggested as the cause of an enhanced pharmacological response, but no reports exist quantitating the kinetics of these displacement interactions. The purpose of this investigation was to examine critically the pharmacokinetic aspects of an interaction between two agents, tolbutamide and sulfadimethoxine, that exhibit a displacement phenomenon *in vitro* (17). This report, describing experiments with sheep, demonstrates the involved complexities and the approaches that can be taken to quantitate drug interactions.

EXPERIMENTAL

Animal Preparation—Vascular catheters, 2.3-mm o.d. Tygon flexible tubing[1], were inserted into either the saphenous vein and the femoral artery or the jugular vein and the carotid artery of 40–50-kg sheep. Between experiments, the catheters were kept patent by filling with heparin[2] and flushing with normal saline periodically. A foley 20 FR catheter[3] was

[1] Type S-54-HL, Port Plastics Inc.
[2] Lipo-Hepin, 1000 units/ml, Riker.
[3] Bardex, 165V.

introduced into the bladder *via* its fundus after ventral midline abdominal incision. Both vascular and bladder catheters were externalized in a pocket attached to the side of the animal to permit ease of chronic sampling.

The sheep diet consisted of alfalfa pellets and water. Throughout all studies, food and water were permitted *ad libitum*.

Arterial blood samples were collected in heparinized or fluoridated Vacutainer tubes. Plasma was separated immediately by centrifugation and either used immediately for analysis or stored at −4° for subsequent analysis. All urine samples were either analyzed immediately or stored in scintillation vials at −4° for subsequent analysis.

Materials—All reagents were of analytical reagent grade. Solvents were generally redistilled prior to use. Tolbutamide[4], ^{14}C-tolbutamide[5], hydroxytolbutamide[4], ^{3}H-tolbutamide, and sulfadimethoxine[6] were administered as sodium salt solutions, diluted with normal saline where appropriate. All solutions were filtered[7] prior to intravenous administration[8].

Analytical Methods—Tolbutamide, hydroxytolbutamide, and carboxytolbutamide were determined in plasma, plasma water, or urine by the analytical method of Matin and Rowland (18).

Sulfadimethoxine and its N^4-conjugated metabolite were determined by the Bratton–Marshall method (19).

Glucose was measured[9] in plasma by the enzymatic glucose oxidase method (20).

Nonesterified (free) fatty acids were determined using the method of Dole (21).

Total plasma protein was measured, using automated equipment[10], by the biuret procedure (22) or the Lowry *et al.* method (23). Plasma electrophoresis was performed on cellulose acetate[11].

Plasma Protein Binding—All three methods were performed at 37°. Equilibrium dialysis was carried out for 6 hr using the dialyzing system[12] and the method of Weder *et al.* (24). Krebs–Henseleit buffer, pH 7.4, was used as the dialyzing fluid. Ultracentrifugation[13] was conducted at 55,000 rpm for 24 hr, after which 0.5–1.0 ml of supernate was removed for analysis. Ultrafiltration was carried out using membrane cones[14]; centrifuging[15] at 2000 rpm for 4 min provided a filtrate that was 10% of the initial plasma volume.

Radioactivity Measurements—Radioactivity was quantitated in biological fluids by scintillation counting[16] using the method of automatic external standardization and employing appropriate quench correction curves.

RESULTS AND DISCUSSION

Intravenous Tolbutamide Bolus—The intravenous bolus administration of tolbutamide in sheep served to determine its metabolic fate, characterize its disposition kinetics, and allow an appraisal of a pharmacological response, namely plasma glucose depression. In an experiment utilizing ^{3}H-tolbutamide (2.0 μCi/mg), 67.8% of the dose (100 mg, 2.9×10^7 dpm) was recovered in urine collected for 9 hr. Of this recovered radioactivity, 86.5% could be removed by exhaustive extraction of acidified urine with 0.5% isoamyl alcohol in dichloromethane. By using the separation method of Matin and Rowland (18), all extracted radioactivity was accounted for as tolbutamide, carboxytolbutamide, and hydroxytolbutamide in a ratio of 2:9:89. In subsequent experiments using unlabeled tolbutamide, 66–80% of the administered dose could be accounted for as the cumulative urinary excretion of these three compounds.

Since carboxytolbutamide represented only 10% of the detectable oxidation products excreted, it was included as part of the hydroxytolbutamide estimate in subsequent analyses of urine collected during the interaction studies. Sheep thus join rats, guinea pigs, rabbits, and humans as species that predominantly eliminate hydroxy- and carboxytolbutamide after tolbutamide administration (25–27). The tolbutamide excretion product(s) unaccounted for remain uncharacterized.

[4] Orinase Diagnostic, The Upjohn Co.
[5] Farbwerke Hoechst AG, Germany.
[6] Hoffmann-La Roche Inc.
[7] Millipore filter, 0.45 μm, 13 mm, Millipore Corp.
[8] Compact infusion pump, Harvard Apparatus Co.
[9] Beckman glucose analyzer, Beckman Instruments.
[10] Technicon, two channel.
[11] Beckman Instruments.
[12] Dianorm, Inno Med, Switzerland.
[13] L2-65B, Beckman Instruments.
[14] Centriflo (CF50A), Amicon Corp.
[15] International model UV
[16] Packard Tri-Carb, Packard Instrument Co.

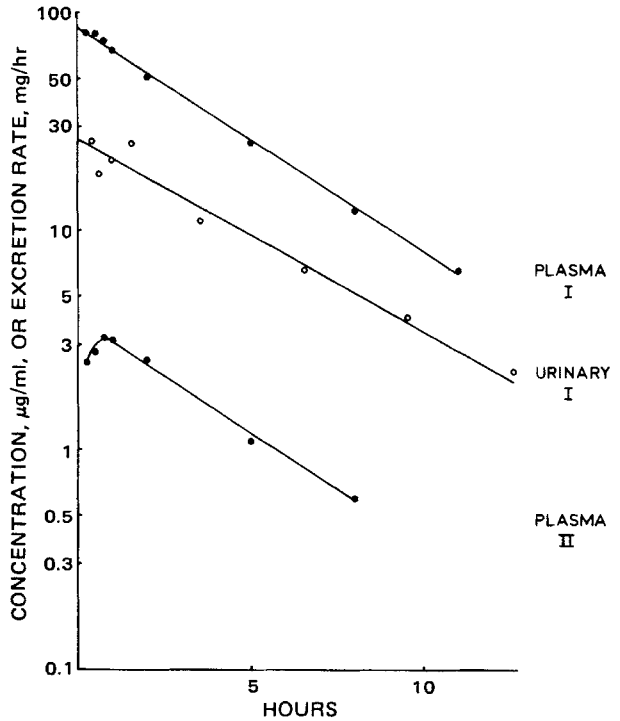


Figure 1—*Plasma concentration and urinary excretion rate of tolbutamide (I) and plasma concentration of hydroxytolbutamide (II) after an intravenous tolbutamide bolus (865 mg).*

Plasma tolbutamide and hydroxytolbutamide levels and the excretion rate of tolbutamide after an intravenous bolus dose of tolbutamide (865 mg) can be seen in Fig. 1. Tolbutamide is removed quickly from the sheep, and the data suggest that the disappearance of hydroxytolbutamide is rate limited by its formation. During some bolus experiments, a distribution phase was occasionally detected. When visible, however, its contribution was always less than 10% of the total area; therefore, less than 10% of the dose was eliminated during the distribution phase. Accordingly, as a reasonable approximation, the elimination of tolbutamide was characterized as occurring from a single compartment.

Repeated intravenous bolus administration of tolbutamide in a single sheep over 4 months provided half-lives, apparent volumes of distribution, and total body clearances (±*SD*) of 3.6 (±0.5) hr, 11.2 (±1.0) liters, and 37.5 (±2.1) ml/min, respectively. Excluded from these values are the results of an experiment that yielded a prolonged half-life of 10.6 hr, a similar apparent volume of distribution (11.7 liters), and a reduced total body clearance (12.7 ml/min). These abnormal kinetic parameters coincided with an infection. The differences could not be explained by changes in total or fractional plasma protein content or by changes in the fraction of tolbutamide bound to plasma proteins. The infectious state apparently decreased the metabolic activity of the liver.

The plasma glucose depression observed after an intravenous tolbutamide bolus (900 mg) can be seen in Fig. 2. A depression occurs shortly after administration, with a rapid return to baseline values within 2 hr. These observations made with sheep are similar to those reported for humans (28).

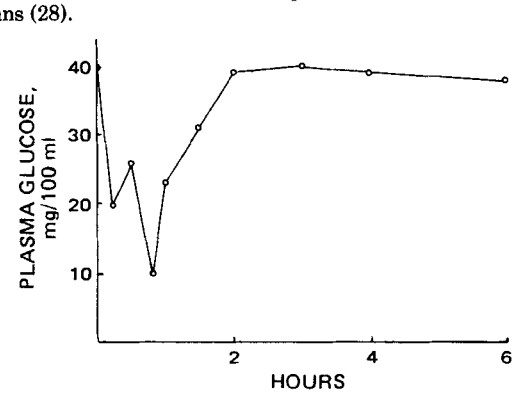


Figure 2—*Plasma glucose concentration in sheep after an intravenous tolbutamide bolus (900 mg).*

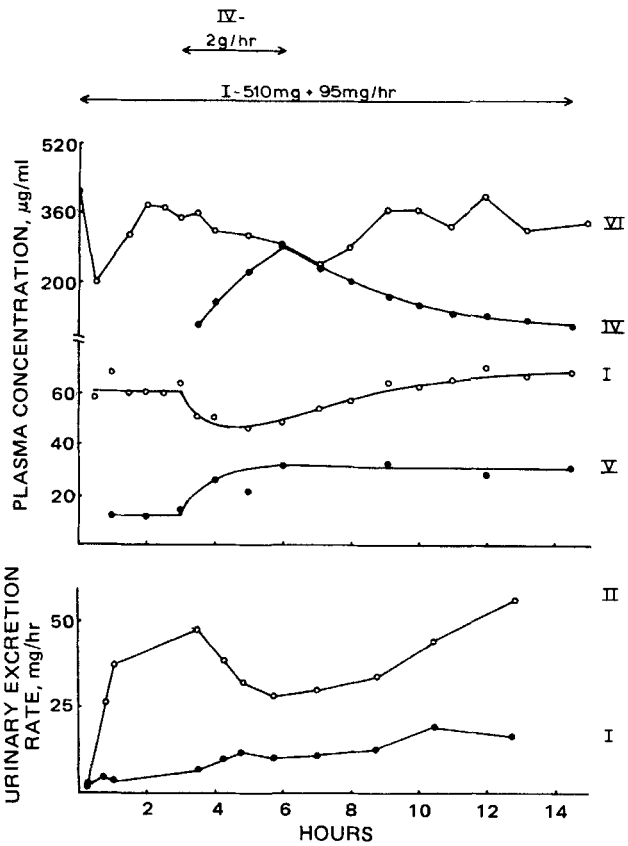


Figure 3—*Interaction Study 1 of tolbutamide and sulfadimethoxine in sheep. Tolbutamide (I) was given as a bolus (510 mg) followed by an infusion (95 mg/hr), while sulfadimethoxine (IV) was administered for 3 hr as an infusion (2 g/hr). The resultant plasma concentrations of glucose (VI), sulfadimethoxine (IV), tolbutamide (I), and unbound tolbutamide (V) and urinary excretion rates of hydroxytolbutamide (II) and tolbutamide (I) are displayed.*

Intravenous Hydroxytolbutamide Bolus—Hydroxytolbutamide is eliminated very rapidly from sheep. An intravenous bolus dose (250 mg) in two sheep provided terminal plasma half-lives of 13.5 and 26 min, apparent volumes of distribution of 15.8 and 26.5 liters, and total body clearances of 1.0 and 1.5 liters/min. These observations confirm the previous suggestion that the rate of removal of hydroxytolbutamide from the plasma after tolbutamide administration is rate limited by its formation (Fig. 1). The entire dose of hydroxytolbutamide was recovered in the urine, 90% as unchanged drug and the remainder as carboxytolbutamide. A 250-mg intravenous bolus dose failed to depress the plasma glucose level. Thus, hydroxytolbutamide appears to lack significant hypoglycemic activity.

Intravenous Sulfadimethoxine Bolus—Sulfadimethoxine exhibited biexponential kinetics, with the distribution and elimination phases having half-lives of 1 and 7.4 hr, respectively. The latter value is similar to a reported half-life of 8.4 hr in sheep (29). Further, the low total body clearance (31.8 ml/min) of sulfadimethoxine was similar to that noted for tolbutamide. However, a 4-g bolus dose failed to produce a significant hypoglycemic effect.

***In Vitro* Plasma Protein Binding**—When using ultracentrifugation, ultrafiltration, and equilibrium dialysis, tolbutamide (100 μg/ml) was bound 85.1, 85.4, and 85.6%, respectively, to sheep plasma at 37°. In light of these similar results, ultrafiltration was used almost exclusively in subsequent *in vivo* studies due to its short analysis time. While all experiments were conducted at 37°, a change to 25° resulted in a maximal mean increase in binding of only 0.7% over the tolbutamide concentration range of 10–100 μg/ml of plasma. At 100, 200, 300, and 400 μg/ml, sulfadimethoxine was bound to the extent of 88.2, 81.2, 73.4, and 66.8%, respectively, in sheep plasma.

Sulfadimethoxine displaces tolbutamide from binding sites in sheep plasma. At a tolbutamide concentration of 50 μg/ml, sulfadimethoxine concentrations of 100, 200, 300, and 400 μg/ml increased the unbound tolbutamide concentration by 52, 102, 128, and 150%, respectively. These observations confirmed the reported effectiveness of sulfadimethoxine

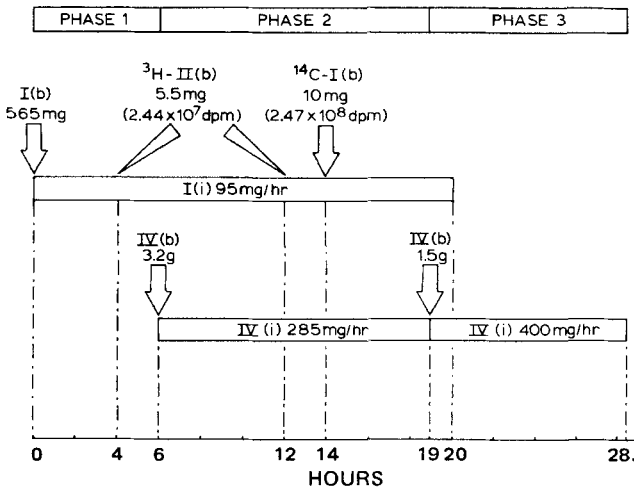


Figure 4—*Flow diagram indicating the times of administration of tolbutamide (I), hydroxytolbutamide (II), and sulfadimethoxine (IV) in Interaction Study 2. All compounds were administered in the form of an intravenous bolus (b) or infusion (i).*

as a displacer of tolbutamide from plasma proteins (17) and made this sulfonamide a logical choice for the investigation of the kinetics of drug interactions involving displacement of drugs from protein binding sites.

Tolbutamide–Sulfadimethoxine Interaction Studies—Significant displacement of tolbutamide from plasma binding sites *in vivo* was ensured by producing the same plasma concentrations of tolbutamide and sulfadimethoxine in the sheep as were used *in vitro*. By utilizing the intravenous bolus data, an infusion rate of tolbutamide was chosen to maintain its plasma concentration at approximately 50 μg/ml and a regimen of sulfadimethoxine was chosen to produce plasma concentrations up to 600 μg/ml. All interaction studies were conducted in a single animal.

In the initial experiment (Interaction Study 1), tolbutamide was administered as a bolus dose followed by a constant infusion for 14.5 hr. Sulfadimethoxine was coadministered for 3 hr by constant infusion. During its infusion, sulfadimethoxine caused a rise in the unbound concentration and a drop in the total plasma tolbutamide concentration (Fig. 3). These observations are in keeping with displacement of tolbutamide from plasma binding sites and redistribution of the unbound drug extravascularly.

At the peak plasma sulfadimethoxine concentration, the unbound tolbutamide concentration increased maximally by 160% (from 12.3 to 31.6 μg/ml). Thereafter, although the plasma sulfadimethoxine concentration fell and the total tolbutamide concentration rose eventually to exceed the presulfonamide steady-state level, the unbound tolbutamide level remained virtually unchanged for the remainder of the experiment. Meanwhile, whereas the urinary excretion rate of hydroxytolbutamide fell and rose during and after the sulfadimethoxine infusion, the tolbutamide excretion rate remained elevated despite a decrease in the plasma sulfadimethoxine concentration. Immediately after the bolus of tolbutamide, plasma glucose levels fell sharply, but they returned to the control value within 2 hr. They fell, albeit more slowly, during sulfadimethoxine administration, reaching a minimum which appeared coincident with the attainment of the maximal unbound plasma tolbutamide concentration.

The interaction study was repeated to examine the kinetics of interactions under steady-state tolbutamide levels in the absence and presence of a steady-state plasma sulfadimethoxine concentration (Interaction Study 2). Figure 4 contains a flow diagram indicating all aspects of drug administration during this study. Generally, the results in Fig. 5 confirm the observations seen during Interaction Study 1. By earlier sampling after an initial sulfadimethoxine bolus (6 hr after initiating tolbutamide administration), the early transient changes in unbound and total plasma tolbutamide concentrations were examined in greater detail. Again they reflected displacement from plasma proteins and rapid extravascular redistribution; similar events were noted in sheep (30) immediately following the displacement of sulfadoxine from binding sites by phenylbutazone.

By the end of phase 2 (19 hr), both unbound and total tolbutamide levels appeared to approach steady state. By then, the unbound tolbu-

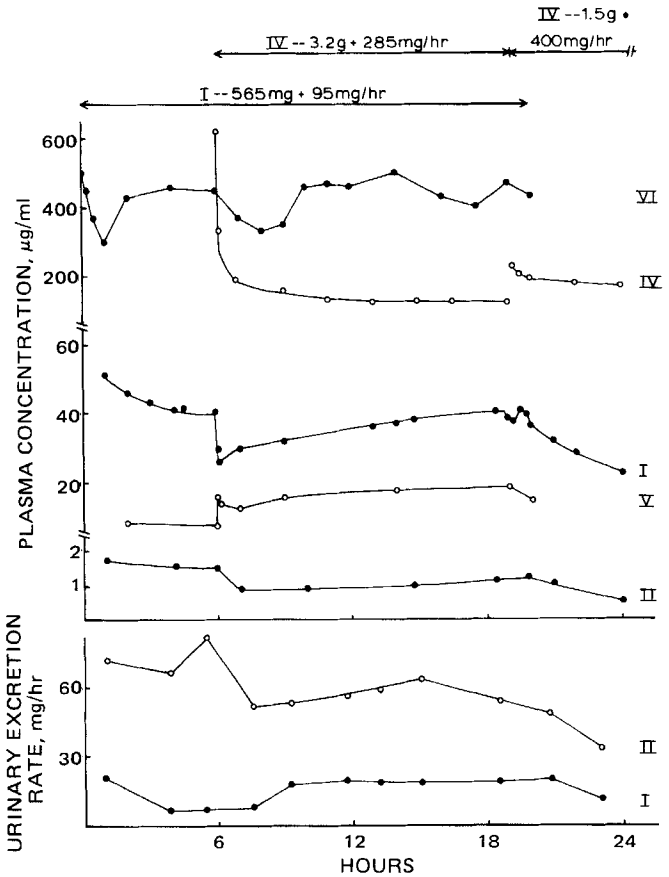


Figure 5—*Interaction Study 2 of tolbutamide and sulfadimethoxine in sheep. Tolbutamide (I) was given as a bolus (565 mg) followed by an infusion (95 mg/hr) until 20 hr. Sulfadimethoxine (IV) was coadministered as a bolus (3.2 g) at 6 hr followed by an infusion (285 mg/hr) until 19 hr, after which an additional bolus (1.5 g) was given followed by a new infusion rate (400 mg/hr) until 28.5 hr. The resultant plasma concentrations of glucose (VI), sulfadimethoxine (IV), tolbutamide (I), unbound tolbutamide (V), and hydroxytolbutamide (II), as well as the urinary excretion rates of hydroxytolbutamide (II) and tolbutamide (I), are exhibited.*

tamide level had increased twofold over the levels seen at the end of phase 1, while total tolbutamide had returned to the preexisting level. An additional bolus and increased infusion rate at the outset of phase 3 produced only minor changes in the total plasma tolbutamide concentration. The half-lives of ^{14}C-tolbutamide and nonradioactive tolbutamide during phase 3 (Figs. 6 and 5, respectively) were both 5.2 hr. This value was longer than the half-life of 3.8 hr observed following a 900-mg intravenous bolus dose of tolbutamide 2 days prior to this interaction study and longer than the ^{14}C-tolbutamide half-life (3.8 hr) observed during phase 2 of this study (Fig. 6).

Plasma hydroxytolbutamide decreased noticeably after the administration of sulfadimethoxine and then gradually increased, approaching a new steady state at 19 hr (Fig. 7). The urinary excretion rate of hydroxytolbutamide decreased temporarily and then increased, while that of tolbutamide remained notably elevated during sulfadimethoxine coadministration. The pattern of changes in plasma glucose was analogous to that seen in Fig. 3; levels were depressed after the first bolus of tolbutamide and sulfadimethoxine, but each time the glucose level returned to the control value.

Displacement of Tolbutamide by Sulfadimethoxine *In Vivo*—The magnitude of the initial changes and the subsequent levels of unbound tolbutamide as seen in Figs. 3 and 5 were greater than anticipated had only displacement from plasma protein sites occurred. By using a mean apparent volume of distribution of 11.2 liters (bolus data), a plasma volume of 1.8 liters, and 85% binding in plasma, it was calculated that only 14% of the drug in the body is bound to plasma proteins. Even if all of this plasma-bound drug had been displaced, to be redistributed throughout the rest of the body, it would fail to increase the unbound concentration to anything like the observed 110% within 1 hr following sulfadimethoxine administration. Therefore, sulfadimethoxine must also displace tolbutamide from an extensive extravascularly bound pool. The early maintenance of these elevated levels of unbound tolbutamide suggests that the distribution space of the unbound drug may, in fact, be limited. A similar explanation was suggested by Jähnchen *et al.* (31) who reported no statistically significant increase in the apparent volume of distribution of dicumarol in the rat, despite extensive displacement of this anticoagulant by phenylbutazone.

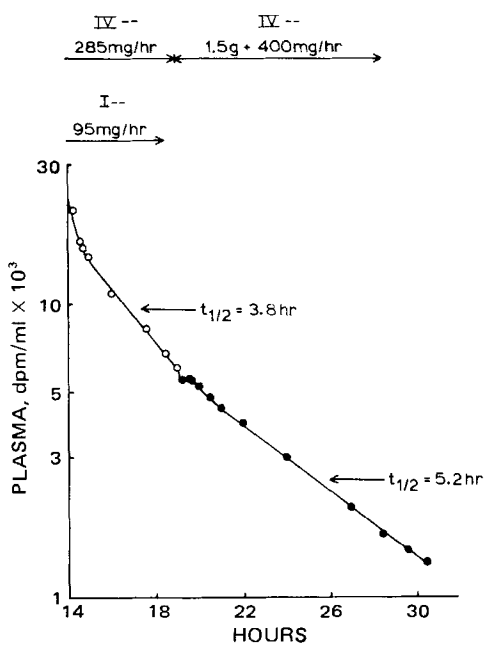


Figure 6—*Total plasma radioactivity after intravenous administration of a pulse of ^{14}C-tolbutamide (2.47×10^8 dpm) during Interaction Study 2.*

The unbound fraction of tolbutamide in plasma (α) depends on several factors, including the total plasma concentrations of the interacting drugs and binding protein(s), as well as the affinities of the binding species for the protein(s). To examine the altered binding during the interaction study, not entirely accounted for by changes in the plasma concentrations of tolbutamide and sulfadimethoxine, the following test was performed. A comparison was made between the values of α determined in samples obtained during Interaction Study 2 with those determined in samples prepared by placing the same concentrations of tolbutamide and sulfadimethoxine into control plasma collected from the animal 2 hr prior to the study.

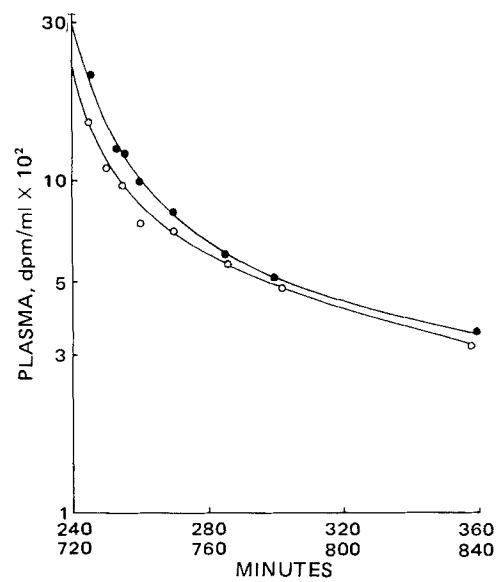


Figure 7—*Total plasma radioactivity determined after intravenous administration of a pulse of ^{3}H-hydroxytolbutamide (2.44×10^7 dpm) before (●) and after (○) sulfadimethoxine administration during Interaction Study 2.*

Table I—Evaluation of Nonesterified Fatty Acid (FFA) and Plasma Proteins in Sheep 1494 Prior or During Interaction Study 2

Hours	α[a] Found	α[a] Expected	FFA, μg/ml	Plasma Proteins, g/100 ml Total	Albumin	α_1-Globulin	α_2-Globulin	β-Globulin	γ-Globulin
Control-a[b]	—	—	—	9.2	2.7	0.5	0.9	2.6	2.5
Control-b[c]	—	—	—	9.2	2.3	0.4	1.1	3.7	1.7
0	—	—	0.66	—	—	—	—	—	—
2.0	0.18	—	0.51	—	—	—	—	—	—
6.0	0.19	—	0.42	—	—	—	—	—	—
7.0	0.43	0.39	0.33	—	—	—	—	—	—
9.0	0.48	0.36	0.44	—	—	—	—	—	—
13.0	—	—	—	9.2	2.7	0.4	1.0	2.4	2.7
14.0	0.47	0.34	—	—	—	—	—	—	—
18.5	—	—	0.52	—	—	—	—	—	—

[a] Unbound tolbutamide fraction in plasma. [b] Sample from the infectious state during which time the tolbutamide kinetics were altered. [c] Sample from the animal 2 months prior to Interaction Study 2.

As seen in Fig. 8, major differences in the value of α between the paired samples were noted throughout most of the interaction study. Measurement of 9-, 11-, and 14-hr samples also showed a similar elevation in the α values for sulfadimethoxine. The possibility that decreases in plasma proteins [*e.g.*, albumin (32–34)] or changes in free fatty acids (35, 36) during the interaction study might have caused the exaggerated unbound fraction was excluded. No significant differences were noted between samples obtained during the study and control samples prior to the study (Table I). The possibility of additional displacement caused by the excessive accumulation of a highly bound N^4-conjugated sulfadimethoxine metabolite (37) also was excluded. No significant increase was noted in the samples between the free and total sulfonamides. The question of the differences remains unresolved.

Tolbutamide Binding and Tolbutamide Renal Clearance—By using total and unbound plasma tolbutamide concentrations, along with the urinary data of Interaction Study 2, the renal clearance of tolbutamide was evaluated. Comparing the data at the end of phases 1 and 2 resulted in renal clearances, based upon total plasma tolbutamide concentrations, of 3.0 and 7.6 ml/min, respectively; the renal clearances based upon unbound tolbutamide levels were 16.2 and 16.5 ml/min, respectively. The similarities of the latter values suggested that the unbound plasma tolbutamide concentration dictated the urinary excretion rate.

Regression analysis on all data of Interaction Study 2 was performed to evaluate the correlation between the rate of urinary tolbutamide excretion and either the total or unbound tolbutamide concentration. As seen in Fig. 9, the correlation coefficient using the unbound data ($r = 0.90$) was higher than when total tolbutamide levels were used ($r = 0.59$). Unfortunately, the unbound tolbutamide data were primarily clustered at two points so the results in Fig. 9 are not conclusive. Nevertheless, the preceding results suggest that a linear relationship exists between the urinary excretion rate of tolbutamide and the unbound plasma tolbutamide concentration. These experiments point out the usefulness of drug interactions in perturbing the normal disposition of an agent in order to evaluate its mode of elimination kinetically.

Hydroxytolbutamide Renal Clearance—The hydroxytolbutamide urinary excretion results observed in Interaction Study 1 (Fig. 3) suggested that sulfadimethoxine possibly reduced the renal clearance of hydroxytolbutamide. This potential interaction was investigated in a separate experiment. The results did not show any significant effect by sulfadimethoxine on the renal clearance of hydroxytolbutamide (738 ml/min) (Fig. 10), and this finding was confirmed in Interaction Study 2. A pulse of ^{3}H-hydroxytolbutamide (5.5 mg, 2.44×10^7 dpm) was administered in the absence (4 hr) and presence (12 hr) of sulfadimethoxine, and the plasma was monitored for radioactivity. No significant change was found in the disposition of the radioactivity (Fig. 7). Since hydroxytolbutamide is cleared almost exclusively by renal excretion, these foregoing investigations indicate that any changes in its urinary excretion rate during the tolbutamide–sulfadimethoxine interaction must have been due to changes in its formation.

Inhibition of Tolbutamide Oxidation—The total plasma clearance of tolbutamide is much lower than the combined flow to the two organs, liver and kidney, responsible for tolbutamide elimination. It is anticipated that the elimination rate of drugs like tolbutamide, which are poorly extracted by the eliminating organs, is dependent on its unbound plasma concentration (35, 38). Certainly the dependence of the renal clearance of tolbutamide on α supports this hypothesis. If this hypothesis is extended to the metabolism of tolbutamide, the sustained elevation of unbound plasma tolbutamide and changes in its total plasma concentration favor a mechanism whereby sulfadimethoxine not only displaces tolbutamide from binding sites but also inhibits its metabolism. Scheme I is the simplest model consistent with the observations. Sulfadimethoxine is depicted as displacing tolbutamide from plasma and tissue binding sites and inhibiting tolbutamide's elimination by nonrenal (metabolic) routes. The basis for these conclusions follows.

In cases where the elimination rate of a drug is proportional to its unbound concentration (Cu), the steady-state unbound concentration (Cu_{ss}) depends only on the infusion rate (R^0) and the clearance based upon the unbound drug (CLu) (35):

$$Cu_{ss} = \frac{R^0}{CLu} \qquad \text{(Eq. 1)}$$

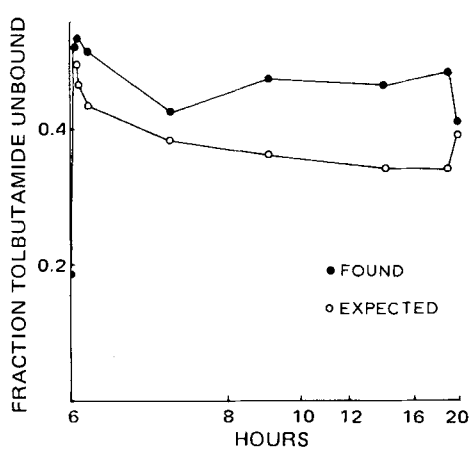


Figure 8—*Comparison of the unbound plasma tolbutamide fraction observed in Interaction Study 2, Fig. 5 (●), with the fraction anticipated (O) based upon* in vitro *displacement experiments using the same total plasma tolbutamide and sulfadimethoxine concentrations.*

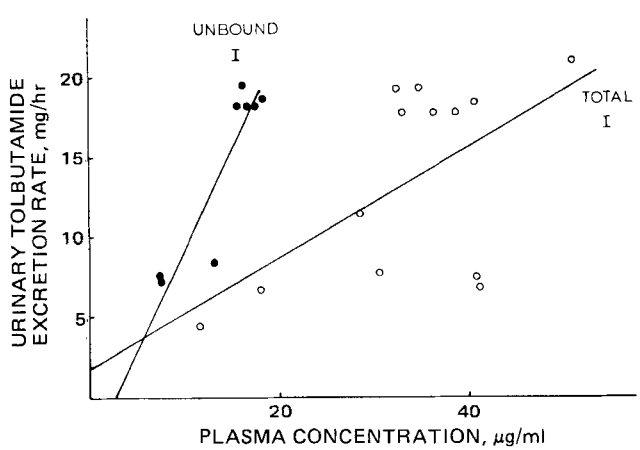


Figure 9—*Regression analysis of the rate of urinary excretion of tolbutamide on the total (O) and unbound (●) plasma tolbutamide (I) concentrations from Interaction Study 2. Correlation coefficients are 0.59 ($0.025 < p < 0.050$) and 0.90 ($p < 0.005$), respectively.*

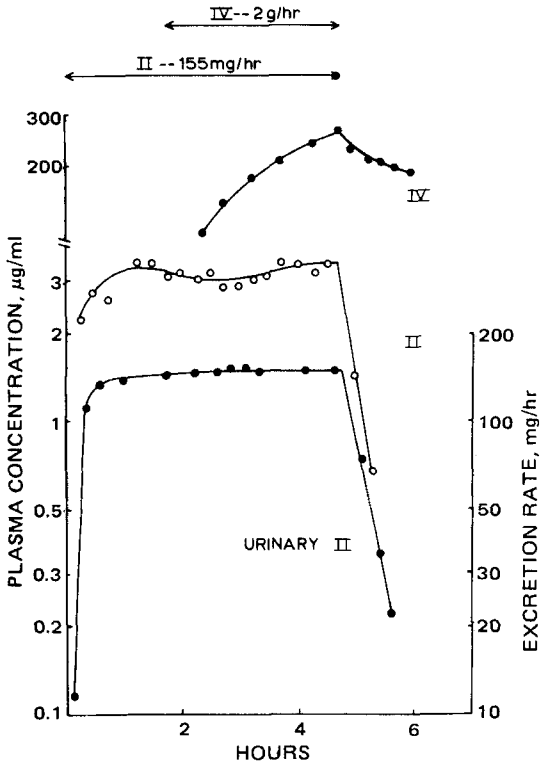


Figure 10—*Interaction of hydroxytolbutamide and sulfadimethoxine in sheep. Hydroxytolbutamide (II) was administered as an infusion (155 mg/hr) for 5 hr while sulfadimethoxine (IV) was coadministered from 2 to 5 hr as an infusion (2 g/hr). The resultant plasma concentrations of sulfadimethoxine (IV) and hydroxytolbutamide (II), as well as the urinary excretion rate of hydroxytolbutamide (II), are displayed.*

By definition, CLu is related to the clearance based on the total plasma concentration (CL) by:

$$CL = \alpha CLu \qquad \text{(Eq. 2)}$$

Thus, CLu, a measure of the inherent ability of an organ to clear a drug, may be regarded as the clearance when all drug has been displaced (α = 1). According to Eq. 1, if a compound acts only as a displacer (α increases), the steady-state unbound concentration of the displaced drug, being independent of α, remains unchanged. But, in the presence of sulfadimethoxine, the Cu_{ss} of tolbutamide rose (Fig. 5). As the infusion rate was fixed, CLu must, therefore, have decreased, with the cause being primarily the inhibition of tolbutamide metabolism.

The kinetics of competitive inhibition for a single metabolic step were described by Gillette (36):

$$\text{rate of metabolism} = \frac{V_{max}[S]}{[S] + K_m(1 + [I]/K_I)} \qquad \text{(Eq. 3)}$$

where V_{max} is the maximal velocity of the reaction, K_m is the Michaelis–Menten constant, $[S]$ is the substrate concentration, $[I]$ is the inhibitor concentration, and K_I is the concentration of inhibitor required to reduce the apparent K_m by 50%. For a compound eliminated by various routes, the rate of metabolism may alternatively be described in terms of its metabolic clearance (CLm) and when $[S] \ll K_m$:

$$\text{rate of metabolism} = \frac{CLm[S]}{1 + [I]/K_I} \qquad \text{(Eq. 4)}$$

This relationship, however, suggests that the metabolism rate is proportional to the total plasma concentration. From Eqs. 1 and 4, a general relationship is derived for the steady-state unbound plasma concentration of an agent during inhibition whose CLu takes place *via* a single metabolic step:

$$Cu_{ss} = \frac{R^0}{CLu}(1 + [I]/K_I) \qquad \text{(Eq. 5)}$$

By denoting $Cu_{ss}{}^1$ and $Cu_{ss}{}^2$ as the unbound steady-state concentration in the absence and presence of an inhibitor, respectively, Eq. 6 applies:

$$Cu_{ss}{}^2 = Cu_{ss}{}^1(1 + [I]/K_I) \qquad \text{(Eq. 6)}$$

When a compound is eliminated by various routes, the metabolic clearance based on unbound drug in the presence of inhibitor (CLu,m^2) can be predicted from the metabolic clearance in the absence of inhibitor (CLu,m^1) and the degree of inhibition:

$$CLu,m^2 = \frac{CLu,m^1}{1 + [I]/K_I} \qquad \text{(Eq. 7)}$$

Rearranging Eq. 7 yields an analytical solution for K_I:

$$K_I = \frac{[I]}{\frac{CLu,m^1}{CLu,m^2} - 1} \qquad \text{(Eq. 8)}$$

Table II—Evaluation of Tolbutamide Kinetics in Sheep 1494

Source	Parameter	Formula	Value				
Intravenous cold tolbutamide bolus data (means)	CL, ml/min	$\frac{\text{dose}}{\int_0^\infty C_p\,dt}$	37.5				
	α	—	0.18[a]				
	CLu, ml/min	CL/α	208				
	$t_{1/2}$, hr	—	3.6				
	V_d, liters	$(1.44CL)(t_{1/2})$	11.2				
			Value				
			Phase 1	Phase 2		Phase 3	
			Cold	Cold	^{14}C	Cold	^{14}C
Interaction Study 2	CL, ml/min	R^0/Ct_{ss}	40.7	38.7	45[b]	32.4[c]	34[d]
	α	Cu_{ss}/Ct_{ss}	0.19	0.45	—	0.45[c]	—
	CLu, ml/min	R^0/Cu_{ss}	214	86	—	72[c]	—
	$t_{1/2}$, hr	—	—	(3.8)	3.8	5.2	5.2
	V_d, liters	$(1.44CL)(t_{1/2})$	—	(12.7)	14.8	14.6	15.3

[a] 50 μg of tolbutamide/ml (*in vitro* determination). [b] Formula: $\text{dose}/[\int_{14}^{19} C_p\,dt + (C_p{}^{19}/K)]$. [c] Assumed or predicted values. [d] Formula:

$$\frac{\text{dose}\left[1 - \frac{\int_{14}^{19} C_p\,dt}{\int_{14}^{19} C_p\,dt + \frac{C_p{}^{19}}{K^2}}\right]}{\frac{C_p{}^{19}}{K^3}}$$

where K^2 and K^3 are the first-order rate constants for elimination during phases 2 and 3, respectively.

Table III—Results Found and Parameters Calculated from the Data of Interaction Study 2 [a]

Parameter	Formula	Phase 1	Phase 2	Phase 3
CLu, ml/min	R^0/Cu_{ss}	214	86	17 + 55 = 72[b]
CLu,r, ml/min	$\frac{\Delta Te}{\Delta t} / Cu_{ss}$	16	17	17[b]
CLu,m, ml/min	*CLu* − *CLu,r* or Eq. 7	198	70	55[b]
[*I*], μg/ml	—	—	120	168
K_I, μg/ml	Eq. 8	—	65	65
α	Cu_{ss}/Ct_{ss}	0.19	0.45	0.45[b]
V_d, liters	—	—	13.8	15.0
$t_{1/2}$ obs., hr	—	—	3.8	5.2
$t_{1/2}$ calc., hr	Eq. 9	—	—	4.9

[a] See text for explanation of abbreviations and equations. [b] Assumed or predicted values.

The elimination kinetics of tolbutamide can be characterized as occurring from a single compartment. Although the half-life in the presence of the displacer–inhibitor ($t_{1/2}^2$) is a function of the half-life in the absence of inhibitor ($t_{1/2}^1$), it also depends on the changes in overall clearance based upon the unbound drug, the apparent volumes of distribution (V_d), and the fractions of the compound in the unbound form according to:

$$t_{1/2}^2 = \frac{t_{1/2}^1 CLu^1 V_d^2 \alpha^1}{CLu^2 V_d^1 \alpha^2} \qquad \text{(Eq. 9)}$$

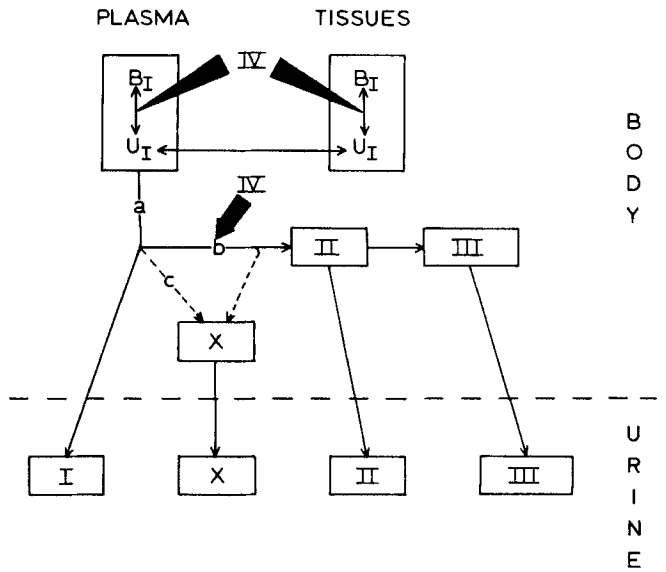
The superscripts 1 and 2 denote the situation in the absence and presence, respectively, of the displacer–inhibitor. Alternatively, they denote the situation at two levels of the displacer–inhibitor.

In Interaction Study 2, following the simplest model (Scheme I), the metabolic clearance based on unbound drug (*CLu,m*) was estimated by subtracting the renal clearance based on unbound drug (*CLu,r*), obtained by dividing the steady-state rate of renal tolbutamide excretion by its steady-state unbound plasma concentration, from the *CLu*, estimated from Eq. 1. The clearance associated with the oxidation of tolbutamide to hydroxytolbutamide, based on unbound drug, was estimated by dividing the steady-state hydroxytolbutamide excretion rate by the steady-state unbound plasma tolbutamide concentration.

Three phases were present in Interaction Study 2 (Figs. 4 and 5). A quantitative explanation for the tolbutamide–sulfadimethoxine interaction might be possible by accounting for tolbutamide's half-life during phase 3. The results of Figs. 5 and 6 revealed a prolongation in the half-life from 3.8 hr in phase 2 to 5.2 hr in phase 3. Table II summarizes the tolbutamide kinetics observed in Sheep 1494 during three nonradioactive (cold) tolbutamide intravenous bolus experiments and during Interaction Study 2. There is close agreement between the bolus results and those observed during phase 1 of Interaction Study 2. The transition from phase 1 to phases 2 and 3 was notably marked by a large reduction in the overall inherent clearance (*CLu*). The cold and ^{14}C-tolbutamide results of phases 2 and 3 are in agreement, providing mean total body clearances (*CL*), half-lives, and apparent volumes of distribution of 41.9 ml/min, 3.8 hr, and 13.8 liters, respectively, during phase 2 and of 33.2 ml/min, 5.2 hr, and 15.0 liters, respectively, during phase 3.

Table III summarizes the calculations made with the model depicted in Scheme I. From the data in phases 1 and 2, the K_I for sulfadimethoxine was calculated to be 65 μg/ml. Substituting this value of K_I and other data into the appropriate equations yielded a predicted half-life of tolbutamide in phase 3 of 4.9 hr. The close proximity between the predicted and observed half-life (5.2 hr) tends to favor the proposed interaction model. The data permitted the investigation of certain modifications in the proposed displacement–inhibition model. Inhibition by sulfadimethoxine at point a (Scheme I) or b and c independently failed to predict tolbutamide's half-life in phase 3 as well as that for the model evaluated. Consideration was also given to a model in which the renal clearance of tolbutamide was dictated by the unbound plasma concentration while the metabolic clearance was linearly related to tolbutamide's total plasma concentration. This model, however, failed to predict any change in tolbutamide's half-life in phase 3.

Tolbutamide Binding and Glucose Depression—The influence of the unbound tolbutamide concentration on glucose depression remains somewhat speculative. *In vitro* studies with pancreatic β-cells demonstrated a decreased islet uptake of tolbutamide in the presence of albumin (39). A sudden increase in unbound tolbutamide *in vivo* might, therefore, evoke an increased insulin release, resulting in a depression of plasma glucose. Indeed, the results of the interaction studies suggest such a possibility.



Scheme I—Sulfadimethoxine (IV) is depicted as altering the bound (B) to unbound (U) ratio of tolbutamide (I) in plasma and tissues. Further, it inhibits the metabolism of tolbutamide (at b) to hydroxytolbutamide (II) and an unknown metabolic product (X). A small portion of hydroxytolbutamide is oxidized to carboxytolbutamide (III).

Insulin release may involve occupancy of tolbutamide on the membrane of the β-cell (39) followed by release of part of the insulin in the labile pool (38). Maximum insulin release might follow the attainment of maximum unbound tolbutamide concentrations, leading to maximum glucose depression. After attainment of maximal unbound levels (maximum β-cell binding), no further stimulus for insulin release would exist. This hypothesis could explain the transient insulin release and glucose depression observed after an intravenous tolbutamide bolus (28).

SUMMARY

The results of the coadministration of sulfadimethoxine and tolbutamide in the sheep point to an interaction not only involving displacement of tolbutamide from proteins, which increases its renal clearance, but also inhibition of its metabolism. This explanation accounts for the changes in the total and unbound plasma tolbutamide concentrations and adequately predicts tolbutamide's half-life during phase 3 in Interaction Study 2. The observation of a simultaneous displacement–inhibition phenomenon in the interaction between tolbutamide and sulfadimethoxine is similar to that reported for the interaction between phenylbutazone and warfarin in humans (14).

This report illustrates some of the complexities of drug–drug interactions. Although gross changes in total plasma drug concentrations during the coadministration of highly plasma protein-bound agents may be absent, their absence may be due to compensative reactions. Considerable coincidental changes in unbound plasma drug concentrations may occur, however, and may provoke exaggerated therapeutic responses during chronic drug coadministration.

This report also presents a steady-state approach for investigating mechanisms of drug interactions and demonstrates that the coadministration of agents may permit investigators to scrutinize mechanisms of drug disposition. Knowledge thus obtained will help to evaluate and predict rationally the clinical significance of drug–drug interactions in humans.

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Mathematical Model for Enteric Film Coating of Tablets

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Abstract □ The enteric film coating of placebo tablets, using a methacrylic acid–methyl methacrylate copolymer as the film former in the coating solution, was studied by statistical techniques. The effects of four independent formulation and process variables on the disintegration time of the coated film in simulated intestinal fluid and on the resistance to disintegration of the coated film in simulated gastric fluid were studied. The results of a statistically designed set of experiments were used as the input data. Regression analysis of these data resulted in two first-order polynomial equations. The linear model obtained for the disintegration time of the coating in the simulated intestinal fluid was analyzed by the steepest descent method to determine the most suitable combination of the independent variables.

Keyphrases □ Tablets—enteric film coating with methacrylic acid–methyl methacrylate copolymer, disintegration time, mathematical model □ Film coating, enteric—with methacrylic acid–methyl methacrylate copolymer, tablet disintegration time, mathematical model □ Models, mathematical—disintegration time of tablets with enteric film coating of methacrylic acid–methyl methacrylate copolymer □ Disintegration time—tablets with enteric film coating of methacrylic acid–methyl methacrylate copolymer, mathematical model □ Methacrylic acid–methyl methacrylate copolymer—enteric film coating of tablets, disintegration time, mathematical model □ Dosage forms—tablets with enteric film coating of methacrylic acid–methyl methacrylate copolymer, disintegration time, mathematical model

Much research in pharmaceutics has concerned the relationship between the controllable formulation and process (independent) variables and the characteristics of the resultant system (response). An empirical relationship can be developed with data from statistically designed experiments. The relationship between the independent