Vehicle Effects on Activity of an Anticonvulsant Compound

DILIP R. SANVORDEKER ˣ and JAMES BLOSS

Abstract □ The effect of several lipid and nonlipid pharmaceutical solvents on the *in vivo* activity of 1-diphenylmethyl-4-[(6-methyl-2-pyridyl)methyleneamino]piperazine (I) was evaluated in the mouse. The intensity of onset and the duration of anticonvulsant activity of the compound were affected depending on the type and form of liquid dosage preparation used. The rate of decline in anticonvulsant activity in the 80–20% response range followed apparent zero-order kinetics. A linear relationship between the observed ED_{50} and the concentration of sorbitol in the dosage form of I was observed. A reduction in the sorbitol content of the dosage form resulted in a proportional increase in the rapidity of onset and the duration of anticonvulsant activity of I. Emulsification restored both the onset and duration of pharmacological activity, which was virtually arrested when the compound was given orally as a solution in oil.

Keyphrases □ Piperazine, substituted—anticonvulsant activity after oral administration, effect of various solvents and dosage forms, mice □ Solvents, various—effect on anticonvulsant activity of a substituted piperazine after oral administration, mice □ Dosage forms, various—effect on anticonvulsant activity of a substituted piperazine after oral administration, mice □ Anticonvulsant activity—1-diphenylmethyl-4-[(6-methyl-2-pyridyl)methyleneamino]piperazine, effect of various solvents and dosage forms, oral administration, mice

To evaluate the pharmacological activity of a new compound, it is commonly dissolved in a suitable vehicle prior to administration to an animal. This approach is particularly useful for poorly water-soluble drugs, since the rate-limiting drug dissolution process is bypassed and penetration of the drug in solution across biological membranes is facilitated. Drugs with aqueous solubility at and below 0.15 mg/ml can pose potential absorption problems (1). To overcome such difficulties, the pharmaceutical scientist often uses water-miscible nonaqueous solvents that can be suitably blended with water and provide the desired solvency for the drug (2).

1-Diphenylmethyl -4- [(6-methyl -2- pyridyl)methyleneamino]piperazine[1] (I) is a potent anticonvulsant compound in animals (3). The saturation solubility of this compound at 37° is estimated to be less than 0.1 mg/ml. This study was designed to evaluate the effect of several lipid and nonlipid pharmaceutical solvents on the *in vivo* activity of I administered *via* the oral route.

EXPERIMENTAL

Materials—Corn oil[2], dimethylacetamide[3], 2,2-dimethyl-1,3-dioxolane-4-methanol[3], polyethylene glycol 400[3], poloxamer 237[4], sorbitol (70% solution[5]), polysorbate 80[3], propylene glycol[3], and normal saline[6] were used as supplied. Compound I[7] was supplied as the pure compound. The crystals of the compound were ground in a mortar and pestle to a fine powder prior to its use.

Preparation of Suspensions of I—Except for drug suspensions in sorbitol solution, an accurately weighed quantity of I was transferred to a 25-ml test tube. Normal saline[6], with and without 1.5 ml of a propylene glycol–polysorbate 80 (50:50 v/v) mixture as a wetting agent, was added to make suspensions of the compound in the 0.2–0.8-mg/ml range. The suspensions were thoroughly mixed with the aid of a portable homogenizer[8] for 5 min. Suspensions of I at the 0.8-mg/ml concentration in sorbitol solutions (7–70%) were prepared by adding a weighed amount of I to 200 ml of the vehicle followed by mixing and homogenizing[9] the suspensions. Prior to oral administration of the dose, all suspensions were shaken vigorously.

Preparation of Solutions of I—Compound I, 100 mg, was dissolved in a nonaqueous solvent. The final volume of this solution was made with distilled water to obtain 1 mg/ml of I in a cosolvent vehicle of a defined solvent composition. These stock solutions and blank vehicles of the same composition were used to prepare diluted solutions of I at 0.1–0.8-mg/ml concentrations.

Stock solutions of I in corn oil and in soya bean oil[2] were prepared by dissolving 100 mg of I in 100 ml of the oil. This solution was then used to prepare additional solutions at lower concentrations. All solutions were prepared fresh each day prior to testing pharmacological activity.

Preparation of Oil-in-Water Emulsions of I—Accurately weighed amounts of 50–500 mg of I were transferred to 100-ml volumetric flasks. The oil was added to make up the volume, and the flasks were placed on a steam bath for approximately 5 min. After cooling, 40 ml of the oil solution of I was transferred to a 250-ml glass container; the volume was increased to 200 ml with a 0.8% solution of a nonionic surfactant[4] in distilled water. The oil and water layers were then mixed and homogenized for 0.5 hr with a lab size homogenizer[9]. Emulsions without the compound were also prepared by this procedure.

For ED_{50} determinations, the emulsions were prepared 1 day prior to the experiment and stored in a refrigerator. All other studies were performed with emulsions prepared on the same day.

***In Vivo* Studies**—Male HAM/ICR mice[10], 20–30 g, were housed in 20.3 × 35.6-cm (8 × 14-in.) stainless steel cages (12 animals/cage) and maintained on a standard pellet diet and tap water prior to each experiment. All formulations of I were given at 0.1 ml/10 g of body weight by the intragastric (oral) route of administration. The electrical current for induction of seizures was generated by a commercially available apparatus[11].

Determination of ED_{50}—Liquid dosage forms of I and their blanks (without the compound) were tested for anticonvulsant activity according to the method of Swinyard *et al.* (4). Thirty minutes after administration of a dose to a group of 20 mice, each animal was challenged with a current of 50 mamp delivered *via* corneal electrodes. Except for 2,2-dimethyl-1,3-dioxolane-4-methanol solutions, this current was sufficient to induce hindlimb tonic extensor seizure in 100% of the control mice. All data were recorded as the number of mice protected per group of 20 animals dosed with a given dosage form. The ED_{50} values were calculated by the method of Litchfield and Wilcoxon (5).

Determination of Duration of Response to I—The method was essentially the same as described previously. Groups of 20 animals were dosed with 8.0 mg/kg of I in the dosage form. The animals were then challenged with electric shock every 0.5 hr up to 5 hr and then every hour up to 8 hr after dosing. The data were recorded as the ratio of animals protected to animals dosed.

RESULTS AND DISCUSSION

Figure 1 illustrates a log dose–response relationship for I administered intragastrically to mice in three different vehicles. The response to this compound followed a previously established semilogarithmic relationship

[1] SC-13504.
[2] Columbus Food Products, Chicago, Ill.
[3] Matheson, Coleman & Bell, Norwood, Ohio.
[4] Pluronic F-87, Wyandotte Chemical Co., Wyandotte, Mich.
[5] Searle & Co., San Juan, Puerto Rico; Emulsion Engineering Inc., Chicago, Ill.
[6] Baxter Laboratories, Morton Grove, Ill.
[7] Lots II-128B and G-136-3, Searle Laboratories, Chemical Development Group, Chicago, Ill.
[8] Model S63C, Tri-R Instruments, New York, N.Y.
[9] Model PCU-2, Polytron, Kinematika GmbH, Luzern, Switzerland.
[10] Charles River Mouse Farms & Co., Wilmington, Mass.
[11] Model 2-C, Hans Technical Associates, Palo Alto, Calif.

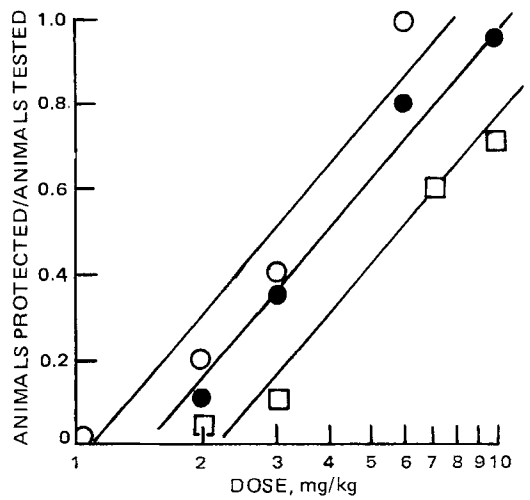

Figure 1—*Linear relationship between log dose and response to I in mice. Key: ○, suspension in saline; ●, solution in polyethylene glycol 400; and □, solution in dimethylacetamide–water (50:50 v/v).*

(6) describable by the following equation:

$$R = m \log D + e \qquad \text{(Eq. 1)}$$

where R is the intensity of the pharmacological response, D is the administered dose, m is the slope of the line illustrating the linear relationship, and e is the intercept on the x (dose) axis. From this plot, it is evident that administration of a solution of I in a water-miscible cosolvent such as dimethylacetamide results in a parallel shift of pharmacological response from lower doses to relatively higher doses.

A least-squares linear regression analysis of the log dose–response data in the 80–20% response range yielded 0.933–1.23 values for the slope. These values provide a good estimate for the free drug occupancy of the receptor sites in the biophase (7). This theoretical consideration implied that practically all of the free concentration of I available in the biophase occupied the receptor sites to provide the desired anticonvulsant response in mice. This observation agrees with the potency of this compound compared to that of its analogs at relatively low doses (1).

Table I lists the effect of several vehicles on the ED_{50} values of I estimated 0.5 hr after intragastric administration of the dosage form. Within 95% confidence limits, no difference was observed for the ED_{50} values estimated after administration of I as a suspension in normal saline and as a solution in polyethylene glycol 400. The apparent twofold increase in ED_{50} values observed for the dimethylacetamide solution is intriguing. Among the possibilities are interference with the bioavailability of I and competition at the receptor site, thus reducing the apparent efficacy by a twofold margin. The log dose–response plot demonstrates that, in the 6–10-mg/kg range, a maximal response was observed at 30 min after oral administration of the drug. With this information, subsequent studies on the duration of drug activity were conducted at the 8-mg/kg dose level to assess the effect of vehicles on this parameter.

Table I—Effect of Vehicles on the ED_{50} of I Estimated 30 min after Intragastric Dosing of Mice

Dosage Form	Vehicle Composition	ED_{50}, mg/kg (95% Confidence Limits)
Suspension	Normal saline	3.0 (2.7–3.7)
Solution	Polyethylene glycol 400–water (60:40 v/v)	3.7 (2.9–4.8)
Solution	Dimethylacetamide–water (50:50 v/v)	6.2 (4.7–8.2)
Solution	2,2-Dimethyl-1,3-dioxolane-4-methanol–water (50:50 v/v)	No effect; vehicle posesses convulsion-blocking activity
Solution	Corn oil	No effect
Emulsion	20% (v/v) corn oil in water emulsified with 0.8% surfactant solution	8.3 (7.9–9.4)

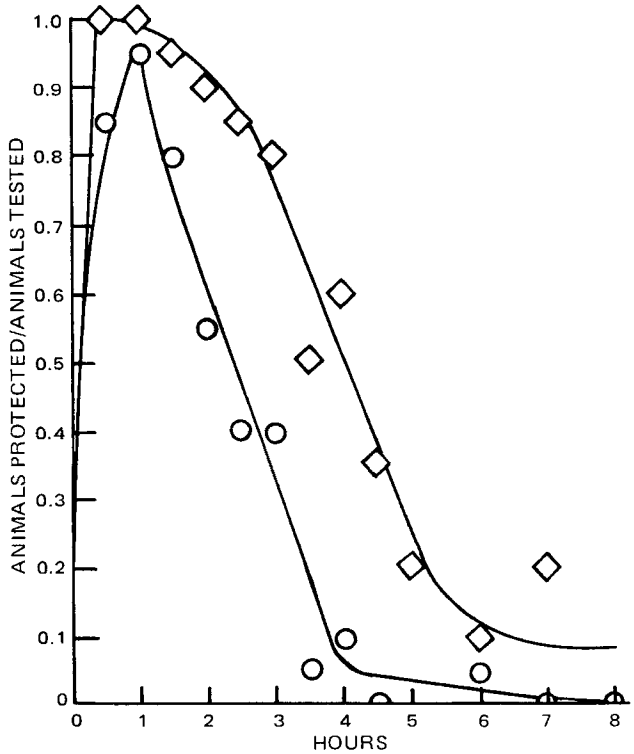

Figure 2—*Time course of pharmacological activity of I after oral administration of a suspension of I without (◇) and with (○) 0.8% surfactant solution in saline.*

Figure 2 illustrates a plot of the intensity, R, of the pharmacological activity of I monitored as a function of time following intragastric administration of an 8-mg/kg dose to mice. The decline in intensity of the anticonvulsant activity in the 80–20% range followed an apparent zero-order process. Since response to a drug is a function of the free drug concentration at the receptor site(s), this observation agrees with previously reported relationships (8–11). It is evident that inclusion of a surfactant (emulsifier) at the 0.8% concentration in the dosage form affects the duration of the anticonvulsant activity of I. However, the rate of decline in intensity of this response remains unaffected as evidenced from the slope of the plot.

The overall inference obtained from these observations is that the availability of the free concentration of I at the anticonvulsant receptor sites may be at a reduced level following its oral administration in the presence of a surfactant. Several investigators have shown that surfactants can affect the rate and extent of drug absorption across the GI tract (12–15). The surfactant solution used in the present study may have modified the extent of absorption of I; consequently, a reduction in the duration of its pharmacological activity can be expected.

Previous investigations (16) showed that absorption of drugs from oily solutions was significantly inhibited. The same authors (16) noted that with an oil-in-water emulsion system where a drug is in solution in the oil phase, the overall absorption rate is significantly large despite a high water-to-oil partitioning property of a drug. In recent years, it has been shown that administration of poorly water-soluble drugs as oil-in-water emulsion dosage forms allows extensive drug absorption as compared with other conventional dosage forms (17–20).

To assess this possibility, the relative pharmacological activity of I was evaluated after oral dosing of an oil-in-water emulsion and an oil solution. As illustrated in Fig. 3, the onset and duration of pharmacological activity of I achieved from an emulsion dosage form were comparable to that of the suspension without the surfactant (Fig. 2). Administration of the compound in a corn oil solution resulted in virtually total arrest of anticonvulsant activity in the mouse. Since the drug has been shown to be very lipophilic[12], it is likely that its transfer rate from large oil globules to intestinal fluid can be insignificant. Consequently, the rate and extent

[12] Lipophilicity of this compound was approximated on the basis of its high partition coefficient (P.C. heptane/pH 7.0 = 34.2). Partitioning data were generated by the Analytical Research Department.

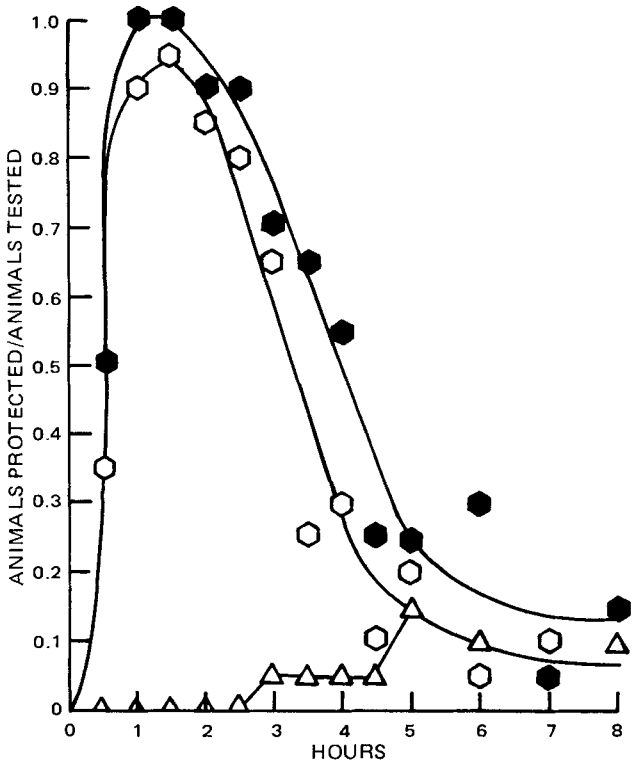

Figure 3—*Time course of pharmacological activity after oral administration of I as an emulsion with corn oil (●), an emulsion with soya bean oil (○), and a solution in corn oil (△).*

of absorption of I can be hindered by this rate-limiting factor. Hence, little or no anticonvulsant effect was observed.

Emulsification of oil in water results in an abundant increase in the surface area of oil, allowing a facile interfacial transport of the anticonvulsant compound from oil to the water phase of the luminal fluid. Under pseudo-sink conditions, rapid absorption of the compound across the intestinal membrane allowed a sufficient concentration to reach the receptor sites, and an optimal duration of anticonvulsant activity was observed.

The foregoing discussion is compatible with the theoretical (16, 21) and observed (17–20, 22) findings on drug delivery from emulsion dosage forms. The medium ED_{50} for a corn oil emulsion was estimated to be 8.3 mg/kg (Table I). It is evident from Fig. 3 that, at 30 min (the time for ED_{50} determination) after oral dosing of the emulsion dosage form, the intensity of the pharmacological response of I was only 50% as compared to the 100% intensity achieved with the drug suspension in normal saline. Thus, to achieve 100% activity at 30 min after dosing, a 16-mg/kg dose of I in the emulsion dosage form is needed. This dose was about 2.6 times higher than that of the suspension dosage form of I. The observed twofold difference between the ED_{50} values for the suspension and the emulsion dosage forms of I may be attributed to the rate-limiting transport of the compound from the oil phase to the aqueous (luminal fluid) phase from which its absorption across the GI tract occurs.

Figure 4 illustrates the effect of sorbitol content (percent w/v) on the apparent ED_{50} of I in mice. A multivariate regression analysis of these data showed that there was an apparent linear relationship (correlation coefficient $r = 0.96$ and residual variance $s^2 = 3.42$) between the observed ED_{50} and the sorbitol content of the dosage form. To test whether or not inclusion of sorbitol in the dosage form modifies the rapidity of onset as well as the duration of anticonvulsant activity of I in mice, experiments were conducted with a suspension dosage form of the compound with varying concentrations of sorbitol (Fig. 5).

The presence of 70% of sorbitol reduced the apparent pharmacological response by as much as 80%. As the concentration of sorbitol in the suspension dosage form was reduced, there was a proportional increase in the rapidity of onset and the duration of pharmacological activity. These results suggest that sorbitol modifies the availability of I for absorption. Animals dosed with distilled water and 70% sorbitol solution and then challenged 0.5 hr later with varying current levels showed no difference in seizure susceptibility. Thus, the possibility of sorbitol interference at the drug receptor sites was ruled out.

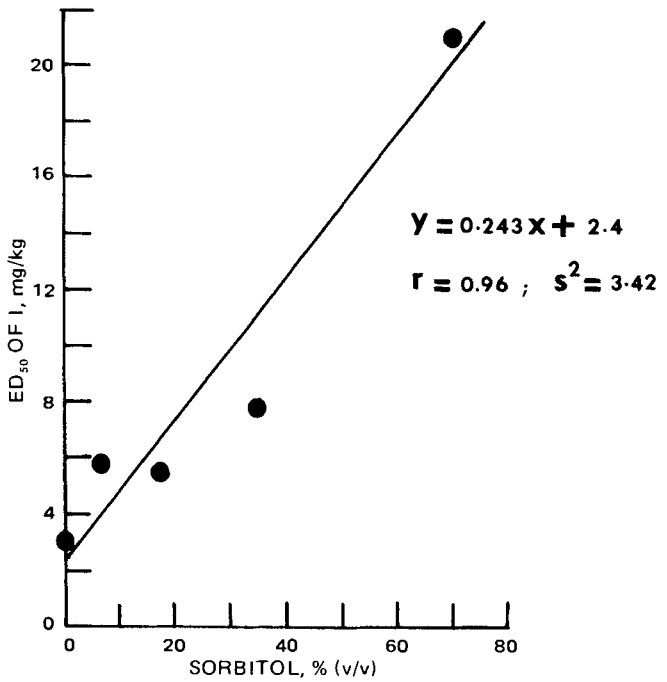

Figure 4—*Effect of sorbitol on the apparent ED_{50} of I in mice.*

Vogel *et al.* (23) reported on the effect of the osmolarity of the instillation fluid on the efficacy and toxicity of drugs given by the intraduodenal route. Administration of drug solutions made hypertonic with mannitol (10–15%) diminished both the efficacy and toxicity of atropine sulfate, pentobarbital sodium, and nicotine. It was concluded that transmucosal movement of fluid from the blood into the intestinal lumen interfered with drug absorption. This finding might explain our observations on the modification of anticonvulsant activity of I in mice in the presence of sorbitol.

Table II illustrates some pharmacokinetic parameters for the anticonvulsant activity of I measured after oral administration of several liquid dosage forms. The time required for peak response of I (T_{max}) showed a range of 0.5–1.5 hr. Emulsification of the drug solution in oil resulted in an increase in this parameter. Whereas the time required for 50% reduction in animal protection ($T_{0.5}$) ranged from 2.45 to 4.0 hr, the presence of a surfactant (emulsifier) at the 0.8% concentration resulted in an appreciable (1.6-fold) decrease in this parameter.

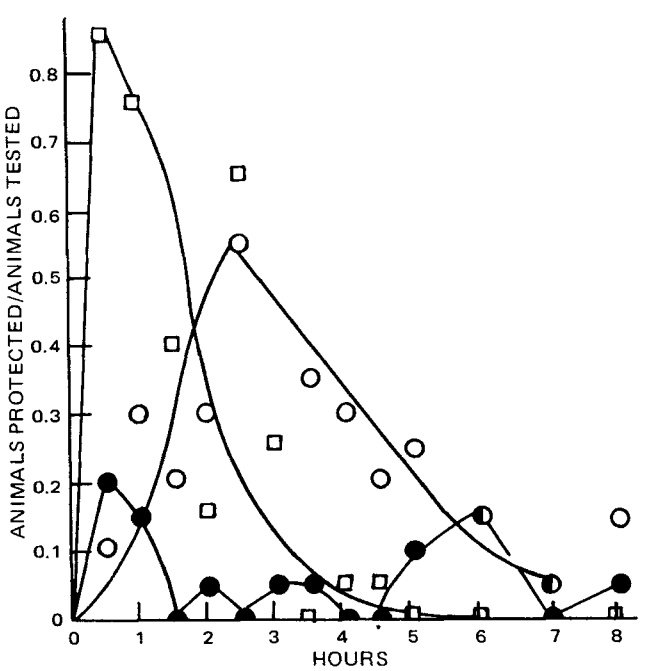

Figure 5—*Time course of pharmacological activity after oral administration of I with 7% (□), 35% (○), and 70% (●) sorbitol in water.*

Table II—Vehicle Effects on the Duration of Anticonvulsant Activity of I

Dosage Form	T_{max}, hr	$T_{0.5}$, hr	$\beta_{apparent}$, $(0.8-0.2)R$
Suspension in normal saline	0.5	4.0	0.265
Suspension in normal saline with 0.8% surfactant	1.0	2.45	0.27
Corn oil emulsion	1.0	3.8	0.26
Soya bean oil emulsion	1.5	3.25	0.24
Corn oil solution	0.5	Not observed	—
Polyethylene glycol (60% v/v) solution	0.5	2.8	0.12
Suspension in 70% sorbitol solution	0.5	Not observed	—

The negative slope, β, of the line (least-squares) drawn between the 80 and 20% response range of the time–response curve was virtually the same except for the polyethylene glycol 400 solutions. The observed constancy of the β-values supports the concept that the rate of decline of the pharmacological effect of a compound is independent of its total amount in the body. The apparent deviation of the β-value estimated for the polyethylene glycol 400 solution is intriguing and may be an artifact of the statistical analysis of the observed data. Binding of I to the polymer (polyethylene glycol 400) may have resulted in erratic absorption of this compound. This could contribute to fluctuations in drug availability at the receptor sites and, consequently, to a decrease in the β-value.

In conclusion, this study has shown that oral administration of I in different liquid dosage forms modified its anticonvulsant activity in mice. The intensity of onset and the duration of the pharmacological activity were affected depending on the liquid vehicle used.

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Use of Anemic Piglet to Assess Bioavailability of Iron from Oral Iron Preparations

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Abstract □ Except for methods using long-lived iron isotopes, there are no reliable means for assessing the bioavailability of iron from oral preparations in human subjects. Use of the anemic piglet as an alternative means was studied. When piglets were made anemic on a commercial milk diet and then dosed with solutions of 1, 2, and 5 mg/kg of ferrous sulfate/day, a dose-related recovery of hematocrit and hemoglobin levels resulted. The most sensitive dose range for use in a bioavailability study of iron was between 1 and 2 mg of iron/kg/day when using these parameters. A study carried out using this method indicated that the iron from a delayed-release capsule and from a ferrous sulfate solution was equally bioavailable. Hemoglobin and hematocrit recovery rates of the anemic piglet were shown to be reliable and sensitive indicators of the bioavailability of iron from various iron dosage forms.

Keyphrases □ Iron—bioavailability, ferrous sulfate delayed-release capsule compared to solution, piglets □ Ferrous sulfate—bioavailability, delayed-release capsule compared to solution, piglets □ Bioavailability—ferrous sulfate delayed-release capsule compared to solution, piglets □ Hematinics—ferrous sulfate, bioavailability, delayed-release capsule compared to solution, piglets

Absorption of iron from the GI tract is regulated by multiple factors, including the amount of iron stored in the body, the degree of hematopoietic activity present, and the amount of iron ingested. For example, an increase in iron absorption takes place if iron is fed orally (1). A study designed to measure the bioavailability of iron from different pharmaceutical iron preparations in human subjects would present many problems, since all of these regulatory factors