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

would have to be controlled to the same extent in all subjects. With a double isotope technique, these problems were circumvented by using each subject as his own control (2, 3). With this technique, differences in iron absorption of various iron salts could be measured. The disadvantages of this method were that radioisotopes had to be used in human subjects and one of the isotopes, iron-55, has a half-life of 2.7 years.

Hemoglobin and/or red cell regeneration studies can be used as an indirect measure of iron bioavailability, but both require the rigid control of iron intake as well as the use of individuals depleted of body iron stores. These conditions are difficult to control in human subjects but can be readily achieved in animals. Several studies (4–6) indicated that suckling pigs rapidly become anemic if they are kept from ingesting soil and maintained solely on a milk diet. Furthermore, this anemia can be satisfactorily controlled by iron supplementation. Kolb (7), in a review of iron metabolism of various domesticated animals, showed that serum iron, iron binding capacity, and serum iron response to iron dosing in the pig is comparable to these parameters in humans. The data in this paper indicate that the anemic pig provides a reasonably alternative means of assessing iron bioavailability that is both reliable and sensitive.

EXPERIMENTAL

Littermate piglets, both male and female, from a crossbred dam (Chester White, Hampshire, Duroc) and a purebred Chester sire[1] were used. Animals were individually housed in galvanized steel monkey cages equipped with nursing bottle holders and rubber mesh floor padding. Room temperature was maintained between 27 and 30°.

A commercial modified milk product[2] at room temperature was placed in disposable-bag, plastic nursing bottles[3]. Piglets were encouraged to suckle by patient hand feeding during the first few days. During this time, the deciduous "needle" teeth were clipped from all animals. In the initial days of the study, it was sometimes necessary to feed this formula to reluctant feeders by stomach tube. The formula was eventually available to the piglets on an *ad libitum* basis.

Blood samples were taken from superficial abdominal skin veins (superficial epigastric) and occasionally, as animals grew older, from ear veins. Enough blood was taken to fill a No. 23, 25-mm disposable needle.

Hematocrit was determined in duplicate using a microhematocrit system[4]. Tubes were spun 4 min and read immediately.

Hemoglobin was measured using a disposable pipet kit[5]. After color development, samples were read on a spectrophotometer at 540 nm, and unknown samples were compared to a standard curve made from a certified cyanomethemoglobin standard[6].

The ferrous sulfate used was the reagent grade heptahydrate[7]. Solutions were made up fresh daily in distilled water. The delayed-release iron formulation used was a commercially available product[8]. The method used was as follows. Piglets, 5 days old, without prior iron supplementation, were fed a commercial modified milk product. When at least two piglets reached the desired level of anemia (approximately 6.5 g/100 ml of hemoglobin and 21% hematocrit) and remained at this level for 2 consecutive days, the animals were placed into the ferrous sulfate or the delayed-release capsule group. If three piglets became simultaneously available, the third animal was placed into a control (no iron) group. Where possible, paired animals of the same sex were used. The selection continued until all animals had been assigned to groups.

[1] H. Schick and Son, Kutztown, Pa.
[2] Similac infant formula, ready to feed, Ross Laboratories, Columbus, OH 43216.
[3] Playtex Ltd., Malton, Ontario, Canada.
[4] Drummond Scientific Co., Broomall, Pa.
[5] Unopette kit No. 5858, Becton Dickinson and Co., Rutherford, N.J.
[6] Hycel, Inc., Houston, Tex.
[7] Merck and Co., Rahway, N.J.
[8] Feosol Spansule Capsules, Smith Kline and French Laboratories, Philadelphia, PA 19101.

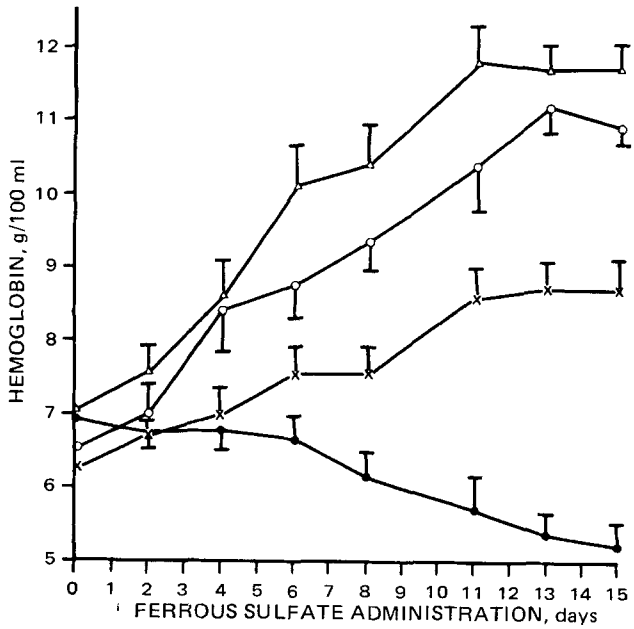

Figure 1—*Mean hemoglobin levels of anemic piglets following oral administration of various doses of iron administered as a ferrous sulfate solution. Each point represents the mean of six piglets* ± SEM. *Key:* △, *5 mg of iron/kg/day;* ○, *2 mg of iron/kg/day;* ×, *1 mg of iron/kg/day; and* ●, *controls (no iron).*

The animals were fed the iron doses daily and, based upon daily body weight, received doses of 1.5 mg of iron/kg as either ferrous sulfate solution or as the delayed-release capsule formulation. The capsule formulation was scaled individually to the weight of each piglet. Blood samples were taken on the 7th and 9th days of iron administration for measurement of both hemoglobin and hematocrit.

RESULTS

Preliminary Studies—To arrive at the final experimental design, preliminary studies had to be carried out to establish the optimum amount of iron for dosing, the iron dosing schedule, and the blood sampling periods necessary to provide a sensitive, comparative assessment of iron bioavailability.

To the uninitiated who desire to use the piglet as an experimental animal, an initial purchase of only two or three animals is suggested to enable laboratory personnel to become familiar with methods of handling, feeding, dosing, and bleeding these animals. With such a starter group, piglets under laboratory conditions were able to survive, grow, and, in the process, rapidly become anemic when fed a diet consisting solely of a commercial modified milk product. Oral administration of sufficient quantities of ferrous sulfate reversed the anemia, as measured by hematocrit and hemoglobin changes.

A second group of piglets was studied when only 24–48 hr old. Animals this age were obtained to avoid the possibility of their having been inadvertently dosed with iron by the supplier. However, an unbelievable amount of constant care was necessary to maintain this age piglet when away from the sow. During this study, one-third of the animals were lost, mostly from severe diarrhea and/or lack of appetite. A few preliminary dosing studies were carried out with the survivors.

When values of both hemoglobin and hematocrit indicated that the animals were anemic, single doses of iron as solutions of ferrous sulfate heptahydrate were administered. A single dose of 2, 10, or 20 mg/kg produced only small, inadequate responses in hematocrit and hemoglobin levels. However, chronic daily dosing of 1, 2, and 5 mg/kg showed promise of a dose–response in both hematocrit and hemoglobin levels. Chronic doses of 10 and 40 mg/kg caused hematocrit and hemoglobin values to rise no higher than did the 5-mg/kg doses. These data indicated that iron between 5 and 10 mg/kg exceeded the iron absorptive rate or the hemoglobin regeneration rate of the piglet and that the desirable dose range for a comparative bioavailability study was 1–5 mg/kg/day.

A third preliminary study was carried out to test this chronic dosing range for a dose–response and sensitivity as well as to determine if slightly older piglets would show an increased survival rate. Twenty-eight piglets were allowed to stay with the sow until 5 days old. These "older" piglets

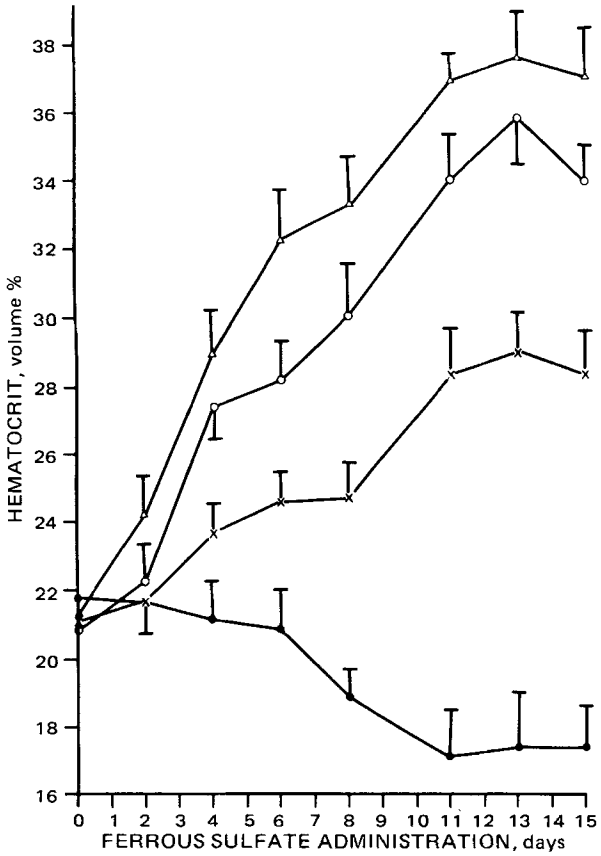

Figure 2—*Mean hematocrit values of anemic piglets following oral administration of various doses of iron administered daily as a ferrous sulfate solution. Each point represents the mean of six piglets* ± SEM. *Key:* Δ, *5 mg of iron/kg/day;* O, *2 mg of iron/kg/day;* ×, *1 mg of iron/kg/day; and* ●, *controls (no iron).*

were healthier and much easier to keep alive; only one of the 28 died. As groups of these piglets became anemic, they were placed into dose groups of 1, 2, and 5 mg of iron/kg. Animals were then dosed daily, and hematocrit and hemoglobin values were determined at frequent intervals.

Figures 1 and 2 show the results obtained. The control values indicated that piglets maintained on the low iron diet remained anemic during the entire experimental period. The data were analyzed statistically, and results are presented in Table I.

The dose of 1.5 mg of iron/kg was chosen for use in a subsequent iron bioavailability comparison study since it fell within the most sensitive detection range. Days 7 and 9 of a daily iron dosing regimen were chosen as hematocrit and hemoglobin measurement periods. Two measurement periods were chosen to assure that hemoglobin and hematocrit levels were monitored during a rising portion of the response curve and not during a plateau period. This criterion is important to establish because a plateau would indicate a maximized hemoglobin and hematocrit response to iron intake, rendering these parameters incapable of rapidly responding to varied levels of iron intake. Once this criterion was established, Day 7 and 9 values could be used individually or averaged for comparative iron bioavailability studies.

Comparison of Iron Bioavailability from Two Different Iron Dosage Forms—Based upon the preliminary studies, a comparative bioavailability study was carried out as outlined under *Experimental.* This study compared the bioavailability of iron from a delayed-release capsule form to similar doses of iron fed as a ferrous sulfate solution.

Thirteen pairs of pigs were assigned to the iron formulations along with six (no iron) controls for each group. Within a pair, both pigs were from the same litter and started on the iron formulation at the same time. Where possible, the same sex was studied as a pair. Table II reports the values obtained.

The results for Days 7 and 9 are reported individually to show that both hematocrit and hemoglobin values were changing in this interval. The differences obtained between Days 7 and 9 were statistically significant for both parameters, confirming that the study did not compare formulations when values were on or near a plateau. The average hemoglobin

Table I—Mean Values of Hematocrit and Hemoglobin following Daily Oral Administration of Iron as Ferrous Sulfate after Statistical Adjustment[a] and Testing for Significance

Iron Dose, mg/kg	Days of Chronic Iron Administration						
	2	4	6	8	11	13	15
	Hematocrit Averages[b], volume %						
1	22.44	23.99	25.25	24.84	27.78	28.07	27.49
2	21.83	27.35	27.69	31.31	34.62	36.36	34.54
5	23.99	28.72	32.22	32.92	37.12	38.10	37.46
	Significance of Hematocrit Averages[c]						
1 *versus* 5	---	**	**	**	**	**	**
2 *versus* 5	---	---	**	---	---	---	*
1 *versus* 2	---	*	---	**	**	**	**
	Hemoglobin Averages[b], g/100 ml						
1	7.11	7.03	7.70	7.53	8.40	8.48	8.44
2	6.88	8.35	8.70	9.49	11.40	11.25	11.07
5	7.30	8.55	10.09	10.37	11.95	11.99	12.02
	Significance of Hemoglobin Averages[c]						
1 *versus* 5	---	*	**	**	**	**	**
2 *versus* 5	---	---	*	---	---	---	**
1 *versus* 2	---	*	---	**	*	**	**

[a] Data adjusted by analysis with a multiple regression model that accounted for differences due to litter and starting baseline. Data prior to statistical adjustment are shown in Figs. 1 and 2. [b] Each value represents average of six piglets. [c] *, $p < 0.05$; **, $p < 0.01$; and ---, not significant.

values of 9.12 g/100 ml following the delayed-release capsule and 8.83 g/100 ml following ferrous sulfate solution were not significantly different. Therefore, one can conclude that the bioavailability of iron from the delayed-release capsule was equivalent to that of the ferrous sulfate solution.

Table II—Hemoglobin and Hematocrit Levels in Anemic Piglets following Daily Administration of 1.5 mg of Iron/kg as Either a Delayed-Release Capsule[a] or a Ferrous Sulfate Solution

	Delayed-Release Pelletized Iron Capsule[a]		Ferrous Sulfate Heptahydrate in Solution	
	Raw Data	Statistically Adjusted Data[b]	Raw Data	Statistically Adjusted Data[b]
	Average Hemoglobin[c], g/100 ml			
Controls (no iron)	5.72		5.47	
Day 7 levels	8.91	8.81	8.32	8.41
Day 9 levels	9.55	9.43	9.12	9.25
Average (Days 7 and 9)	9.23	9.12	8.72	8.83
	Average Hematocrit[c], volume %			
Controls (no iron)	19.4		19.1	
Day 7 levels	31.9	31.8	29.7	29.8
Day 9 levels	33.1	33.0	32.2	32.4
Average (Days 7 and 9)	32.5	32.4[d]	31.0	31.1

[a] Feosol Spansule Capsule, Smith Kline & French Laboratories. [b] To account for design variables and to adjust all results to a common baseline, all data were analyzed with a multiple regression model that accounted for differences due to litter and starting baselines. This produced the "adjusted means." Differences between Day 7 and 9 levels were significant in all cases. [c] All control values represent the mean of six piglets. All other values represent the mean of 13 piglets. [d] This value was significantly higher than hematocrit produced by ferrous sulfate administration, $p < 0.05$. Hemoglobin levels following either dose form were not significantly different. A difference of only 0.44 g of hemoglobin/100 ml would have been significant.

Comparison of hematocrits showed that average values obtained following the delayed-release capsule (32.4%) and following ferrous sulfate (31.1%) were statistically significant ($p < 0.05$). This finding indicated that the bioavailability of iron from the delayed-release capsule was equivalent to, and perhaps somewhat better than, ferrous sulfate solution when using hematocrit regeneration as an index of bioavailability. A difference of 1.2 hematocrit units (only 3.6% of the average hematocrit) would have been statistically significant. The careful selection of a dose of iron that was below the maximum capable of being absorbed by the gut and the regenerating capacity of the bone marrow was probably an important reason for the success of this approach to iron bioavailability methodology. The method demonstrated that delaying the release of iron in the pelletized iron preparations tested in no way impaired its bioavailability.

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Antibacterial Structure–Activity Relationships Obtained with Resistant Microorganisms I: Inhibition of R-Factor Resistant *Escherichia coli* by Tetracyclines

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Abstract □ Apparent partition coefficients and inhibitory activities against sensitive and resistant *Escherichia coli* were determined for 14 tetracyclines. The difference in the kinetics of inhibition of the two organisms is discussed in terms of their permeabilities. The partition coefficients were determined in an octanol–buffer system. Values for eight compounds were in general agreement with the literature; values for the remaining six compounds had not been reported previously. Growth of the organisms was determined by a single-point turbidimetric method in the presence and absence of tetracyclines. Inhibitory activities were obtained by a kinetic treatment. Derived rate constants for the sensitive organism were linearly related to antibiotic concentration. For the resistant organism and 12 compounds, the derived rate constants and antibiotic concentration were related in a manner typical of saturation kinetics. These inhibitory activities were related to the partition coefficients, while activities against the sensitive strain were not. These findings suggest that activity against the resistant strain is permeability controlled but that activity against the sensitive strain has a different rate-determining step.

Keyphrases □ Antibacterial activity—various tetracyclines, related to partition coefficients, resistant and sensitive microorganisms, structure–activity relationships □ Tetracyclines, various—antibacterial activity, related to partition coefficients, resistant and sensitive microorganisms, structure–activity relationships □ Partition coefficients—various tetracyclines, related to antibacterial activity, resistant and sensitive microorganisms □ *Escherichia coli*—resistant and sensitive, inhibition by various tetracyclines, related to partition coefficients, structure–activity relationships □ Structure–activity relationships—various tetracyclines, antibacterial activity, resistant and sensitive microorganisms

The development of safe, effective antimicrobial drugs has revolutionized medicine in the last 30 years. Unfortunately, microorganisms are highly versatile, and the brilliance of the chemotherapeutic achievement has been dimmed by the emergence of microbial strains resistant to these drugs. This emergence of resistant pathogens is becoming an increasingly important problem. In addition to limiting the use of antibiotics, two possible approaches to the problem would be to seek entirely new antibacterial agents and to modify existing chemotherapeutic agents. The latter approach has the advantage that derivatives of present chemotherapeutic agents are likely to retain the high benefit to risk ratios of the parent drugs.

The general mechanisms by which microorganisms may become resistant to antibiotics were summarized (1), and the one where drug modification might effectively overcome drug resistance is the loss of cell permeability to the drug. Changes in the physicochemical nature of a drug resulting in decreased or increased cell permeabilities were described for many systems (2).

Davies (3) stated that, as far as can be ascertained, all tetracycline-resistant strains obtained from clinical situations are resistant because they carry an R-factor. The nature of R-factor resistance to tetracyclines was investigated extensively and reviewed (4, 5). These studies indicate that tetracycline resistance is related to a decreased uptake of tetracyclines by resistant cells. Several investigators showed that cell-free protein-synthesizing systems from sensitive and resistant strains were equally inhibited by tetracyclines (6–8).

Escherichia coli accumulated oxytetracycline at high concentrations (9), and this accumulation was inhibited by azide and 2,4-dinitrophenol and was dependent upon the energy source in the medium. Similar observations