Drug Absorption V: Influence of Food on Oral Absorption of Phenobarbital in Rats

S. KOJIMA*, R. B. SMITH, and J. T. DOLUISIO

Abstract □ This paper reports the effect of food on the oral absorption of phenobarbital in the rat. The presence of food was found to lower significantly the serum levels of intact drug and to reduce dramatically (and at low doses, 100 mg./kg., to eliminate) sleeping times induced by phenobarbital. The amounts of drug remaining unabsorbed in the stomach and intestines were determined in fasted and nonfasted rats. Greater than 90% of the amount unabsorbed was found in the stomach in both test conditions, but the amounts unabsorbed were greater in the nonfasted rats. *In situ* studies indicate that phenobarbital is primarily absorbed from the intestines. Thus, it is concluded that the presence of food decreased the pharmacological activity of phenobarbital by decreasing the rate of absorption and that this decreased absorption rate is due primarily to slowed gastric emptying. Since the drug is rapidly absorbed in the intestine, the extent of absorption is the same in the nonfasted rat as in the fasted rat.

Keyphrases □ Absorption kinetics—oral phenobarbital, effect of food, rats □ Phenobarbital—effect of food on oral absorption, rats □ Barbiturate absorption—effect of food, rats

A number of investigations have been concerned with the absorption characteristics of barbiturates. For example, Kakemi *et al.* (1, 2) studied the *in situ* absorption of various barbiturates from the rat stomach and intestine and the effect of pH, Sjogren *et al.* (3) studied absorption from various dosage forms, and Bush *et al.* (4) studied the serum level profiles for ultrashort-acting barbiturates. The effect of concomitant administration of alcohol on oral administration was also studied (5). Even though many of the factors affecting oral absorption have been characterized, little work has been done on the effect of food. The paucity of reports on the effect of food is not limited to barbiturates but extends to most drugs. The effect of food on barbiturate absorption is of particular interest, since barbiturates are in widespread use as sedatives and hypnotics in situations where food consumption is not controlled.

The results of the studies done on the effect of food showed that the presence of food in the GI tract influences significantly the oral absorption of drugs. In a previous paper (6), the authors reported that the rate of dicloxacillin absorption in man had been markedly decreased (approximately 200%) by food, but the extent of dicloxacillin ultimately absorbed decreased by a small percentage (14%). MacDonald *et al.* (7) found that food delayed absorption but did not decrease the amount of four sulfonamides eventually absorbed. Bush *et al.* (4) found that food delayed onset and significantly increased the duration of hypnosis for thiopental in a single subject who broke protocol by eating a cheese sandwich. The effect of food may result from hindered diffusion to the mucosal absorption surface, decreased dissolution rate of solid dosage forms, or delayed stomach emptying for drugs absorbed primarily in the intestine.

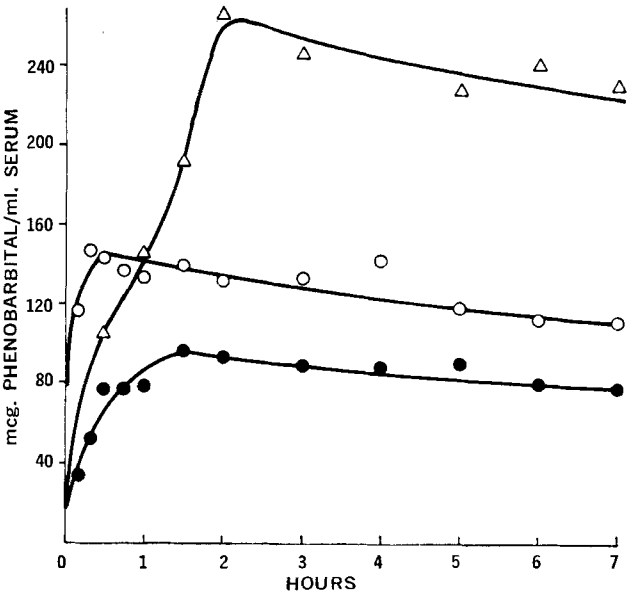


Figure 1—*Serum levels of phenobarbital following oral administration of phenobarbital in fasted and nonfasted rats. Key:* ○, *fasted, 100 mg./kg.;* ●, *nonfasted, 100 mg./kg.; and* △, *nonfasted, repeated dosing of two 150-mg./kg. doses 1 hr. apart.*

The purpose of the present investigation was to study the influence of food on the absorption rate, extent of absorption, and pharmacological action of phenobarbital when administered in aqueous solution to the rat. It was expected that food would delay and/or decrease the apparent rate of absorption of phenobarbital, consequently delaying onset and perhaps increasing duration of hypnosis. If this were the case, a human subject that consumed food before taking a hypnotic dose of barbiturate may experience an abnormal delay in the onset of action and take more drug, with the consequent hazard of toxicity.

This paper reports the animal results obtained thus far. A future publication will deal with human studies on the effect of food on barbiturate absorption.

EXPERIMENTAL

Reagents and Equipment—All chemicals were reagent grade except phenobarbital USP and mephobarbital NF XII. A gas chromatograph[1] and a scintillation counter[2] were used.

Test Animals—Male Sprague-Dawley albino rats, weighing between 200 and 490 g., were used. The rats were fasted 48 hr. prior to experimentation, but drinking water was allowed *ad libitum*. The rats were kept in cages having wide mesh floors to minimize

[1] Varian Aerograph Series 1700.
[2] Packard Tri Carb model 3375.

Table I—Onset and Duration of Action of Phenobarbital following Oral Administration with and without Food

	Number of Rats	Dose, mg./kg.	Onset of Action, min.	Duration of Action, min.
Fasting (p.o.)	11	100	28 ± 7.2	227 ± 23.6
Nonfasting (p.o.)	10	100	—[a]	—[a]
	12	300	76 ± 35.7	>540
	12	300[b]	79 ± 11.4	>600

[a] All rats did not lose the righting reflex. [b] Repeated dosing of two 150-mg./kg. doses 1 hr. apart.

coprophagy. For studies on the effect of food, the rats were allowed food[3] for about 16 hr. after fasting 48 hr. The rats consumed about 0.08–0.1 g. food/g. body weight.

***In Situ* Rat Model**—The procedure for studying drug transfer kinetics in the *in situ* rat model was described previously (8).

Drug Administration—Phenobarbital solutions administered to the rats were freshly prepared by the addition of 1.1 equivalents of sodium hydroxide to the acid. The solutions were of such concentration that the rats received 0.01 ml. orally/g. body weight. The oral administration was carried out by use of a stomach catheter.

Duration of Action of Phenobarbital—The duration of action (hypnosis) of phenobarbital was defined as the time between the loss of the righting reflex after administration of the drug and the time the animal regained the righting reflex.

Experimental Procedures—The rats were sacrificed periodically after administration of phenobarbital. The serum was collected immediately after the animals were sacrificed. The contents of the gut (stomach and intestines) were washed out using 15–20 ml. of water, and the washings were centrifuged.

To determine the initial passage of solution into the duodenum in fasted rats, 0.01 ml./g. body weight of a solution containing ^{14}C-polyethyleneglycol as a nonabsorbable marker was administered. After administration, the rats were immediately sacrificed by decapitation and opened by midline incision. The stomach was tied off at the pyloric junction, and then both the stomach and intestines were removed. This process required from 2 to 3 min. to complete. The contents of both the stomach and intestines were washed into separate Kimex tubes with 5–10 ml. of water and centrifuged. Samples of the supernatant were then analyzed by scintillation counting.

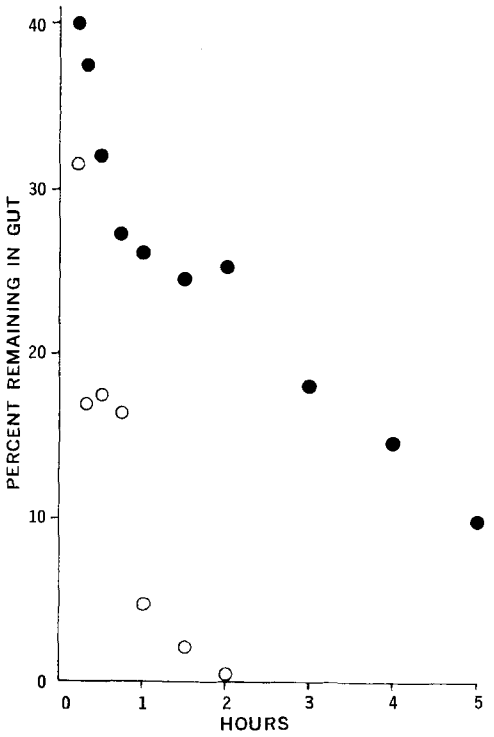


Figure 2—*Percent of phenobarbital remaining in the gut (stomach and intestines) following oral administration of phenobarbital in fasted and nonfasted rats. Key: ○, fasted, 100 mg./kg.; and ●, nonfasted, 100 mg./kg.*

[3] Purina Lab Chow.

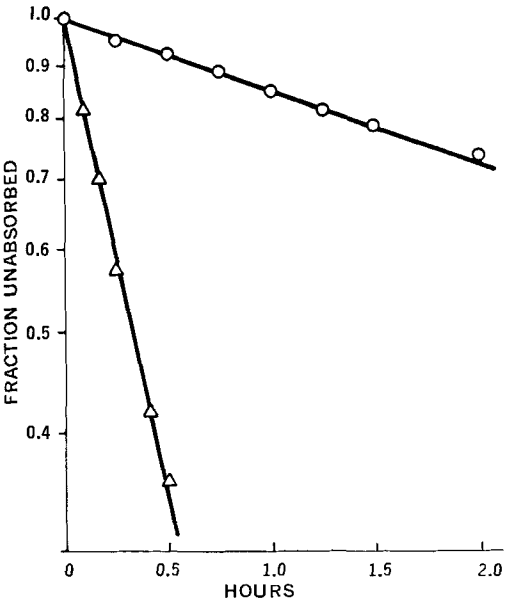


Figure 3—*Semilogarithmic plots of the disappearance of phenobarbital from the rat stomach and intestine* in situ. *Average values for three rats. Key: ○, stomach, pH 3.0; and △, intestine, pH 6.0.*

Analytical Procedures—To 1.0 ml. of serum or gut sample in a 15-ml. Kimex tube was added 1.0 ml. of 0.2 *M* phosphate buffer (pH 5.85). The mixture was extracted with 6, 6, and 2 ml., successively, of ethyl ether by shaking on a mixer for 2 min. The combined extract was evaporated at about 45°. The resulting residue was dissolved in an adequate volume of 2.5 mg. % mephobarbital–chloroform solution and submitted to GLC.

Conditions of GLC—A gas chromatograph equipped with a hydrogen flame-ionization detector was used. The carrier gas was nitrogen. The column was 1.8 m. (6 ft.) × 0.63 cm. (0.25 in.) (o.d.) glass, containing a packing of 3% PPE-20 on 100–120 mesh Supelcoport.

The calibration curve for phenobarbital was prepared as follows. The sample solutions (5–80 mcg./ml.) were prepared by dissolving different amounts of phenobarbital in a chloroform solution of mephobarbital (2.5 mg.%) as the internal standard. At the fixed range and attenuation of the instrument, 2 μl. of sample solution was injected into the gas chromatograph. The peak area was determined by triangulation. The calibration curve was obtained by plotting the concentration of phenobarbital against the peak area ratio of phenobarbital to mephobarbital.

RESULTS AND DISCUSSION

The onset and duration of hypnotic action of phenobarbital for doses of 100 mg./kg. body weight in fasted and nonfasted rats are shown in Table I. Food had a significant effect on the hypnotic action of phenobarbital. For the 100-mg./kg. dose, the fasted rats had an average onset time of 28 min. and an average duration of 227 min.; however, the same dose in nonfasted rats produced no hypnotic effect, *i.e.*, not a single rat lost the righting reflex.

The serum levels of phenobarbital at various times following oral administration of this same dose are shown in Fig. 1. The percent remaining in the gut at various times was also found for this dose and is shown in Fig. 2. The serum level curves demonstrated that the presence of food concomitant to oral administration of phenobarbital delayed the attainment and decreased the magnitude

Table II—Percent Phenobarbital Remaining in the Stomach, Intestines, and Cecum following Oral Administration

	Hours					
	0.16	0.5	1.0	2.0	3.0	5.0
100 mg./kg. Fasted						
Stomach	28.3	7.7	13.0	2.0	0.7	0.1
Intestines	1.2	2.6	0.9	0.3	0.4	0.3
Cecum	0.2	0.2	0.2	0.2	0.3	0.3
Total	29.6	10.6	14.0	2.5	1.3	0.7
100 mg./kg. Nonfasted						
Stomach	42.3	40.5	24.0	15.5	18.0	16.5
Intestines	0.2	0.3	0.5	0.4	0.4	0.4
Cecum	0.1	0.2	0.4	0.7	1.2	1.1
Total	42.5	41.1	24.9	16.6	19.6	18.0

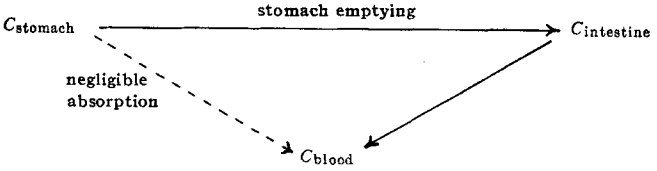
of the maximum serum level. It is evident from the plots of percent remaining to be absorbed that the presence of food decreased the rate of absorption. For the 100-mg./kg. dose, the percent remaining to be absorbed at 1 hr. was 4.5% in fasted rats as compared to 26% in nonfasted rats. These data indicate that the presence of food decreases the rate of absorption and, consequently, the prolonged absorption alters the serum level profile. If a minimum concentration in serum necessary to elicit a response is assumed, these data can be correlated to the hypnotic action described earlier. The presence of food delays attainment of this minimum serum level and, hence, delays onset; depending on the relationship between the maximum level obtained and the minimum effective level, the duration of action may be increased or decreased.

The absorption of phenobarbital from the rat stomach and intestine was studied using an *in situ* technique (8). The fractions unabsorbed for the stomach and intestine are plotted in Fig. 3. It is apparent that the rate of absorption is much greater in the intestine, $t_{1/2}$ = 19 min. for intestinal absorption (pH 6.0) and $t_{1/2}$ = 255 min. for gastric absorption (pH 3.0). This difference in absorption rates was also observed by Kakemi *et al.* (1, 2). This result suggests that phenobarbital absorption is similar to the absorption of other weak acids reported previously (9). In the previous investigation, the conclusion was that the small intestine may be the main site of absorption for both weak acids and bases, possibly due to the large surface area, blood supply, and longer residence time. Since negligible absorption may occur in the stomach, the effect of food may have resulted from delayed stomach emptying and, hence, a delay before the drug reaches the primary absorption site—the small intestine.

To test this hypothesis, the determination of the percent of drug remaining was repeated; however, in this experiment the percents in the stomach, small intestine, and large intestine (including cecum) were determined separately (Table II). As can be seen from Table II, greater than 90% of the drug remaining to be absorbed at any given time was contained in the stomach in both fasted and nonfasted rats. Virtually no drug reached the large intestine. This finding not only supports the hypothesis but also suggests that since intestinal absorption is efficient, the presence of food will not significantly decrease the extent of phenobarbital absorption unless degradation occurs in the gastric fluid.

It is also possible that the apparent difference in gastric emptying time between fasted and nonfasted rats was due to rapid movement of solution through the stomach during dosing rather than following drug administration. The solution may immediately pass into the duodenum in fasted animals due to the volume of the drug solution, whereas the presence of food may inhibit this passage. This possibility was tested by administering a solution containing a nonabsorbable marker, ^{14}C-polyethyleneglycol, and determining the percentage of solution that had passed into the duodenum immediately (within 3 min.) after intubation in fasted rats. It was found that an average of 10.4% (a range of from 5.6 to 18.3%) was passed into the duodenum in eight rats. These data indicate that the initial emptying in fasted rats will not account for the difference in stomach emptying between fasted and nonfasted rats.

Further studies were carried out to measure the onset and duration of action and the serum level in nonfasted rats when 150 mg./kg. of phenobarbital was administered orally and then a second



Scheme I

150-mg./kg. dose was administered 1 hr. later (Fig. 1 and Table I). The time of onset of action was 79 min., that is, about 20 min. after the administration of the second dose. The duration of action was greater than 10 hr.; seven of the 12 rats studied slept for over 29 hr. and three rats died. When a single dose of 300 mg./kg. of phenobarbital was administered orally to nonfasted rats, the onset and duration of action were approximately the same as for those of the repeated dosing (Table I). The deviation of onset of action was greater for the single 300-mg./kg. dose than for the repeated dosing, and one rat did not lose the righting reflex. As shown in Fig. 1, the serum levels in the repeated-dosing case increased greatly after the administration of the second dose, reaching a maximum in about 1 hr. after the second dose, *i.e.*, 2 hr. after the first dose. These data suggest that the absorption of the second dose is faster and the rapid rise in the 1st hr. after the second dose reflects the faster absorption of the second dose along with residual absorption of the first dose. More importantly, however, these data indicate that in man if a second dose is self-administered, there is a significant hazard of intoxication due to a delayed onset of action.

This study demonstrated the effect of food on the oral absorption of phenobarbital. It was shown that absorption was negligible from the stomach and, consequently, that the primary site for absorption was the small intestine. The presence of food altered absorption, primarily by delaying stomach emptying and hence increasing the time for the drug to reach its primary absorption site. Since the intestine may be the primary absorption site for a great number of drugs (9), this example may represent a model for the effect of food in general on absorption (Scheme I). Thus, if intestinal absorption is very fast, the rate of stomach emptying may become rate limiting in the absorption process in the presence of food. The increased time that the drug is in the stomach may also reduce the amount of drug eventually absorbed for drugs that are unstable in gastric fluid.

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