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Factors influencing Absorption and Excretion of Drugs. I. Effect of Food on Gastrointestinal Absorption of Amobarbital in Rats

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The effect of food on the gastrointestinal absorption of amobarbital was studied in the rats. The presence of food in the gastrointestinal tract decreased significantly the serum level and brain level of amobarbital and reduced the sleeping time induced by the drug. The amounts of amobarbital remaining to be absorbed in the stomach and intestines were determined in fasted and nonfasted rats. Most of the drug unabsorbed was contained in the stomach in both experimental conditions, but the amounts unabsorbed were greater in the nonfasted rats. It was clarified by the *in situ* experiment that the primary absorption site of amobarbital was the small intestine. Thereafter, it was demonstrated that the presence of food in the gut decreased the pharmacological activity of amobarbital by the decrease of absorption rate based on delayed gastric emptying. Since amobarbital is very rapidly absorbed in the small intestine, the presence of food will not decrease the extent of absorption of the drug.

It has been generally recognized that the oral absorption of a drug is influenced by various physiological factors such as gastric emptying, intestinal motility, gastrointestinal blood flow, interaction of the drug with food, interaction of the drug with components of the gastrointestinal fluids, *etc.* as well as physico-chemical properties of the drug. In general, the presence of food in the gastrointestinal tract may result in decreased diffusion rate of drugs to the mucosal absorption surface, decreased dissolution rate of solid dosage forms, delayed gastric emptying for drugs absorbed primarily in the intestine, or provision of substances to which the drug can bind.

Many investigators²⁻¹⁰⁾ have studied the effects of foods on the gastrointestinal absorption of various drugs. The effects of food on the gastrointestinal absorption of barbiturates, which are in widespread use as sedatives and hypnotics in situations where food consumption is not controlled, is of particular interest. Although there are a number of investigations concerning with the absorption characteristics of barbiturates, however, little work has been done on the effect of food.

In a previous paper,¹¹⁾ the author reported that the presence of food decreased the pharmacological activity of phenobarbital, as a long-acting barbiturate, by decreasing the rate of absorption and that this decreased absorption rate is due primarily to slowed gastric emptying.

The present study reports the effect of food on the rate of absorption, extent of absorption, and pharmacological action of amobarbital as an intermediate duration hypnotic in rats.

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Experimental

Materials—All chemicals were reagent grade except amobarbital JP VIII and allobarbital JP VIII.

Test Animals—Wistar male rats weighing between 200 and 280 g were used. The rats were fasted 48 hr (24 hr in an *in situ* experiment) prior to use, but drinking water was allowed *ad libitum*. The rats were kept in cages having wide mesh floors to prevent coprophagy. For studies on the effect of food, the rats were allowed food¹²⁾ for about 15 hr after fasting 48 hr. The rats consumed about 0.08–0.12 g food/g body weight.

Administration of Drug—Amobarbital solution (10 mg/ml), which were freshly prepared by the addition of 1.1 equivalents of sodium hydroxide to the acid, was administered to rats. The oral administration was carried out by use of a stomach catheter.

Duration of Action of Amobarbital—The duration of action (hypnosis) of amobarbital was defined as the time between the loss of the righting reflex after administration of the drug and the regaining of the righting reflex.

In Situ Rat Experiment—The procedure for studying drug absorption in the *in situ* rat gut was carried out according to the method of Doluisio, *et al.*¹³⁾ The sample solutions used were 20 mg% amobarbital in isotonic citrate-phosphate buffer (pH 3.0) for the stomach experiment and 100 mg% amobarbital in isotonic phosphate buffer (pH 6.0) for the small intestine experiment. Phenol red, which was expected to be unabsorbed, was dissolved in both sample solutions described above to indicate any volume change.

Experimental Procedures—A. Collection of Blood Samples by a Cardio-puncture Method: After oral administration of amobarbital to rat secured on its back on an animal board, 0.1 ml of blood sample was taken by performing a cardio-puncture at periodic intervals.

B. Collections of Serum, Brain, and Gut Contents after Sacrificing Rat: The rats were sacrificed by decapitation at various times after oral administration of amobarbital. The serum and brain were collected immediately after the animals were sacrificed. After removing the gut (stomach and intestines), the contents of the gut were washed out using 20–30 ml of water and the washings were centrifuged.

Analytical Procedures—To 1.0 ml of serum¹⁴⁾ or gut sample in a 10 ml stoppered tube was added 1.0 ml of 0.2M phosphate buffer (pH 5.85). The mixture was extracted with 5, 5, and 2 ml, successively, of ethyl ether by shaking on a mixture for 2 min. The combined extract was evaporated at about 45°. The resulting residue was dissolved in an adequate volume of 20 mg% allobarbital-chloroform solution and submitted to gas-liquid chromatography (GLC). When amobarbital was added to rat serum and gut contents, the analytical method described above gave recoveries of 94.0 to 98.0% for the serum and 93.0 to 96.0% for the gut contents.

Each rat brain (weight 1.50–1.85 g) was homogenized with 0.5M NaH₂PO₄ in a glass homogenizer, the total volume of 0.5M NaH₂PO₄ used in homogenizing and washing being about 10 ml. The homogenate was extracted with 8 and 6 ml of ethyl ether. The resulting extract was combined, concentrated to about 5 ml at about 45°, and shaken with 2 ml of 0.4N NaOH. After withdrawing the upper ether phase, the lower aqueous phase was shaken with 2 ml of ethyl ether, the ether being discarded. To the NaOH extract was added 1 ml of 1N HCl and the mixture was extracted with 5, 5, and 2 ml, successively, of ethyl ether. The ether extract was treated by the same procedure above mentioned and submitted to GLC. When amobarbital was added to rat brain, the method described above gave recoveries of 90.0 to 95.0%.

Apparatus and Experimental Conditions of GLC—A Shimadzu Model GC-3BF gas chromatograph equipped with a hydrogen flame ionization detector was used. The carrier gas used was nitrogen. The column was 2.1 m × 3 mm (i.d.) glass spiral-tube, containing a packing of 3% OV-17 on 80–100 mesh Shimalite W. The column temperature was maintained at 220°. The flow rate of carrier gas was 39 ml/min.

The calibration curve for amobarbital was prepared as follows. The sample solutions (5–200 µg/ml) were prepared by dissolving different amounts of amobarbital in a chloroform solution of allobarbital (20 mg%) as the internal standard. At the fixed range and sensitivity of the instrument, 2 µl of sample solution was injected into the gas chromatograph. The calibration curve was obtained by plotting the concentration of amobarbital against the peak height ratio of amobarbital to allobarbital.

Result and Discussion

The onset and duration of hypnotic action of amobarbital were compared in fasted and nonfasted rats. As shown in Table I, food had a significant effect on the hypnotic action

- 12) "CE-2," Nippon Clea Co., Ltd. was used. This food consisted of moisture (6.0%), crude protein (24.0%), crude fat (3.5%), crude fiber (4.5%), crude ash (6.0%), and nitrogen free extract (56.0%).
- 13) J.T. Doluisio, N.F. Billups, L.W. Dittert, E.T. Sugita, and J.V. Swintosky, *J. Pharm. Sci.*, **58**, 1196 (1969).
- 14) When blood sample was taken by a cardio-puncture, 0.1 ml of whole blood was used.

of amobarbital. When administering orally a single dose of 100 mg amobarbital per kg body weight, the fasted rats had an average onset time of 4.8 min and an average duration of 112 min. However, the nonfasted rats receiving the same dose produced no hypnotic effect only resulting in a sedative effect.

TABLE I. Onset and Duration of Hypnotic Action of Amobarbital following Oral Administration with and without Food

	No. of rats	Dose (mg/kg)	Onset of action (min)	Duration of action (min)
Fasting	10	100	$4.8 \pm 1.2^a)$	$112.1 \pm 30.8^a)$
Nonfasting	6	100	— ^{b)}	— ^{b)}

a) mean \pm standard deviation

b) All rats did not lose the righting reflex.

In order to clarify the transition of blood level of amobarbital, blood samples of fasted and nonfasted rats receiving the same dose of amobarbital were taken by a cardio-puncture periodically. The blood levels of amobarbital at various times are shown in Fig. 1. The blood level curves demonstrated that the presence of food concomitant to oral administration of amobarbital delayed the attainment and decreased the magnitude of the maximum blood level.

Riegelman, *et al.*¹⁵⁾ have proposed that it is mathematically and physiologically more acceptable to conceive the body to be a two-compartment open system. Such a model, which includes an extravascular tissue compartment, is shown in Fig. 2 and should be of great value in describing the *in vivo* dynamics of amobarbital. In this model, k_{12} is the first-order rate constant controlling distribution of the drug from blood to tissues, k_{21} is the first-order rate constant controlling return of the drug from tissues to blood, and k_{e1} is the sum of the first-order rate constants for the simultaneous processes of metabolism and excretion. The above two-compartment open model results in the following equation (1) describing the blood level-time curve.

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where C_p is the blood level of the drug, β is the slope of the terminal linear segment on the semilogarithmic plots of blood level *vs.* time, and B is the intercept of this line. If this line is extrapolated to time = 0, and the differences between the actual data line and the extrapolated line are plotted on the logarithmic scale against time, A and α are the intercept and slope, respectively, of the residual line. The parameters of equation (1) are given in Table II. The individual rate constants, namely k_{12} , k_{21} , and k_{e1} , in this model were calculated by the method of Riegelman, *et al.*¹⁵⁾ As shown in Table II, the values of C_p^0 , α , k_{12} , and k_{21} in the nonfasted rats were smaller than those in the

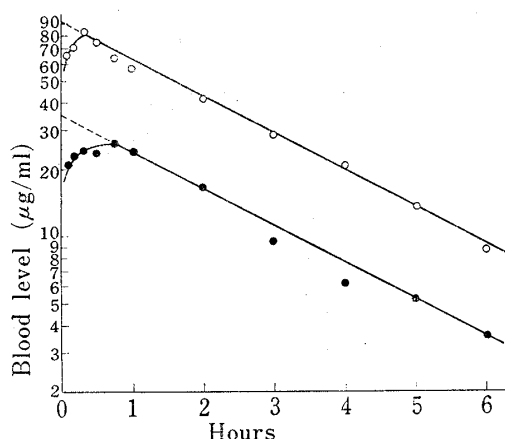


Fig. 1. Mean Blood Levels of Amobarbital following Oral Administration of Amobarbital (100 mg/kg) in Fasted and Nonfasted Rats

The values in the fasted rats (○) represented the mean of 6 experiments, and in the nonfasted rats (●) the mean of 5 experiments.

15) S. Riegelman, J.C.K. Loo, and M. Rowland, *J. Pharm. Sci.*, **57**, 117 (1968).

fasted rats, whereas the values of β and k_{e1} in the nonfasted rats were the same as those in the fasted rats. Furthermore, Loo and Riegelman¹⁶⁾ have developed a method for calculating absorption rate constant for such drug when the parameters of the two-compartment model are known. The last column of Table II showed the results of the Loo-Riegelman calculation of absorption rate constants (k_a) for amobarbital in the fasted and nonfasted rats. The absorption rate of the drug in the nonfasted rats was much slower than that in the fasted rats. This result suggested that the presence of food decreased the absorption rate of amobarbital from the gastrointestinal tract.

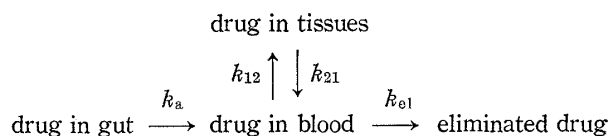


Fig. 2. The Two-Compartment Open Model

TABLE II. Kinetic Data obtained after Oral Administration of Amobarbital (100 mg/kg) in the Fasted and Nonfasted Rats

	$C_p^0 (A+B)$ ($\mu\text{g/ml}$)	α (hr^{-1})	β (hr^{-1})	k_{12} (hr^{-1})	k_{21} (hr^{-1})	k_{el} (hr^{-1})	k_a (hr^{-1})
Fasting	184	11.55	0.385	5.21	5.99	0.74	6.93
Nonfasting	71	6.30	0.385	2.62	3.35	0.73	3.20

These values were calculated from the data given in Fig. 1.

Furthermore, the brain levels of amobarbital at various times were determined in the fasted and nonfasted rats following oral administration of the same dose of amobarbital. The serum level-time curves and brain level-time curves shown in Fig. 3 were obtained by grouping together the data from several rats, since these rat experiments yielded only one experimental point per rat in determining the serum level or brain level. The maximum brain level of amobarbital in the fasted rats was attained at approximately 30 min after oral administration of amobarbital as well as the maximum serum level. This result may indicate that the equilibrium of amobarbital between the blood and brain is established very rapidly and that the drug produces the hypnotic action quickly after the attainment of a definite brain level. The onset of hypnotic action of the drug reflects the time required for penetration of the drug from the blood to the brain and the intensity of the effect corresponds closely with the concentration of the drug present in the brain at the time. Although the maximum brain level of amobarbital in the nonfasted rats was attained at about 30–45 min after oral administration of the drug, however, the presence of food in the animal gut resulted in the decreased magnitude of the maximum brain level in which no hypnotic action was induced. Also, the decrease of brain level of amobarbital was almost in parallel with that of the serum level, and the elimination rates of the drug from serum and brain were relatively rapid.

The percent of amobarbital remaining unabsorbed in the gut at various times was also determined in the same fasted and nonfasted rats used to determine the brain level of the

TABLE III. Absorption Rates of Amobarbital from Rat Stomach and Small Intestine, *in Situ*

	Buffer ^{a)}	Half-lives (min)	Mean k_a (min^{-1})
Stomach	A	173, 169, 178	0.004
Small intestine	B	12, 11.5, 13	0.057

a) A: isotonic citrate-phosphate buffer (pH 3.0)
B: isotonic phosphate buffer (pH 6.0)

16) J.C.K. Loo and S. Riegelman, *J. Pharm. Sci.*, **57**, 918 (1968).

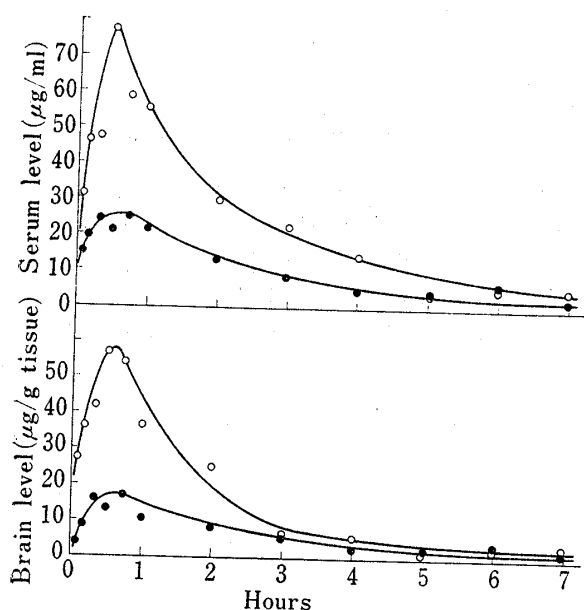


Fig. 3. Serum Levels and Brain Levels of Amobarbital following Oral Administration of Amobarbital (100 mg/kg) in Fasted and Nonfasted Rats

○: fasted rats
●: nonfasted rats

These curves were obtained by grouping together the data from several rats.

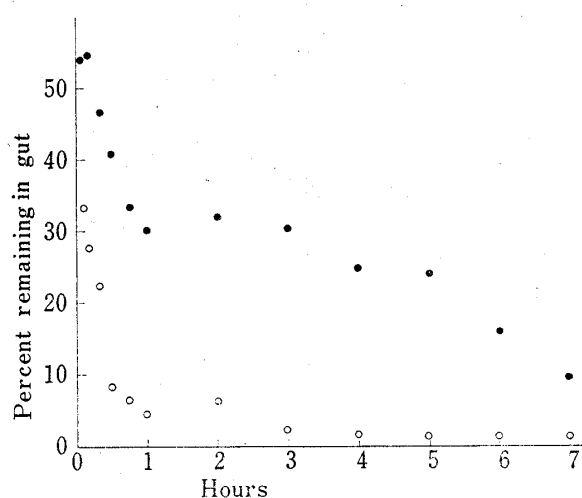


Fig. 4. Percent of Amobarbital Remaining in the Gut (Stomach, Small Intestine, Cecum, and Large Intestine) following Oral Administration of Amobarbital in Fasted and Nonfasted Rats

○: fasted rats ●: nonfasted rats

drug. As can be seen from Fig. 4, after oral administration of 100 mg/kg of amobarbital, the percent of the drug remaining to be absorbed at 1 hr was 4.4% in the fasted rats as compared to 30% in the nonfasted rats. This result demonstrated that the presence of food in the gut decreased the absorption rate of amobarbital and retarded the attainment of the minimum serum level available for the production of the hypnotic action. Moreover, the absorption of amobarbital from rat stomach and small intestine was investigated using the *in situ* method of Doluisio, *et al.*¹³⁾ As indicated in Table III, the absorption rate of the drug was much greater in the small intestine, namely $k_a=0.057 \text{ min}^{-1}$ for the small intestinal absorption (pH 6.0) and $k_a=0.004 \text{ min}^{-1}$ for the gastric absorption (pH 3.0). These data indicate

TABLE IV. Percent of Amobarbital Remaining in the Stomach, Small Intestine, and Cecum^{a)} following Oral Administration of Amobarbital (100 mg/kg)

	Percent remaining, hr							
	0.16	0.33	0.5	1	2	3	4	5
Fasting								
stomach	23.2	20.9	7.0	3.7	4.7	1.0	0.7	0.6
small intestine	4.0	0.9	0.9	0.4	1.3	0.9	0.2	0.2
cecum	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.2
total	27.5	22.1	8.1	4.4	6.2	2.1	1.1	1.0
Nonfasting								
stomach	53.7	45.8	40.5	29.8	31.8	29.9	23.5	9.1
small intestine	0.9	0.7	0.2	0.3	0.2	0.2	0.2	0.2
cecum	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
total	54.8	46.7	40.9	30.3	32.2	30.3	23.9	9.5

^{a)} included the large intestine

that the absorption of amobarbital is negligible from the stomach and, consequently, that the major site for the drug absorption is the small intestine. Thus, the presence of food in the gut altered the gastrointestinal absorption of amobarbital by delaying the gastric emptying and hence increasing the time for the drug to reach its major absorption site.

The percents of amobarbital remaining in the stomach, small intestine, and cecum (including large intestine) were also determined separately (see Table IV). As shown in Table IV, a major portion of the drug remaining to be absorbed at any given time was contained in the stomach in both fasted and nonfasted rats. Actually only an extremely small amount of the drug reached the cecum and large intestine in both fasted and nonfasted rats. These results demonstrate that the primary site available for the absorption of amobarbital is the small intestine and, consequently, that the presence of food will not significantly decrease the extent of absorption of the drug.