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Effect of Fasting on the Elimination of Barbital and Phenobarbital in Rabbits[1]

Nobuyoshi Kaneniwa, Mitsuru Hiura, and Tomoo Funaki

School of Pharmaceutical Sciences, Showa University[2]

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The effect of fasting on the elimination of barbital and phenobarbital was studied using non-fasted, partly fasted, and fasted rabbits. The renal excretion rate constants of both barbiturates were markedly low during fasting, while the metabolic rate constants were little affected. To clarify the mechanism of reduction of the renal excretion rate of these drugs, creatinine clearance and barbital clearance were simultaneously measured in fasted and non-fasted rabbits. The magnitude of apparent tubular reabsorption rate of barbital increased on fasting without any change of the glomerular filtration rate. The urinary pH, which affects the fraction of undissociated species in the renal tubules, was measured. The urinary pH decreased slowly from 8.4 in freely fed rabbits to 6.0 at 24 hr and 4.7 at 48 hr after fasting, and it recovered to the original state within 4 hr after refeeding. Therefore, the fraction of undissociated species of both barbiturates in the tubular urine during fasting becomes much larger than that in fed rabbits. Consequently, the relative tubular reabsorption rate should be higher during fasting. Therefore, it can be concluded that the lower renal excretion rate constant during fasting is due to enhanced reabsorption of undissociated species in the acidic tubular urine.

Keywords——barbiturate; fasting; starvation; ketoacidosis; urinary pH; glomerular filtration rate of barbital; tubular reabsorption rate of barbital; elimination kinetics

It is well known that fasting significantly alters the drug-metabolizing enzyme activity in liver microsomes in various animal species. Dixson *et al.*[3] reported depression of the oxidation pathways in male mice fasted for 36 hr, whereas Kato and Gillette[4] reported that starvation increased the activity of drug-oxidizing enzymes in female rats. Zannoni *et al.*[5] reported increased hepatic microsomal enzyme activity in guinea pigs fasted for 3 days. In contrast, Reidenberg and Vesell[6] reported that a significant change occurred in the apparent volume of distribution of a drug without any change in the metabolic clearance in obese volunteers fasted for 7 days. On the other hand, there have been few reports on the effect of fasting on renal clearance of drugs, except for the study by Reidenberg *et al.*[7] They indicated that the reduced renal clearance of sulfisoxazole in the starved obese volunteers was caused by metabolic ketoacidosis.

The important sites of elimination of drugs in the body are the liver and kidneys. If the drug-clearing ability of these organs changes during fasting, the elimination of a drug from the body may be affected. In our preceding study on the elimination of barbiturates in rabbits,[8] the elimination of barbital and phenobarbital from the plasma was greater when food was provided than during fasting, although the reason for this is not known. The present study was therefore undertaken to clarify the mechanism of the reduction of elimination of barbital and phenobarbital on fasting.

1) Presented at the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April 1978.
2) Location; *Hatanodai 1-5-8, Shinagawa-ku, Tokyo, 142, Japan.*
3) R.L. Dixson, R.W. Shultice, and J.R. Fouts, *Proc. Soc. Exp. Biol. Med.*, **103**, 333 (1960).
4) R. Kato and J.R. Gillette, *J. Pharmacol. Exp. Ther.*, **150**, 285 (1960).
5) W.G. Zannoni, E.J. Flynn, and M. Lynch, *Biochem. Pharmacol.*, **21**, 1377 (1972).
6) M.M. Reidenberg and E.S. Vesell, *Clin. Pharmacol. Ther.*, **17**, 650 (1975).
7) M.M. Reidenberg, H. Kostenbauder, and W.P. Adams, *Metabolism*, **18**, 209 (1969).
8) N. Kaneniwa, M. Hiura, and S. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **27**, 1501 (1979).

Experimental

Feeding Conditions for Rabbits and Drug Administration——Adult male albino rabbits weighing 2.5—3.0 kg were used; the rabbits were used repeatedly at intervals of 2 weeks to test various feeding conditions. Non-fasted rabbits were given a commercial solid diet (CR-1, Nihon Clea, Co., Tokyo) freely before and after injection of the drug. Partly fasted rabbits were fasted for 24 hr before receiving the drug, then food was given at 8 hr for barbital and 12 hr for phenobarbital after intravenous injection of the drug. Fasted rabbits were fasted for 24 hr before receiving the drug and until 60 hr after injection of the drug. However, coprophagy was not prevented, and they were allowed water *ad libitum* in all cases. The rabbits were injected with 80 mg/kg of barbital or 20 mg/kg of phenobarbital as a solution into the aural vein. The preparation of drug solutions and sampling of plasma and urine were as described in our preceding report.[8]

Biliary Excretion of Barbital——Three male albino rabbits were fasted for 24 hr before the experiment. A portion of their abdomen was locally anesthetized with 1% procaine hydrochloride, and was opened through a midline incision downward extending about 2 cm from the diaphragm. A polyethylene catheter (Intramedic, PE-60, Clay Adams, U.S.A.) was inserted into the bile duct by the usual method, and the abdominal incision was sutured after confirmation of bile draining. After injection of 80 mg/kg of barbital into these cannulated rabbits, plasma and bile samples were collected at selected intervals.

Renal Clearance of Barbital and Creatinine——Standard renal clearance techniques[9] were employed. Three male albino rabbits were locally anesthetized with 1% procaine hydrochloride. After the mid-abdominal incision, a polyethylene catheter (Intramedic, PE-50, Clay Adams, U.S.A.) was inserted into the ureter for urine collection. To ensure a constant concentration of the drug in plasma, creatinine was administered at 10 mg/kg, and after 10 min, a maintenance dose was infused at 9 mg/min. However, since the elimination of barbital is very slow and the plasma concentration change was very small during the period of sampling of plasma and urine for measurement of renal clearance, barbital was initially injected at a dose of 40 mg/kg and no further dose was infused. Barbital and creatinine clearances were determined simultaneously every 10 min. Urine was collected during this period, and plasma samples were obtained from the femoral artery at the midpoint of the urine collection period. Barbital and creatinine clearances (C) were calculated as $C=UV/P$, where U, and P indicate the urine and plasma concentrations, respectively, of barbital or creatinine in mg/ml, and V indicates the urine flow rate in ml/min.

Determination of Drug in Plasma, Urine, and Bile——The concentrations of barbiturate in plasma, urine, and bile were determined by gas chromatography with a hydrogen flame ionization detector, as described in our preceding report.[8] The concentrations of creatinine in plasma and urine were measured by photometry, using the method of Hare.[10]

Measurement of Urinary pH——Urine was collected *via* a catheter from rabbits at selected intervals. The pH of urine samples was measured with a pH meter equipped with a glass electrode (model HM-5A, Toa Electronic Ltd., Tokyo) within 10 min after collection.

Results and Discussion

Effect of Fasting on the Elimination of Barbital and Phenobarbital

The plasma concentration decay and cumulative urinary excretion of barbital and phenobarbital were determined after intravenous injection of 80 mg/kg of barbital or 20 mg/kg of phenobarbital into rabbits kept under three feeding states; non-fasted, partly fasted, and fasted.

As shown in Fig. 1, the semilogarithmic plots of plasma concentration of both barbiturates as a function of time were linear for the non-fasted and fasted rabbits. The slopes for both drugs were greater in the non-fasted state than in the fasted state. In the partly fasted state, the slopes before receiving food were nearly equal to those in the fasted state, while those after receiving food increased immediately and were nearly equal to those in the non-fasted state.

Since the first plasma sample at 1 hr deviated from the β phase line in Fig. 1, early plasma samples were taken during the first 1 hr and the biphasic data were analyzed in terms of a two-compartment model with elimination occurring from the central compartment. As described in our preceding report,[8] however, the pharmacokinetic behavior of both barbitu-

9) M. Fujimoto, *Nippon Rinsho*, **25**, 1154 (1967).
10) R.S. Hare, *Proc. Soc. Exp. Biol. Med.*, **70**, 148 (1950).

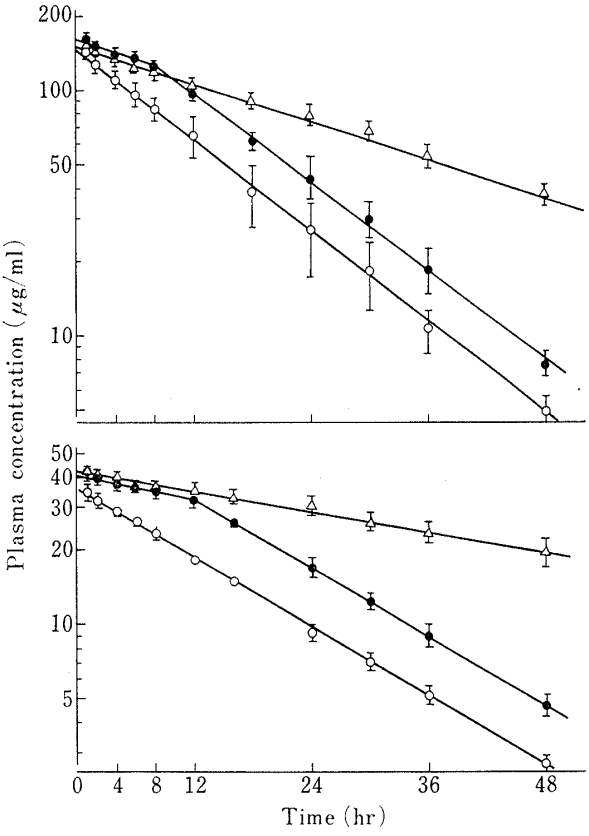

Fig. 1. Plasma Concentration-Time Plots of Barbital (upper) and Phenobarbital (lower) after Intravenous Administration of 80 mg/kg of Barbital or 20 mg/kg of Phenobarbital to Feed-Controlled Rabbits

○; non-fasted, ●; partly fasted, △; fasted.
Each point represents the mean of three rabbits and the vertical bars indicate the standard error of the mean.

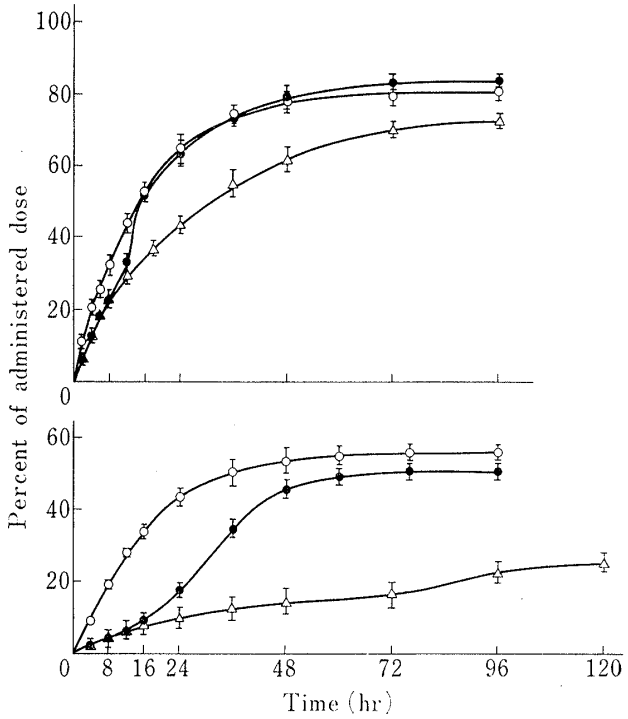

Fig. 2. Cumulative Urinary Excretion of Unmetabolized Barbital (upper) and Phenobarbital (lower) after Intravenous Administration of 80 mg/kg of Barbital or 20 mg/kg of Phenobarbital to Feed-Controlled Rabbits

○; non-fasted, ●; partly fasted, △; fasted.
Each point represents the mean of three rabbits and the vertical bars indicate the standard error of the mean.

rates could be adequately approximated by a one-compartment model based on the plasma data in the linear segment.

As shown in Fig. 2, the cumulative urinary excretion of barbital and phenobarbital before receiving food in the partly fasted rabbits was nearly equal to that in the fasted rabbits; the cumulative urinary excretion increased slowly after receiving food and increased markedly at 4 hr after receiving food. The cumulative amount of barbital ultimately excreted in the urine hardly differed between non-fasted and partly fasted rabbits, but it was slightly lower in the fasted rabbits. The cumulative amount of phenobarbital excreted in the urine increased in the order non-fasted, partly fasted, and fasted. The magnitude of the reduction of urinary excretion of phenobarbital in the fasted rabbits was strikingly large compared to other feeding conditions.

The fraction of the cumulative amount of unmetabolized drug excreted in the urine relative to the intravenous dose is lower than unity, so the elimination of barbital and phenobarbital from the body should also occur by hepatic metabolism, biliary excretion, or both processes. The cumulative biliary excretion and plasma concentration of barbital were measured using fasted rabbits with bile-duct cannulation, and the results are shown in Fig. 3. The cumulative amount of unmetabolized barbital excreted in the bile was about 4.3% of the dose up to 20 hr, and the slope of the plasma concentration decay was similar to that for rabbits without bile-duct cannulation. Therefore, it is considered that biliary excretion eliminates little barbital from the body, and is negligible as an excretory process of barbital.

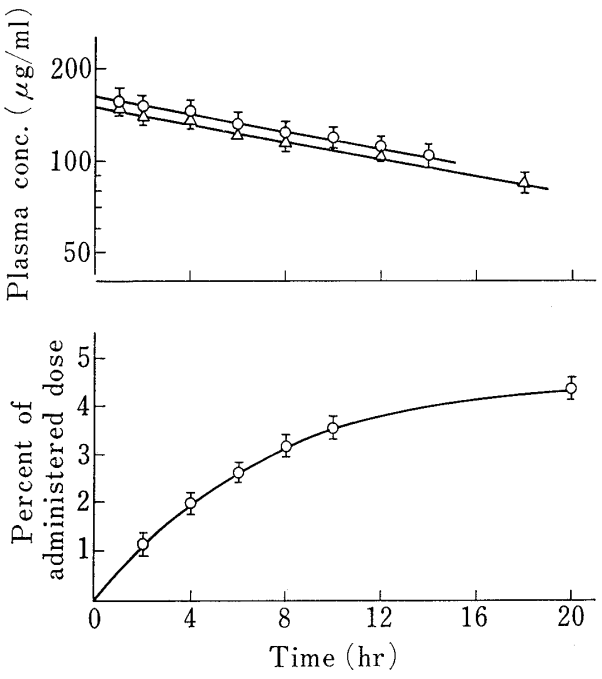

Fig. 3. Plasma Concentration-Time Plots of Barbital (upper) in Bile-duct Cannulated and Bile-duct Non-cannulated Rabbits, and Cumulative Biliary Excretion of Unmetabolized Barbital (lower) in Bile-duct Cannulated Rabbits after Intravenous Administration of 80 mg/kg of Barbital

○; bile-duct cannulated, △; bile-duct not cannulated. Each point represents the mean of three rabbits and the vertical bars indicate the standard error of the mean.

This may also apply to phenobarbital, because unmetabolized phenobarbital is excreted into bile in very low concentrations.[11]

To study the changes of drug-clearing ability of the liver and kidneys on fasting, the elimination rate constant (K_{el}) of barbital and phenobarbital under the three feeding conditions was calculated from the slope of linear segments in the semilogarithmic plots of the plasma concentration of three barbiturates as a func tion of time. The urinary excretion rate constant (K_e) was calculated by the sigma-minus method. Oxy-barbiturates such as barbital and phenobarbital are metabolized only by the liver, and the drugs not metabolized are excreted slowly in the urine.[11] Accordingly, the difference between the elimination rate constant and excretion rate constant should correspond to the metabolic rate constant (K_m). The calculated elimination rate constants, excretion rate constants, and metabolic rate constants for barbital and phenobarbital are listed in Table I.

The metabolic rate constants of barbital and phenobarbital were approxi-

Table I. Elimination Rate Constants (K_{el}), Excretion Rate Constants (K_e), and Metabolic Rate Constants (K_m) of Barbital and Phenobarbital in Non-fasted, Partly Fasted, and Fasted Rabbits

Rate constant (hr^{-1})		Fasted	Partly fasted		Non-fasted
			Before[a]	After[a]	
Barbital	K_{el}	0.0354	0.0422	0.0623	0.0656
		(0.0043)	(0.0026)	(0.0039)	(0.0037)
	K_e	0.0257	0.0354	0.0526	0.0557
		(0.0072)	(0.0043)	(0.0025)	(0.0044)
	K_m	0.0097	0.0068	0.0097	0.0099
		(0.0014)	(0.0021)	(0.0054)	(0.0018)
Phenobarbital	K_{el}	0.0243	0.0263	0.0537	0.0628
		(0.0005)	(0.0033)	(0.0015)	(0.0038)
	K_e	0.0032	0.0058	0.0324	0.0373
		(0.0010)	(0.0010)	(0.0040)	(0.0005)
	K_m	0.0212	0.0205	0.0213	0.0255
		(0.0013)	(0.0043)	(0.0043)	(0.0039)

a) Before and after administration of food.
Each value represents the mean of three rabbits and figures in parentheses give the standard error of the mean.

11) L.S. Goodman and A. Gilman, "The pharmacological Basis of Therapeutics," Macmillan Publishing Co., Inc., New York, 1975, p. 102.

mately constant in all feeding states, but the urinary excretion rate constants of both barbiturates were markedly lower in fasted than in fed rabbits. During fasting, therefore, barbital and phenobarbital stay in the body for a relatively long period and are much more subject to hepatic metabolism. As shown in Fig. 2, it is thus reasonable that the cumulative amount of phenobarbital excreted in the urine increased in the order non-fasted, partly fasted, and fasted rabbits. On the other hand, since barbital has a very low metabolic rate constant compared to the excretion rate constant, the cumulative amount excreted in the urine is little affected by fasting.

It can be concluded that fasting depresses the renal clearing ability without affecting the hepatic clearing ability in the overall elimination processes of barbital and phenobarbital.

Effect of Fasting on Renal Clearance

Barbiturate filtering through the glomeruli is largely reabsorbed passively from tubular urine into the renal blood capillaries.[12] To elucidate whether the tubular reabsorption rate of barbiturate is altered by fasting, renal clearances of barbital and creatinine were determined in non-fasted and fasted rabbits. Barbital clearance (C_b), creatinine clearance (C_{cr}), and the ratio of barbital clearance to creatinine clearance (C_b/C_{cr}) are listed in Table II.

Table II. Creatinine Clearance (C_{cr}), Barbital Clearance (C_b), and the Ratio of Barbital Clearance to Creatinine Clearance (C_b/C_{cr}) in Fasted and Non-fasted Rabbits

	C_b(ml/min)	C_{cr}(ml/min)	C_b/C_{cr}
Non-fasted	0.601±0.120	15.47±1.65	0.0381±0.0075
Fasted	0.190±0.026	13.40±3.64	0.0150±0.0022

Each value represents the mean ± S.E. of three rabbits.

Creatinine is excreted in the urine only by glomerular filtration, without tubular secretion or reabsorption, so that if drug molecules are not significantly bound to plasma proteins in the blood, the difference in the ratio of drug clearance to creatinine clearance from unity reflects the fraction of tubular reabsorption rate to glomerular filtration rate.

Creatinine clearance did not differ markedly in the non-fasted and fasted rabbits. Therefore, the glomerular filtration rate should not be affected by fasting for 24 hr in rabbits. Barbital clearance was significantly smaller than creatinine clearance, in spite of the low protein binding of barbital,[13] and the C_b/C_{cr} value was far from unity under both conditions. Furthermore, the C_b/C_{cr} ratio in the non-fasted rabbits was about 2.5 times that in the fasted rabbits. These results indicate that barbital filtering through the glomeruli is largely reabsorbed from renal tubules, and that fasting facilitates the apparent tubular reabsorption rate of barbital without affecting the glomerular filtration rate.

Effect of Fasting on the Urinary pH

Changes in the concentration of the undissociated species of weakly acidic and basic drugs accompanying changes in the urinary pH can have an important effect on passive reabsorption in the renal tubules. The urinary pH was measured before and 8, 24, 36, and 48 hr after fasting, and 2, 4, and 6 hr after receiving food at 48 hr. As shown in Fig. 4, the urinary pH decreased slowly with prolongation of the fasting period, and it recovered to the original state within 4 hr after refeeding. Such a reduction of urinary pH during the

12) A. Giotti and E.W. Maynert, *J. Pharmacol. Exp. Ther.*, **101**, 296 (1951); W.J. Waddell and T.S. Butler, *J. Clin. Invest.*, **36**, 1217 (1957).

13) It is well known that plasma protein binding of barbital is less than 5%.

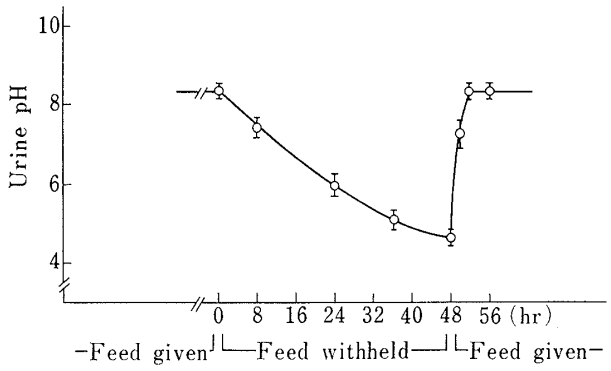

Fig. 4. Relationship between Urinary pH and Fasting Period

Each point represents the mean of three rabbits and the vertical bars indicate the standard error of the mean.

fasting period may be caused by metabolic ketoacidosis, because starved animals and man frequently suffer from metabolic ketoacidosis.[14]

The fraction of undissociated species of barbital and phenobarbital in the tubular urine can be determined from the urinary pH and the pK_a of the drug (barbital, 7.8; phenobarbital 7.4) by employing the Henderson-Hasselbalch equation. The urinary pH remains constant at about 8.4 under free feeding conditions, and the fraction of undissociated species in the tubular urine of non-fasted rabbits is about 24% for barbital and 9% for phenobarbital. Since the urinary pH changes to about 6.0 at 24 hr after fasting, the fraction of undissociated species just after drug injection is about 99% for barbital and 97% for phenobarbital in the partly fasted and fasted rabbits. The urinary pH decreases slowly until feeding, but the fraction of undissociated species of barbital and phenobarbital is little altered by urinary pH lower than 6.0 (>99%). In the partly fasted state, the fraction of undissociated species of both barbiturates in the tubular urine after refeeding should suddenly decrease, reaching the same order as that of freely feeding rabbits within 4 hr.

There was a large difference in the fraction of undissociated species of both barbiturates between fasted and freely fed rabbits. Since the undissociated species of barbital and phenobarbital are preferentially reabsorbed from the renal tubules,[12] the relative tubular reabsorption rate should be higher in fasted rather than in fed rabbits. Therefore, it can be concluded that the lower renal excretion rate constants of barbital and phenobarbital (Table I) and the lower renal clearance of barbital (Table II) in fasted rabbits are due to enhanced reabsorption of undissociated species in the acidic tubular urine. Similarly, the higher urinary excretion rates of barbital and phenobarbital (Fig. 2) in the partly fasted rabbits after receiving food are related to the relative depression of the tubular reabsorption rate of the drug in the alkaline tubular urine.

Reidenberg *et al.*[7] reached a similar conclusion regarding the depression of renal clearance of sulfisoxazole in obese volunteers who fell into mild metabolic ketoacidosis when receiving only water, potassium, and vitamin supplements for 8—12 days. However, it should be noted that a change of renal clearance based on the reduction of urinary pH in rabbits was produced by fasting for 24 hr before drug administration, which is a common regime in animal experiments. The change of renal clearance arising from the reduction of urinary pH during fasting may produce discernible changes in the elimination of other weakly acidic and basic drugs. Furthermore, a reduction of urinary pH during mild fasting may also occur in other animal species. Therefore, the fasting period should be kept constant in studies on the pharmacokinetics and pharmacological effectiveness of drugs, in order to ensure that the data are comparable.

14) A. White, P. Handler, and E.L. Smith, "Principles of Biochemistry," MaGraw-Hill Book Company, New York, 1964, p. 458.