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# 197. Kiichiro Kakemi, Takaichi Arita, Ryohei Hori, and Ryoji Konishi*1 : Absorption and Excretion of Drugs. XXX.*2 Absorption of Barbituric Acid Derivatives from Rat Stomach.*3

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The absorption of the unionized forms of barbituric acid derivatives from rat stomach was found to be a first order process and varied with the differences in the chemical structures. N-methylation and O-S displacement markedly affected the gastric absorption. The mediated physicochemical factor was partition coefficient to organic solvent. Effect of pH of drug solution also influenced the absorption of especially readily absorbed derivatives. In respect to ionized forms, the similarity in rate constants was observed among all series of derivatives, and the mechanism involved was assumed to be a physical diffusion through pores. From these observations, it was concluded that all types of barbituric acid derivatives are absorbed from rat stomach by a relatively simple physical process, which is in good agreement with pH-partition process suggested by Brodie, *et al.*

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Since Karel[1] suggested that the stomach has an ability to absorb drugs in addition to its essential nature as a digestive organ, a number of biopharmaceutical investigations[2~5] have been carried out concerning the gastric absorption of various drugs. Gastric absorption of barbituric acid derivatives have also been reported by several investigators.[2~6] However, they have not examined adequate number of derivatives enough to elucidate a reliable relationship between the characteristics of gastric absorption and chemical structures or physicochemical properties. This report deals with the absorption of sixteen barbituric acid derivatives from rat stomach, and shows that the rate of their absorption are correlated with such factors as lipid solubility, ionization constant, and diffusion coefficient, known to affect drug absorption.

## Experimental

**Materials**——Barbituric acid derivatives used in this paper are summarized in Table I. Most of them were obtained from commercially available sources and recrystallized from ethanol-water mixture. Probarbital, 5-allyl-5-ethylbarbituric acid were prepared by the condensation of urea and the corresponding diethyl dealkylmalonates,[7,8] and N-methylated derivatives of allobarbital, amobarbital and cyclobarbital were prepared according to the method described by Butler.[9] All other materials were of analytical grade.

**Procedure of Absorption Experiments**——i) Ligation method : The *in situ* technique of Schanker, *et al.*[2] was adopted. Male Wistar rats, weighing 130~170 g., were fasted for 20 hr. prior to operation but

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*2 Part XXIX : This Bulletin, **15**, 172 (1967).

*3 Presented in part to the 83rd Annual Meeting of the Pharmaceutical Society of Japan (April, 1963).

1) L. Karel : Physiol. Rev., **28**, 433 (1948).
2) L.S. Schanker, P.A. Shore, B.B. Brodie, C.A.M. Hogben : J. Pharmacol. Exptl. Therap., **120**, 528 (1957).
3) K. Kakemi, T. Arita, H. Yamashina : Arch. Pract. Pharm., **21**, 97 (1961).
4) K. Kakemi, T. Arita, S. Ohashi : Yakugaku Zasshi, **82**, 348 (1962).
5) T. Koizumi, T. Arita, K. Kakemi : This Bulletin, **12**, 413 (1964).
6) H. Weese : Arch. exptl. Pathol. Pharmakol., **181**, 46 (1936).
7) R. Adams, R.M. Kamm : "Org. Syntheses" coll. vol. **1**, 250 (1941). John Wiley & Sons, Inc., New York.
8) M. Kopp, B. Tschoubar : Bull. soc. chim. France, **1951**, 30.
9) T.C. Butler : J. Pharmacol. Exptl. Therap., **108**, 474 (1953).

had access to drinking water at all times. The animals were anesthetized with urethane (175 mg./kg., intraperitoneal administration) and were maintained under anesthesia for the entire course of the experiment. As mentioned later in discussion, a first order reaction kinetics is applicable to gastric absorption of barbituric acid derivatives, the absorption rate constant was calculated from the following equation where $K$ is the absorption rate constant,

$$K=-2.303\ \log\left(\frac{Cf}{Ci}\cdot\frac{Pi}{Pf}\right)\qquad(hr^{-1})$$

$Ci$ and $Cf$ are the concentrations of a drug at the initial and the final states respectively, and $Pi$ and $Pf$ are those of phenol red used as an indicator for volume change during the absorption period.

ii) Perfusion method: The technique developed by Schanker, *et al.*[10] and modified in our laboratory[4,11] was adopted in the gastric perfusion *in situ*. Rats are treated similarly as in the ligation method and cannulated with polyvinyl cannula from pylorus and cardia into the stomach, and ligated with care to exclude major blood vessels. Twenty ml. of drug solution was perfused at a rate of approximately 6 ml./min. for 1 hour. Samples are withdrawn from the reservoir of drug solution at an interval of 15 min. and analyzed.

**Preparation of Drug Solutions**——The components of isotonic buffer solutions used as the medium are listed in Table II. The concentrations of drugs were as follows except in the case of examining the concentration dependency in absorption of ionized form of drug: 200 μg./ml. for oxy series, 100 μg./ml. for N-methyl series, and 25~50 μg./ml. for thio series. Phenol red was dissolved in the drug solution to the concentration of 20 μg./ml. All derivatives did not reveal any appreciable decomposition under the experimental conditions.

**Analytical Methods**——i) Barbituric acid derivatives: In all absorption experiments, the extraction method with organic solvents was used to minimize the contamination of gastric contents. An aliquot of the organic phase was then shaken with alkaline media, and the optical density of the latter phase was determined spectrophotometrically. Oxy series, N-methyl series, and thio series were determined according to the methods of Goldbaum, Butler, and Brodie, *et al.* respectively.[12~14] Phenol red did not interfere with the determination. For the determination of the samples other than absorption experiments, direct spectrophotometric measurements are made after alkalinization.

ii) Phenol red: Phenol red was determined colorimetrically (550 mμ) by the addition of $N$ NaOH solution. The presence of barbituric acid derivatives showed no interference.

iii) Chlorine ion: In the determination of diffusion coefficients, cell constants were determined with potassium chloride or hydrochloric acid as a standard. Chlorine ion was determined by the method of Iwasaki, *et al.*[15].

**Determination of Physicochemical Properties**——i) Apparent partition coefficients: Barbituric acid derivatives were dissolved in the same isotonic buffer solution as used in the absorption experiments. Six ml. of the buffered drug solution was added to an equal volume of organic solvent previously saturated with the same buffer solution, and equilibrated at 37° by vigorous shaking. The separated aqueous phase was analyzed. The apparent partition coefficient of a drug was calculated from the decrease of concentration in the aqueous phase. Carbon tetrachloride, chloroform, and isopentyl acetate were used as the organic solvents. Some of N-methyl derivatives and thio derivatives have extremely high coefficients. In such cases, ratio of aqueous phase to organic phase was increased to obtain an appropriate optical density in the determination. This was corrected in the calculation.

ii) Diffusion coefficients: Diffusion coefficients of barbituric acid derivatives in water at 37° were determined according to the method of McBain and Liu[16] using glass porous filter. The diffusion cell, full of drug aqueous solution (50~100 μg./ml.), was inserted in a given volume of distilled water at 37° for one or two hours. After the preliminary diffusion, the subsequent proper diffusion was continued for 10 to 12 hours. Diffusion coefficients were calculated by the method of Northrop and Anson.[17] The cell constant was standardized by potassium chloride and hydrochloric acid.

iii) Apparent ionization constants: Apparent ionization constants for the designated derivatives by an asterisk(*) in Table I were determined spectrophotometrically using the buffer solutions of a constant ionic strength $\mu=0.15$. Other listed values are from the papers by Krahl[18] and Sato.[19]

10) L.S. Schanker, D.J. Tocco, B.B. Brodie, C.A.M. Hogben: J. Pharmacol. Exptl. Therap., **123**, 81 (1958).
11) T. Koizumi, T. Arita, K. Kakemi: This Bulletin, **12**, 421 (1964).
12) L.R. Goldbaum: Anal. Chem., **24**, 1604 (1952).
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14) B.B. Brodie, L.C. Mark, E.M. Papper, P.A. Lief, E. Bernstein, E.A. Rovenstine: J. Pharmacol. Exptl. Therap., **98**, 85 (1950).
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17) J.H. Northrop, M.L. Anson: J. Gen. Physiol., **12**, 543 (1929).
18) M.E. Krahl: J. Phys. Chem., **44**, 449 (1940).
19) Y. Sato: Nippon Kagaku Zasshi, **78**, 382 (1957).

## Results and Discussion

Normal pH of the gastric lumen in the rat is reported to be 1~2.[20] The absorption of barbituric acid derivatives listed in Table I was examined at this physiological pH. The rate constants of gastric absorption, partition coefficients at pH 1.1 to various organic solvents at 37°, and diffusion coefficients in water at 37° were summarized in Table I. At pH 1.1, all derivatives exist solely as unionized molecules, since $pK_{a1}$ values for the derivatives investigated are ranged from 7.41 to 8.34. It is apparent from the data in Table I that absorption patterns of the unionized form of drugs from the rat stomach can be classified by the series described above. N-methylation of oxy series at 1 (or 3)-position increased the absorption rate markedly. Barbital and methar-

Table I. Rate Constants of Gastric Absorption and Some Physicochemical Properties of Barbituric Acid Derivatives

| Series | Barbiturate[a] | $pKa_1$ | Absorption rate[b] constant (1/hr.) | Partition coefficient[c] | | | Diffusion[d] coefficient ($cm^2$/sec.) |
|---|---|---|---|---|---|---|---|
| | | | | $CCl_4$, ×10 | $CHCl_3$ | Isopentyl acetate | |
| Oxy-series | Barbital | 7.91 | 0.053 | 0.35 | 0.72 | 3.82 | 1.271 |
| | Probarbital | 8.01 | 0.082 | 0.61 | 1.60 | 8.81 | 1.099 |
| | 5-Allyl-5-ethyl-barbituric acid* | 7.68 | 0.036 | 0.63 | 1.31 | 9.59 | — |
| | Allobarbital | 7.79 | 0.092 | 1.09 | 2.13 | 16.8 | 0.788 |
| | Phenobarbital | 7.41 | 0.135 | 2.33 | 4.44 | 34.4 | 1.049 |
| | Cyclobarbital | 7.50 | 0.142 | 2.97 | 3.80 | 4.14 | 0.815 |
| | Pentobarbital | 8.11 | 0.194 | 9.27 | 24.1 | 106 | 0.994 |
| | Amobarbital | 7.94 | 0.195 | 9.44 | 33.8 | 113 | 0.901 |
| N-Methyl-series | Metharbital* | 8.17 | 0.178 | 20.2 | 34.7 | 20.6 | 1.178 |
| | Hexobarbital | 8.34 | 0.276 | 76.0 | 129 | 73.2 | 0.882 |
| | 5-Cyclohexen-1-yl-5-ethyl-1-methylbarbituric acid* | 8.14 | 0.276 | 308 | 301 | 187 | — |
| | 5,5-Diallyl-1-methylbarbituric acid* | 8.06 | 0.290 | 68.9 | 140 | 85.5 | — |
| | Mephobarbital | 7.70 | 0.354 | 63.6 | 95.5 | 55.8 | 1.051 |
| | 5-Ethyl-5-isopentyl-1-methyl-barbituric acid* | 8.31 | 0.421 | 895 | 545 | 402 | — |
| Thio-series | Thiopental | 7.45 | 0.475 | 378 | 321 | 991 | 0.939 |
| | Thiamylal | 7.48 | 0.417 | 689 | 688 | 1700 | 0.868 |

*a*) Nomenclature of the derivatives is the synonym used in Chemical Abstracts except those which chemical names are written.
*b*) pH 1.1, average of at least three rats. *c*) pH 1.1, 37°. *d*) 37°, in water, ×$10^5$.

bital, phenobarbital and mephobarbital are typical examples. Displacement of oxygen atom to sulfur atom in oxy series also increased the absorption as seen in the case of pentobarbital. These changes in absorption rates are reflected well to the differences in their apparent partition coefficients. Futhermore, in each series, the substituents at 5-position were found to be influencing the gastric absorption. These differences are well reflected by the partition characteristics. On the other hand, the estimated diffusion coefficients of barbituric acid derivatives were proportional to the molecular weights as suggested by Thovert.[21] Diffusion coefficient which was suggested as an influencing factor in transport across heterogeneous membranes by Higuchi[22] and applied in gastro-

20) M.H.F. Friedman: Proc. Soc. Exptl. Biol. Med., **54**, 42 (1943).
21) J. Thovert: Compt. rend., **135**, 579 (1902).
22) W.I. Higuchi, T. Higuchi: J. Am. Pharm. Assoc., **49**, 598 (1960).

intestinal absorption of sulfonamides[5,23] can not be a predominant property to interprete the differences observed in the gastric absorption of barbituric acid derivitives. These results indicate that in the absorption of barbituric acid derivatives from the rat stomach, the process of partition in the lipid components in gastric mucous membrane is a rate-determining step, which is in good agreement with pH-partition hypothesis developed by Brodie and his colleagues.[2,10,24,25] Although partition into organic solvent is apparently differ from that into a supramolecular lipids in biological membrane, as shown in Fig. 1 and Fig. 2, a logarithmic relathionship between absorption rate constants and partition coefficients was found to exist. The significance of this relationship will be discussed further in the succeeding report. In this limited sense, partition coefficients to organic solvent is a practically useful clue to the prediction of the rate of gastric absorption from chemical structures, and to the elucidation of absorption mechanism. In order to examine the existence of an active mechanism involved in the gastric absorption of the unionized forms, time course of gastric absorption was studied with thiopental as the typical of readily absorbed derivatives. In this case, the perfusion method was used instead of the ligation. As shown in Fig. 3, a first order process was predominant without a specific concentration dependence in gastric absorption. This suggests that the type of absorption of the unionized forms is of passive nature, and a specialized

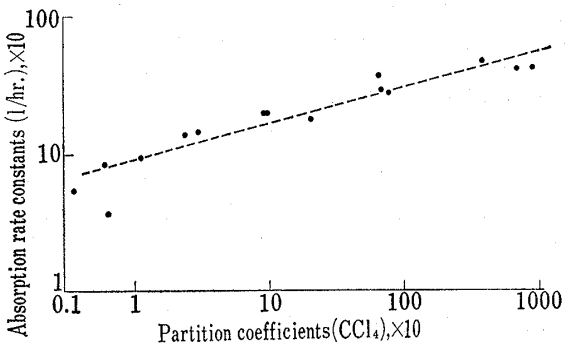


Fig. 1. Logarithmic Relationship between Absorption Rate Constants and Partition Coefficients for Organic Solvent (A)

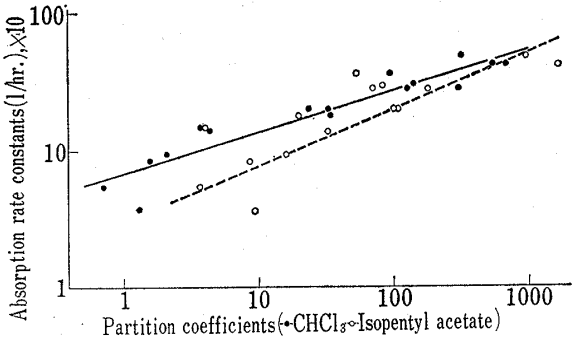


Fig. 2. Logarithmic Relationship between Absorption Rate Constants and Partition Coefficients for Organic Solvents (B)

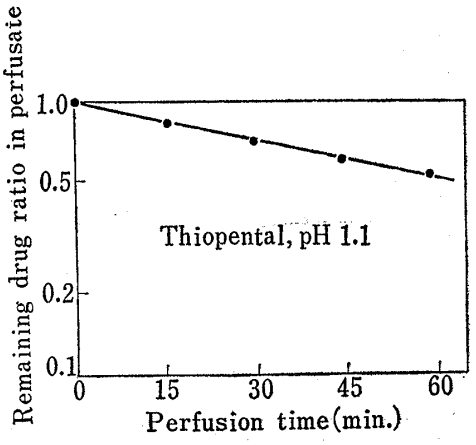


Fig. 3. First Order Process of Gastric Absorption during Continuous Perfusion *in situ*

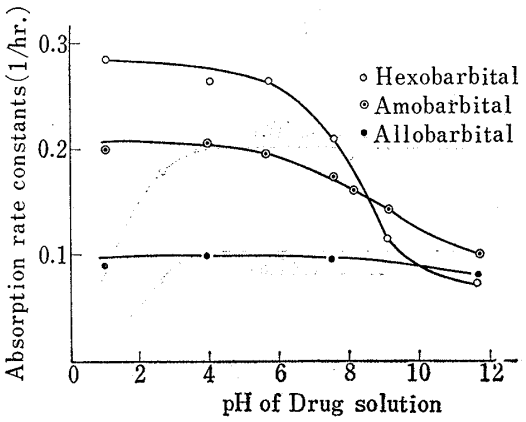


Fig. 4. pH-Gastric Absorption Rate-Profiles of Some Barbituric Acid Derivatives
Each point represents the average of at least 3 rats.

23) K. Kakemi, T. Arita, S. Muranishi : This Bulletin, **13**, 861 (1965).
24) B.B. Brodie : J. Pharmacol. Exptl. Therap., **120**, 540 (1957).
25) B.B. Brodie, L.S. Schanker : *Ibid.*, **125**, 275 (1959).

Table II. Components for Isotonic Buffered Systems

| pH range | Salt components | pH range | Salt components |
|---|---|---|---|
| 1.1 | HCl-KCl | 8.0 | $KH_2PO_4$-$NaHCO_3$ |
| 3.8～6.0 | Citric acid-$Na_2HPO_4$ | 9.0 | Boric acid-KCl-NaOH |
| 7.0～7.6 | $KH_2PO_4$-$Na_2HPO_4$ | 11.5 | $Na_2HPO_4$-NaOH |

active mechanism is not mediating. In respect of ionization constant, an attempt was made to clarify quantitatively the effect of ionization on the gastric absorption. Absorption rate constants of three derivatives, allobarbital, amobarbital and hexobarbital, were estimated at various pH ranging from 1.1 to 11.7. Isotonic buffer solutions used in this experiment are shown in Table II. It is clear from Fig. 4 that preferential absorption occurs at the acidic region where these compounds are almost completely unionized. It is pertinent to consider that the decreasing pattern of absorption with pH was noted to correspond to their individual $pK_{a1}$, suggests little pH change of the drug solution during absorption experiment. The characteristics in pH-absorption profile was found to correlate to that in pH-partition profile shown in Fig. 5. These phenomena are predictable from Brodie's hypothesis. However, it is noted that poorly absorbed de-

Table III. Absorption Rate Constants of Ionized Forms of Barbituric Acid Derivatives and Their Ratios to Unionized Forms

| Barbiturate | Ionized[a] form | Ionized form / Unionized form [b] | Barbiturate | Ionized[a] form | Ionized form / Unionized form [b] |
|---|---|---|---|---|---|
| Barbital | 0.062 | 1.17 | Amobarbital | 0.108 | 0.55 |
| Probarbital | 0.119 | 1.45 | Metharbital | 0.082 | 0.46 |
| Allobarbital | 0.089 | 0.97 | Hexobarbital | 0.089 | 0.32 |
| Phenobarbital | 0.112 | 0.83 | Mephobarbital | 0.153 | 0.43 |
| Cyclobarbital | 0.082 | 0.58 | Thiopental | 0.184 | 0.39 |
| Pentobarbital | 0.126 | 0.65 | Thiamylal | 0.144 | 0.35 |

*a*) Absorption Rate Constants at pH 11.5～11.7. *b*) Data in Table I were used.

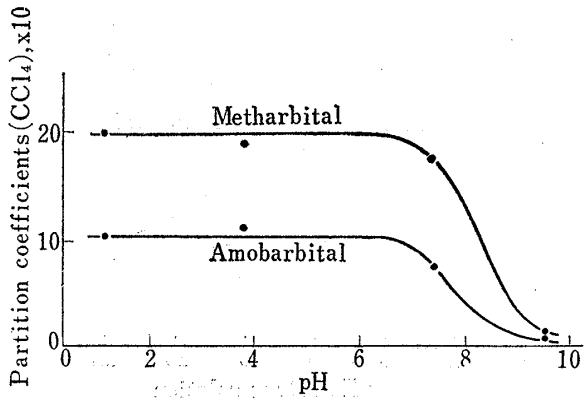


Fig. 5. pH-Partition-Profiles of Two Barbituric Acid Derivatives
●: Experimentally determined
———: Theoretically calculated from $pKa_1$ values and partition coefficients at pH 1.1 (unionized form) using the following equation.
$P_0=P(1+10^{pH-pKa_1})$
$P_0$: Partition coefficients at pH 1.1.
P: Apparent partition coefficients.

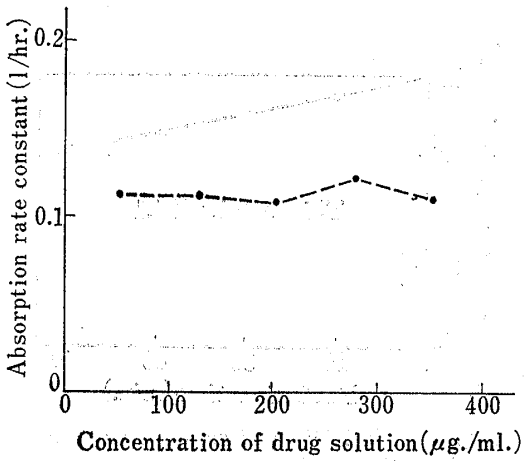


Fig. 6. Concentration Dependence of Absorption Rate Constant[a] of Ionized Amobarbital
*a*) pH 11.7

rivatives, for example, allobarbital illustrated in Fig. 4, were absorbed uniformally at all pH range studied. At the alkaline region where the drugs are ionized, the absorption rate constants are similar for all derivatives, and relatively large compared to the reported data on other drugs.[2,3] This is apparent from Table Ⅲ showing absorption rate constants of the ionized forms of barbituric acid derivatives and their ratios to those of the unionized forms. At present state, it is reasonable to assume that ionized forms of weak acids and bases penetrate a biological membrane predominantly through pores in the membrane as exemplified by water filled pore,[26] although other processes than pore route is contributing to some extent. Molecular dimensions resulted from diffusion studies also support this interpretation. Participation of an active mechanism was not observed in the absorption of ionized forms of barbituric acid derivatives, as shown in Fig. 6.

26) J.F. Danielli, H. Davson : " The Permeability of Natural Membranes " 61 (1952). Cambridge Univ. Press.