

240. Kiichiro Kakemi, Takaichi Arita, Ryohei Hori, and Ryoji
Konishi*¹: Absorption and Excretion of Drugs. XXXII.*²
Absorption of Barbituric Acid Derivatives
from Rat Small Intestine.*³

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Absorption of barbituric acid derivatives from rat small intestine was investigated systematically, and it was found that the absorption did not depend on chemical structure and lipid solubility so much as in the gastric absorption, but no specialized absorption mechanism was demonstrated. The pH-partition hypothesis was partially operative, but pH absorption profile had an optical pH range in all derivatives investigated and N-methyl series which have higher lipid solubility than the corresponding oxy series were absorbed slowly than expected. These phenomena of *in situ* experiments which are assumed exceptional to pH-partition hypothesis were correlated significantly with the *in vitro* binding to mucosal preparations of rat small intestine.

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Previous works in our laboratory have shown that absorption of various barbituric acid derivatives from rat stomach depends on lipid solubility¹⁾ of the unionized forms, and was interpreted in connection with chemical structure.*² This fact suggests that pH-partition hypothesis developed by Brodie, *et al.*³⁾ is valid in the gastric absorption of wide variety of barbituric acid derivatives. In this report, the similar systematical investigations were carried out on the absorption of seventeen barbituric acid derivatives from rat small intestine which is assumed generally to be a potentially important site of drug absorption. And the correlation between absorption characteristics and physico-chemical properties was considered on the basis of pH-partition hypothesis which was operative in the gastric absorption.

Experimental

Materials—Barbituric acid derivatives used in this paper were prepared as previously described.*² All other materials were of analytical grade. The nomenclature and abbreviation is same as in the previous paper.

Procedure of Absorption Experiments—The procedure of absorption experiments from rat small intestine was the same as those reported in the papers³⁾ from our laboratory except that rats were anesthetized by urethane (175 mg./kg., intraperitoneal injection), and samples were withdrawn from the reservoir of perfusing drug solution at an interval of 15 min. for 1 hr. 15 min. lag perfusion was set in each run.

Drug Solution—Isotonic buffer systems and concentration of the perfusing drug solution were the same as previously described.¹⁾ First order rate constants were calculated from the slopes of the curves relating perfusion time and remaining concentration ratio of the drug in the perfusion fluid.

Analytical Methods—The same spectrophotometric methods were used as previously described.¹⁾

Measurement of % Binding of Various Barbituric Acid Derivatives to Mucosa of Rat Small Intestine—The mucosal preparation of rat small intestine was generally prepared by the modified method of

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*² Part XXXI: This Bulletin, 15, 1705 (1967).

*³ Presented in part to the 84th Annual Meeting of the Pharmaceutical Society of Japan (November, 1963).

1) K. Kakemi, T. Arita, R. Hori, R. Konishi: This Bulletin, 15, 1534 (1967).

2) C.A.M. Hogben, D.T. Tocco, B.B. Brodie, L.S. Schanker: J.Pharmacol. Exptl. Therap., 125, 275 (1959).

3) K. Kakemi, T. Arita, S. Ohashi: Yakugaku Zasshi, 82, 348 (1962). T. Koizumi, T. Arita, K. Kakemi: This Bulletin, 12, 421 (1964).

Dickens and Weil-Malherbe.⁴⁾ Rat small intestine washed as in the absorption experiment was isolated, and both the mucosal and serosal sides were rinsed well with saline. Cut open a length of approximately 9 cm., and spread on a glass plate with the mucous membrane facing upward. Carefully blot with filter paper to remove adhering moisture and solid matters, and scraped off the mucosa with the edge of a microscope slide glass, and the pooled mucosa was weighed. A portion of the mucosa was dried at 110° immediately for the determination of dry weight/wet weight ratio. One % (dry wt.) homogenates was prepared in a Potter-Elvehjem teflon homogenizer with the buffer solution used in the absorption experiment. Ionic strength was adjusted to 0.05 in this experiment. Equilibrium dialysis method employed by Klotz⁵⁾ was adopted to estimate the binding. Thiopental, pentobarbital and hexobarbital were dissolved in the buffer solution at the concentration of $3.3 \times 10^{-4}M$. Eight ml. of the drug solution was placed in a test tube (20 ml. volume) as the outer fluid, cellulose tubing (Visking Co. 8/32#) containing 4 ml. of the mucosal homogenates was immersed in the test tube and equilibrated for 72 hr. at 5°. The absorption of the drugs to the tubing was negligible. Percentage of binding was calculated from the difference of drug concentration in the outer fluid in the presence of mucosa from that in the absence of mucosa.

Results and Discussion

In order to clarify the process of absorption of barbituric acid derivatives from rat small intestine, the effect of drug concentration on absorption rate constant was examined. The result with barbital is represented in Fig. 1. There is no apparent effect of the drug concentration in perfusion solution. The linearity of time course suggests that the absorption from small intestine is probably mediated by passive process fitting a first order reaction kinetics. Other barbituric acid derivatives than barbital were all similar in this point as typically shown in Fig. 2. The extent of absorption was

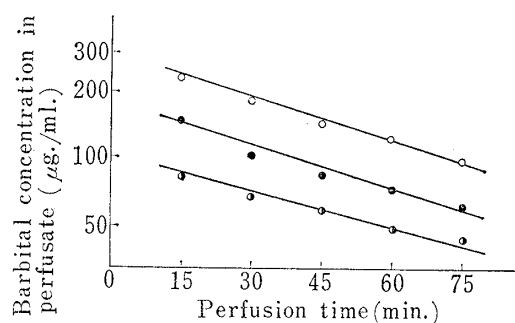


Fig. 1. Effect of Initial Concentration on Absorption from Rat Small Intestine

- 300 µg./ml.
- 200 µg./ml.
- 100 µg./ml.

Each point represents an average of at least two rats. Initial pH of drug perfusion fluid was 5.5.

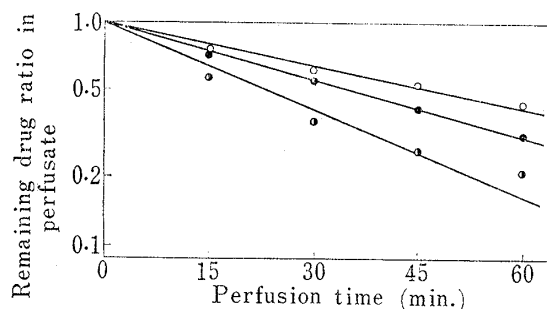


Fig. 2. First Order Process of Absorption from Small Intestine during Continuous Perfusion *in situ*

- Metharbital
- Secobarbital
- Thiopental

0 min. represents 15 min. after the start of perfusion.

expressed by the first order rate constant in this paper. The rate constants at pH 5.5 where all derivatives are assumed to exist in the unionized formes are listed in Table I. The correlation between absorption rate constant and partition coefficient was not satisfactory and the dependence of absorption on partition coefficients was not so significant as compared to the case of gastric absorption as shown in Fig. 3. Since the effect of pH on partition coefficient is confirmed in the previous paper to correspond to the value of pK_{a1} , the partition coefficients at pH 5.5 are almost identical to those at pH 1.1. In spite of the tendency above, thio series having high lipid solubility were highly absorbed, and this can be considered rational on the basis of pH-partition hypothesis.

4) F. Dickens, H. Weil-Malherbe : Biochem. J., **35**, 7 (1941).

5) I.M. Klotz, F.M. Walker, R.B. Pivan : J. Am. Chem. Soc., **68**, 1486 (1946).

TABLE I. Rate Constants of Absorption of Barbituric Acid Derivatives from Rat Small Intestine

Barbiturates	Absorption Rate Constant at pH 5.5 (1/hr.)	Barbiturates	Absorption Rate Constant at pH 5.5 (1/hr.)
Barbital	1.014	Metharbital	1.014
Probarbital	1.223	Mephobarbital	1.124
5-Allyl-5-ethylbarbituric acid	1.205	N-Methylallobarbital	1.155
Allobarbital	1.320	Hexobarbital	0.894
Phenobarbital	1.569	N-Methylcyclobarbital	0.749
Cyclobarbital	1.094	N-Methylamobarbital	1.101
Pentobarbital	1.279	Thiopental	1.808
Amobarbital	1.260	Thiamylal	1.663
Secobarbital	1.188		

Each constant is an average value from at least three rats.

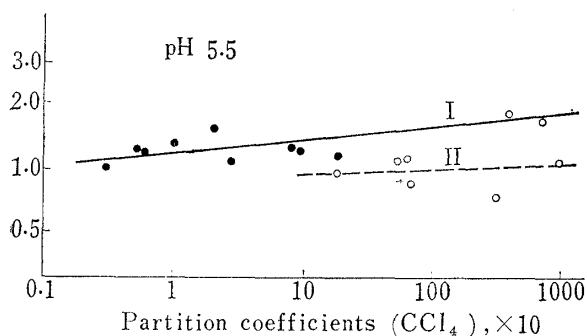


Fig. 3. Relationship between Absorption from Small Intestine and Partition Coefficients

- Oxy Series
- N-Methyl Series
- Thio Series

Partition Coefficients were reported previously.* The calculated correlation coefficients for I and II are 0.704* (statistically significant) and -0.187 (not significant) respectively.

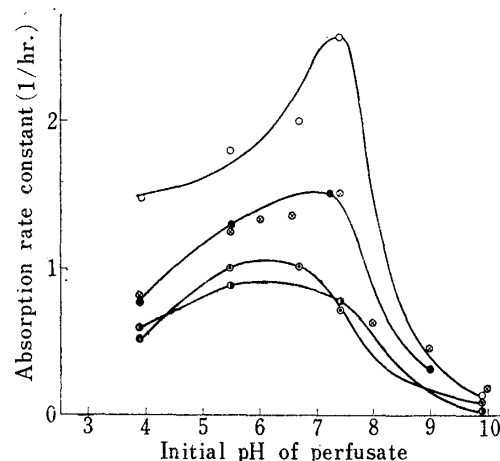


Fig. 4. pH-Absorption-Profiles of Some Barbituric Acid Derivatives

- Thiopental ● Pentobarbital
- ⊙ Barbital ● Hexobarbital
- ⊗ Amobarbital

However, in comparison of oxy, thio series and N-methyl series, it was found that the latter which are more lipid soluble than the corresponding oxy series, were absorbed at the rate almost identical to the oxy series. The combination of barbital and metharbital is one of the typical, and the absorption rate constants for barbital and metharbital are identical, but the partition coefficients to organic solvents increased in favor of metharbital. Other N-methyl derivatives than metharbital are contrarily decreased a little in the absorption compared to the corresponding oxy series. This specific behavior of N-methyl series of barbituric acid derivatives in the absorption from small intestine is in agreement with the partition studies reported in the previous paper, and it appears that absorption of barbituric acid derivatives from rat small intestine is mediated by the processes favoring hydrogen bonding or similar postulates rather than partition, but at present, this remains as a speculation. It was also apparent from the results that in each series of derivatives, the effect of substituent on 5-position of barbituric acid ring is not significant, and the classification of the extent of absorption by chemical structure was not demonstrated as simply applied in the stomach. This fact also suggests the speculated mechanism. In order to examine further the specific aspects of absorption of barbituric acid derivatives from rat small intestine, the effect of perfusion

fluid pH was examined with five derivatives; barbital, pentobarbital, amobarbital, hexobarbital and thiopental. If partitioning were predominant in absorption process, the absorption rate constants at various pH should be proportional to the unionized form defined by pK_{a1} as seen in gastric absorption. However, as shown in Fig. 4, the maximal pH of absorption was observed at pH from 6.5 to 7.5 in all derivatives investigated. The average pH of rat small intestine is known to be approximately 6.6,⁶⁾ and the virtual pH of approximately 5.3 is verified to exist at the mucosal surface of small intestine,²⁾ the difference in pH between the initial and the final perfusion fluid was relatively small in the experimental conditions employed, so that in Fig. 4, pH of the initial fluid was plotted. At the higher pH than pK_{a1} , absorption was decreased according to the fraction of unionized form computed from pK_{a1} , and this indicates that the unionized form is preferentially absorbed. But at the acidic range where no significant difference is considered in the fraction of the unionized moiety, the absorption rate constants of all derivatives studied decreased gradually with the decrease in the pH of perfusion fluid. And it was also noted that the absorption of hexobarbital, a N-methyl derivative, was limited as described above at the pH range examined, although the pattern in pH-profile of the absorption of hexobarbital was similar to those of the other series of derivatives. To examine the possibility that the absorption mechanism changes with pH, the absorption rate constants were similarly determined at pH 3.8 and correlated with the partition coefficients. The result is shown in Fig. 5 and no essential

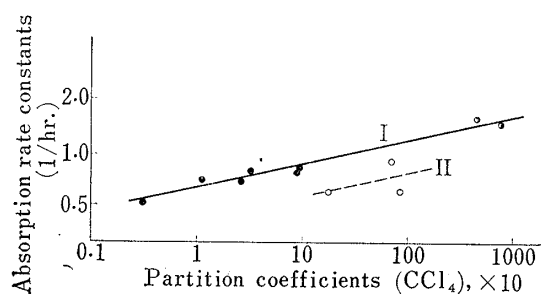


Fig. 5. Relationship between Absorption from Small Intestine and Partition Coefficients

- Oxy Series
- N-Methyl Series
- Thio Series

Partition Coefficients were reported previously.*² The calculated correlation coefficients for I and II are 0.973** (statistically very significant) and 0.437 (not significant) respectively.

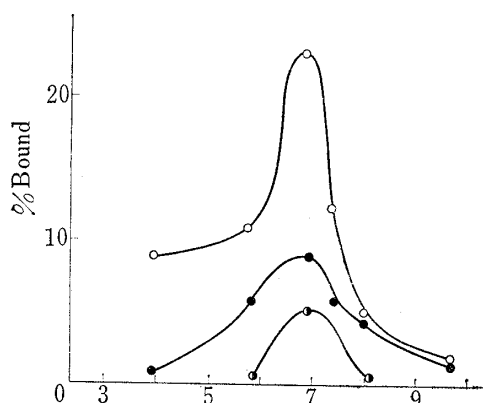


Fig. 6. pH-Binding to Mucosa^{a)}-Profiles of Some Barbituric Acid Derivatives

- a) Equilibrium Dialysis for 72 hr.
Drug Solution $3 \times 10^{-4} M$
Mucosal Suspension 1% (w/v) dry weight
- Thiopental
 - Pentobarbital
 - Hexobarbital

deviation in the absorption characteristics from that at pH 5.5 was observed. These results lead to the suggestion that the absorption of barbituric acid derivatives from rat small intestine is different to some extent in the mediating mechanism from the transport system specialized by pH-partition hypothesis. It was felt from these results that it may be possible to reconcile these phenomena by finding out some known factor participating in the absorption mechanism. Previous investigations of drug absorption have dealt almost exclusively with the rather phenomenal problems and there is little report at present concerning with absorption of drugs from rat small intestine from the two points; effect of pH, and N-methylation which is frequently encountered in drug

6) G. Rendina, E.J. Sarcinone, C.J. Lee, H.W. Barrett: Proc. Soc. Exptl. Biol. & Med., **95**, 350 (1957).

molecules. During the course of searching the more important factor than the ionization state of molecule and lipid solubility, our interests in drug interactions with proteins had led to the suggestion that a kind of interaction or complexation of barbituric acid derivatives to the proteins of absorptive mucosal surface imply more exactly the reality of affinity for the membrane than the partition. A higher concentration of a drug at the membrane surface has an advantage to absorption across the mucosal membrane. In this suggestion, the interacted complex with proteins was considered to be weak in the strength of binding and dissociated easily in favor of the serosal direction. The structural and chemical features of rat small intestine are not yet essentially elucidated, but the existence of protein molecules layer generalized by Danielli is considered to hold true also in the present system. Although this interaction may possibly be a step in absorption from various parts of the gastrointestinal tract, the experimental data of absorption from stomach,¹⁾ colon,^{7,8)} and rectum⁹⁾ indicate this kind of interaction is more influencing in the small intestine. In respect to barbituric acid derivatives, the binding to plasma protein or purified bovine serum albumin has been studied with special interest in the effect on the activity or potentiation by Goldbaum and Smith,⁹⁾ but little experiment has been reported in connection with membrane transport. In our preliminary experiments, as the protein solution, the homogenates (1% w/v dry wt.) of mucosa of rat small intestine prepared by the method of Dickens and Weil-Malherbe was used in the equilibrium dialysis with pentobarbital, hexobarbital and thiopental. The effect of pH on binding of these derivatives was investigated. The result shown in Fig. 6, is similar in shape to the absorption pattern. In addition to this, hexobarbital, a N-methyl derivative, was bound less than the oxy series of derivatives. This finding is also correspond with the order in the absorption from rat small intestine. These findings are assumed to be one of the evidences for the concept that the interaction as expressed by the ability of *in vitro* binding to mucosa is a limiting factor in the absorption of barbituric acid derivatives from rat small intestine. The problem arises from that the preparations of mucosa used in this experiment is crude and structural in nature, so that the binding must be mediated by a complex or overlapped process. However, the tendency of the binding reported here is similar to the results of Goldbaum and Smith with bovine serum albumin. The experiments are proceeding on the extension and confirmation of this suggestion concerning the absorption from rat small intestine.

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7) L.S. Schanker : J. Pharmacol. Exptl. Therap., **126**, 283 (1959).

8) The paper is in preparation for publication, a part of the results was reported at the 86th Annual Meeting of Pharmaceutical Society of Japan in Toyama (April, 1966).

9) L.R. Goldbaum, P.K. Smith : J. Pharmacol. Exptl. Therap., **111**, 197 (1954).