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Absorption and Excretion of Drugs. XXXIV.[1] An Aspect of the Mechanism of Drug Absorption from the Intestinal Tract in Rats[2]

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Some qualitative discrepancies from the pH–partition theory demonstrated with barbituric acid derivatives previously were examined with various types of drugs and the confirmation of the suggestion that the binding process to the mucosal surface is an important factor of the absorption from the small intestine was made. The absorption of the ionized form of drugs was also interpreted by this binding factor. Furthermore, the dispositions of the small intestine were compared with that of the other parts of the gastrointestinal tract. The effect of the N-methyl configuration on the absorption was also found to persist the importance of the binding process.

In the previous papers,[1,4] it was clarified that the absorption of barbituric acids from the rat small intestine is correlated to their abilities to bind with the intestinal mucosa, and some observed discrepacies from pH-partition hypothesis developed by Brodie, *et al.*[5] are interpretable by this binding factor. In this paper, the concept that the binding to the mucosal surface is a determining step in the absorption of drugs from the small intestine was further extended. And whether these phenomena are also demostrated on the other parts of the alimentary tract was investigated to elucidate systematically the specificity of the mechanism of drug absorption.

Experimental

Materials——All drugs listed in Table I and the reagents were obtained from the commercially available sources of analytical grade, and used without further purification. The artificial membrane used in the binding experiments were cut from the cellulose dialyzer tubing (Visking Co., Ltd., 8/32 inch, 0.7 cm diameter).

Animals——Male Wistar rats weighing 130—170 g were used in all the absorption experiments. The intestinal mucosa in the binding experiments were prepared from the duodenum part of fresh rat or bovine small intestine by the method of Dickens and Weil-Malherbe.[7] In some binding experiments, the acetone-powdered mucosa of the rat small intestine was prepared by the method of Sasagawa,[8] and used as the mucosal preparation.

1) Part XXXIII: K. Kakemi, T. Arita, R. Hori, R. Konishi, and K. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 248 (1969).
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3) Location: *a*) *Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto*; *b*) *Nishi-7-chome, Kita-15-jo, Sapporo.*
4) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1883 (1967).
5) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **123**, 81 (1958).
6) L.S. Schanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957).
7) F. Dickens and H. Weil-Malherbe, *Biochem. J.*, **35**, 7 (1941).
8) T. Sasagawa, "Kosokenkyuho," Vol. 1, ed. by S. Akabori, Asakura Publishing Co., Tokyo, 1962, p. 14.

Table I. Drugs investigated and Their pK_a's

	Drugs	pK_a[a]			Drugs	pK_a[a]	
1	Salicylic acid	3.0	13.4[b]	11	*p*-Nitroaniline	1.0	
2	*p*-Hydroxybenzoic acid	4.5[c]	9.3[d]	12	Aminopyrine	5.0	
3	Benzoic acid	4.2		13	Antipyrine	1.4	
4	Thiopental	7.6	12.3[e]	14	*p*-Toluidine	5.3	
5	Barbital	7.8	12.7[f]	15	Ephedrine	9.6	
6	*p*-Hydroxypropiophenone	7.8		16	Xanthine	0.7[g]	9.9[g]
7	Phenol	9.9		17	Theophylline	0.7	8.4[h]
8	5-Nitrosalicylic acid	2.3		18	Theobromine	0.7[g]	10.0[g]
9	Aniline	4.6		19	Caffeine	0.8	
10	*m*-Nitroaniline	2.5		20	Hexacol[i]	7.9[j]	

a) The pK_a values are cited from the papers of Brodie, *et al.*,[5,6] except those for which the references are indicated.
b) A.V. Willi and J.F. Stocker, *Helv. Chim. Acta*, **38**, 1279 (1955)
c) S.G. Vandenberg, C. Henrich, and J.M. Vundennbelt, *Anal. Chem.*, **25**, 726 (1954)
d) B.N. Mattoo, *Trans. Faraday Soc.*, **52**, 1462 (1956)
e) Y. Sato, *Nippon Kagaku Zasshi*, **78**, 921 (1957)
f) J. Fox and D. Shruger, *Bull. Soc. Chim. Belges*, **61**, 44 (1953)
g) J.K. Wood, *J. Chem. Soc.*, **83**, 568 (1903); *idem, ibid.*, **89**, 1831 (1906); *idem, ibid.*, **89**, 1839 (1906)
h) A. Turnner, Jr. and A. Osol, *J. Am. Pharm. Assoc.*, **38**, 158 (1949)
i) A trade name (Fujisawa Pharmaceutical Co.) for 2-allyloxy-4-chloro-N-(2-diethylaminoethyl)benzamide hydrochloride
j) K. Kakemi, H. Sezaki, and S. Horiuchi, *Yakuzaigaku*, **27**, 229 (1967)

Absorption Experiments——The perfusion method employed in the absorption experiments was almost identical with those described in previous papers[4,9] from our laboratory. The amount of the accumulation in the small intestine *in situ* was also determined as previouly.[1]

Determination of the Binding to the Mucosal Preparation——The binding of the drugs to the mucosa of the small intestine was determined by both the equilibrium dialysis and the ultrafiltration method as described in the previous paper.[1]

Determination of the Partition Coefficients of Several Xanthine Derivatives to Chloroform——The partition coefficients of xanthine derivatives were determined by the same method as described in the previous report.[10]

Analytical Methods——The spectrophotometric determination was applied to all the drugs investigated.

A: Aniline, *m*-nitroaniline, *p*-nitroaniline, *p*-toluidine, aminopyrine, 5-nitrosalicylic acid, salicylic acid, benzoic acid, phenol red, and sulfaguanidine——These drugs were anlayzed by the methods described or cited by Brodie, *et al.*[6,11]

B: *p*-Hydroxybenzoic acid, phenol, and *p*-hydroxypropiophenone——One-fifth ml of 1N HCl and 10 ml of chloroform were added to 1 ml aqueous sample of the drug. The mixture was shaken for 25 min and then centrifuged. An 8 ml aliquot of the chloroform layer was shaken with 5 ml of 1N NaOH for 20 min. The optical density of the separated aqueous layer was determined at 280 mμ for *p*-hydroxybenzoic acid, 288 mμ for phenol, and 325 mμ for *p*-hydroxypropiophenone.

C: Caffeine——One-half ml aqueous sample of the drug was shaken with 10 ml chloroform for 30 min, and the separated chloroform layer was directly determined at 276 mμ.

D: Xanthine——One ml aqueous sample of the drug was shaken with 10 ml ethyl acetate for 30 min, and then centrifuged. An 8 ml aliquot of the ethyl acetate layer was shaken with 5 ml of 1N NaOH for 20 min. The separated aqueous layer was determined at 283 mμ.

E: Theophylline and theobromine——One-fifth ml of 1N HCl and 10 ml of chloroform containing 3% (v/v) isoamyl alcohol were added to 1 ml aqueous sample of the drug. The mixture was shaken for 30 min. An 8 ml aliquot of the chloroform layer was then shaken with 5 ml of 1N NaOH for 20 min. The resulting aqueous layer was determined at 275 mμ for both drugs.

F: Hexacol——One ml of 1N NaOH and 5 ml of chloroform were added to 1 ml aqueous sample of the drug. The mixture was shaken for 30 min, and then centrifuged. The chloroform layer was determined at 293 mμ.

9) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **13**, 7 (1965).
10) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1534 (1967).
11) C.A.M. Hogben, D.J. Tocco, B.B. Brodie, and L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **125**, 275 (1959).

G: Ephedrine, antipyrine, and barbituric acid derivatives——Ephedrine was determined by the method described by Aoki, *et al.*[12] Antipyrine was determined by the method of Hahn, *et al.*[13] Barbituric acid derivatives were determined as previously.[10]

Results and Discussions

In order to confirm with various drugs the suggestion that the binding to the mucosa is an important factor in the absorption from the small intestine, the binding of 15 of those listed in Table I was determined at pH 7.2 with 1% (dry weight) mucosal homogenates from the fresh bovine duodenum by the equilibrium dialysis method, and correlated with the absorption rate from the rat small intestine. As a preliminary experiment to compare the rat and bovine mucosa, the pH–binding profiles of salicylic acid and ephedrine were examined, however any significant difference was not observed. The data of the absorption rates used here were cited from the papers of Brodie, *et al.*[4,11] The correlation was shown in Fig. 1. The number beside the point corresponds to that in Table I. The correlation coefficient of this relationship was estimated to be 0.82. The relation of the absorption rates to the partition coefficients was also examined with the data reported by Brodie, *et al.*[4,11] The partition coefficients of drugs at pH 7.2 were calculated from their pK_a values and the coefficients of the respective unionized forms by the equation used in the previous paper.[10] However, the calculated correlation coefficient for this relation was 0.24 and the mutual dependnece between the absorption rate and the partition coefficient seemed to be excluded. These results suggest that the binding to the mucosal surface is also an important factor in the absorption of the wide variety of drugs including acidic, and basic drugs, and therefore the binding properties seem to be useful to the significant prediction of the absorption characteristics. And it was also found that these are in agreement with the previous results obtained with barbituric acid derivatives.[1] Furthermore, the fact that an almost completely ionized form of drug at pH 7.2, for example salicylic acid, was absorbed to a considerable extent, was interpreted preferentially by intervening this binding effect rather than the pH–partition hypothesis, although the binding determined here is not well characterized. In order to confirm these phenomena more precisely, the pH–profiles of the following five drugs were examined on both the absorption rates from the small intestine and the binding to the mucosal preparation, and compared with the molecular states and the partition coefficients. The drugs investigated were salicylic acid, 5-nitrosalicylic acid as the acidic drug, *p*-toluidine, ephedrine as the basic drug, and caffeine as the practically neutral drug. The data obtained with salicylic acid are represented in Fig. 2. The theoretical curve for the unionized fraction is drawn arbitrarily according to the extent of the absorption or the binding. Salicylic acid exists solely as the ionized form at the pH range higher than 5. The absorption still takes place to a considerable extent at these pH values, and the pH–profile of the absorption rate is inconsistent with the partition characteristics which was confirmed to correspond to the theoretical molecular species pattern calculated from pK_a. This observation is not well interpreted by the pH–partition theory. However, as shown in the figure, the binding exhibited an almost identical trend to the absorption. The absorption of salicylic acid has

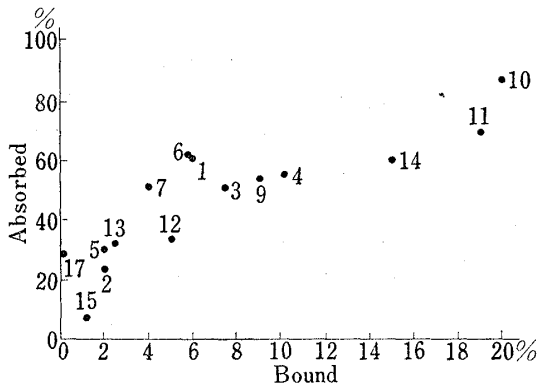

Fig. 1. Relation between Absorption Rates and Binding to Mucosa

12) H. Aoki, Y. Iwayama, and N. Yada, *Yakugaku Zasshi*, **82**, 918 (1961).
13) M. Hahn, J. Kolsek, and M. Perpar, *Z. anal. Chem.*, **151**, 104 (1956).

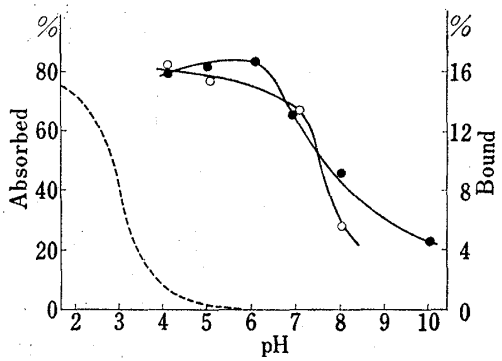

Fig. 2. pH–Profiles of Absorption Rate and Binding to Mucosa (A) Salicylic Acid

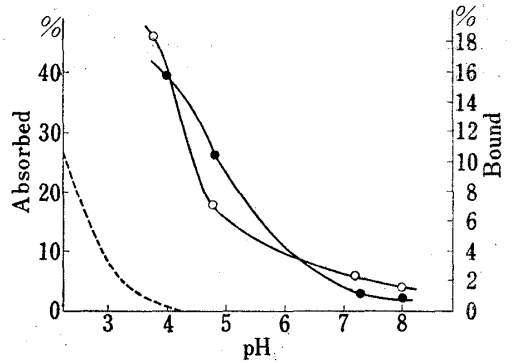

Fig. 3. pH–Profiles of Absorption Rate and Binding to Mucosa (B)[a] 5-Nitrosalicylic Acid

a) The symbols are same as in Fig. 2.

been examined extensively by several investigators,[5,11,14] but the mechanism with which the ionized form is absorbed has not been well elucidated. It may be suggested from these results that the binding process refered in the previous paper is effective also in the absorption of the ionized form. The virtual pH for the small intestine, reported to be 5.3 by Brodie, *et al*,[5] is not useful to the quantitative considerations. The pattern of 5-nitrosalicylic acid is shown in Fig. 3. The absorption of the drug is also irrelevant to the molecular species difference and rather correlative to the binding. In addition to these acidic drugs, the be-

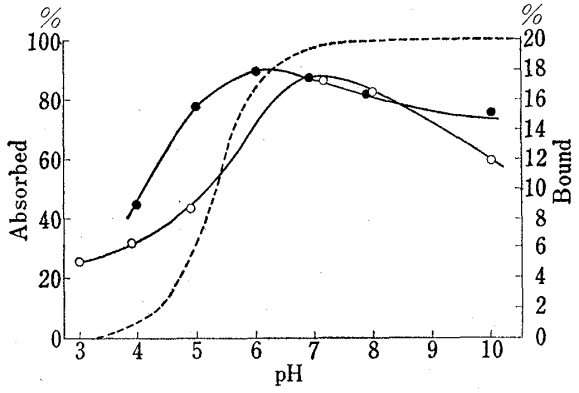

Fig. 4. pH–Profiles of Absorption Rate and Binding to Mucosa (C)[a] *p*-Toluidine

a) The symbols are same as in Fig. 2.

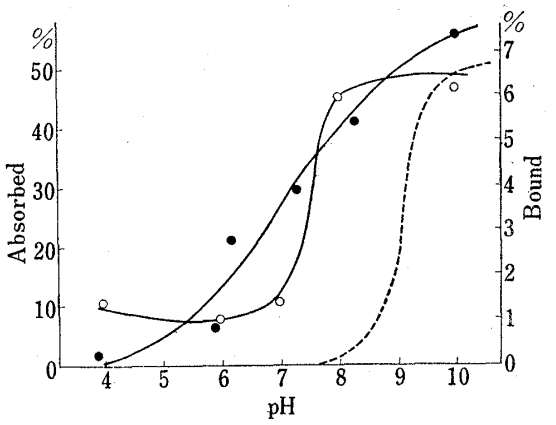

Fig. 5. pH–Profiles of Absorption Rates and Binding to Mucosa (D)[a] Ephedrine

a) The symbols are same as in Fig. 2.

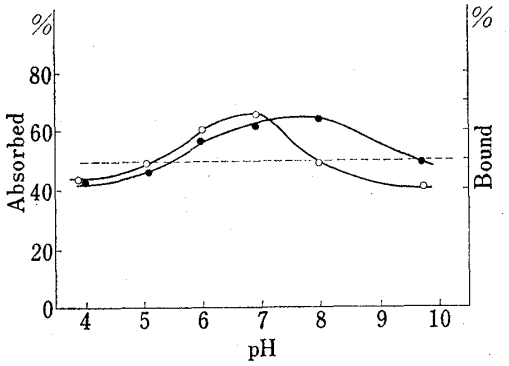

Fig. 6. pH–Profiles of Absorption Rates and Binding to Mucosa (E)[a] Caffeine

a) The symbols are same as in Fig. 2.

haviors of the basic drugs were also examined. The results obtained with *p*-toluidine and ephedrine are shown in Fig. 4 and Fig. 5 respectively. In the case of *p*-toluidine, the significant deviations as demonstrated above were not observed, but the tendency that the absorption and the binding decrease as the pH becomes higher than 7 is contrarily relative to the results with barbituric acid derivatives. Ephedrine was bound and absorbed significantly in the ionized form. As a neutral drug, caffeine which is not ionizable at the physiological pH of intestines, was examined similarly. The results are shown

in Fig. 6. The absorption exhibited an optimal range near pH 7—8, and is correlative to the binding characteristics which had also an optimal pH near 7. From these results, it was suggested that the binding of the drugs to the mucosa is an influencing factor to the absorption from the small intestine of both the ionized and the unionized form, and this is generally applicable to the wide variety of drugs which are different in the chemical structures and in the nature as the electrolytes. The absorption mechanism of the ionized drug molecule can not be precisely to be similar to that of barbituric acid derivatives; that is, the ionized form of drug is adsorbed onto the mucosal surface, and the adsorbed molecule then behaves as an absorbable one, which diffuses rapidly through the membrane of the small intestine into the blood stream. The rapid diffusion through the membrane was confirmed by clarifying that the accumulation in the intestine tissue is small and constant during the perfusion period. The amount of ephedrine accumulated in the whole small intestine was 170 μg at 15 min after the beginning of perfusion, 165 μg at 75 min after the beginning of perfusion, when 40 ml of 100 μg/ml ephedrine solution was perfused. The kinetic treatment revealed apparently that the absorption process of the ionized drug fits a first order kinetics at the conditions employed, so some specific carrier system such as demonstrated by Levine and Pelikan[15] seems not to be included. The specific effect of N-methyl configuration on the absorption was demonstrated with barbituric acid derivatives in the previous papers, and in the present studies, the examination was extended to the basic drugs, xanthine derivatives. The derivatives are xanthine as the non-methylated, theophylline and theobromine as the dimethylated, and caffeine as the trimethylated. The absorption from the rat small intestine, and the binding to the acetone-powdered rat mucosa were determined as usually. The results are summarized in Table II.

Table II. Absorption Rates, Partition Coefficients and Binding to Mucosa of Xanthines

Xanthines	% Absorbed	P.C. ($CHCl_3$)	% Bound
Xanthine	61.3	0.01	18.6
Theophylline	40.6	0.28	9.7
Theobromine	37.6	0.46	8.6
Caffeine	46.6	20.3	9.3

The partition coefficients to chloroform are increased by N-methylation, but the absorption is reduced in spite of this increase in lipid solubility. The degree of binding was found to be a more reliable property to dictate and predict the absorption. These results were found to be in good agreement with those reported with a structurally related drug series, barbituric acid derivatives. From the results hitherto, it appeared of interest to investigate the difference in the absorption by the part of the gastrointestinal tract; it is necessary for the development of drug availability to investigate the specificity or potentiality of the part of the tract. In this field, however, little information is now available. In the previous papers from our laboratory, it was reported that in the case of barbituric acid derivatives, the stomach and the small intestine behaved differently and the specific phenomena inconsiderable from the pH-partition theory seemed to occur only in the small intestine, and with sulfonamides the mechanism of the absorption from the rectum is simply related to the pH-partition theory. To demonstrate clearly the specificity of the small intestine by comparing

14) K. Kakemi, T. Arita, and H. Yamashina, *Yakuzaigaku*, **21**, 97, (1961); *idem*, *ibid.*, **21**, 100 (1961); H. Nogami and T. Matsuzawa, *Chem. Pharm. Bull.* (Tokyo), **9**, 532 (1961); H. Nogami, M. Hanano, and H. Yamada, *Chem. Pharm. Bull.* (Tokyo), **16**, 389 (1968).

15) R.R. Levine and E.W. Pelikan, "Annual Review of Pharmacology," Vol. 4, ed. by W.C. Cutting, Annual Reviews, Inc., Carifornia, 1964, p. 69.

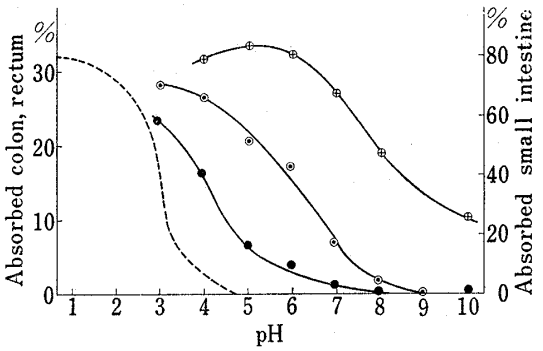

Fig. 7. pH–Profiles of the Absorption Rates from the Small intestine, the Colon and the Rectum (A) Salicylic Acid

—⊕— Absorption rates from the small intestine, the data illustrated are taken from those in Fig. 2.
—⊙— absorption rates from the colon
—●— absorption rates from the rectum
-------- theoretical unionized fraction

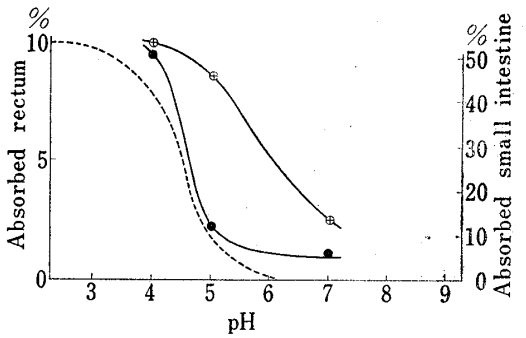

Fig. 8. pH–Profiles of the Absorption Rates from the Small Intestine and the Rectum (B)[a] *p*-Hydroxybenzoic Acid

a) The symbols are same as in Fig. 7.

with the colon and the rectum, four ionizable drugs; two acidic and two basic drugs, were examined on their pH–profiles of the absorption rates from the small intestine, the colon and the rectum. The difference in the effect of N-methyl configuration with the absorption sites was also investigated with some xanthine derivatives. In Fig. 7, the results of salicylic acid are shown. It is apparent that the patterns of the pH–absorption profiles differ from part to part. At the pH range higher than 7 where the drug exists completely as the ionized form, the absorption rates from the small intestine is 24—66%, but from the rectum, the absorption was not observed and furthermore the pH–profile of the rectal absorption had a similar pattern to that of the theoretical unionized fraction. In respect to the absorption of the ionized form of the drug, the small intestine was found to behave specifically. The virtual pH's for these sites are not useful for the interpretation of these observations, because at the virtual pH's which are 5.3,[5] 6.5,[16] 5.4[9] for the small intestine, the colon, and the rectum respectively, salicylic acid exists almost as the ionized form. The other acidic drug, *p*-hydroxybenzoic acid, was similar as shown in Fig. 8. The ionized form of *p*-hydroxybenzoic acid was also absorbed significantly from the small intestine. The behaviors of the basic drugs, ephedrine and Hexacol were similarly examined and the results are represented in Fig. 9, and Fig. 10, respectively. These drugs exhibited the same specificity for the absorp-

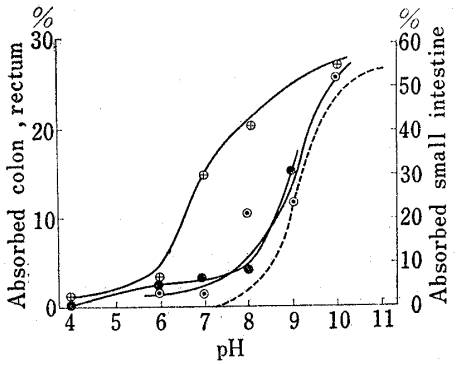

Fig. 9. pH–Profiles of the Absorption Rates from the Small Intestine, the Colon and the Rectum (C)[a] Ephedrine

a) The symbols are same as in Fig. 7.

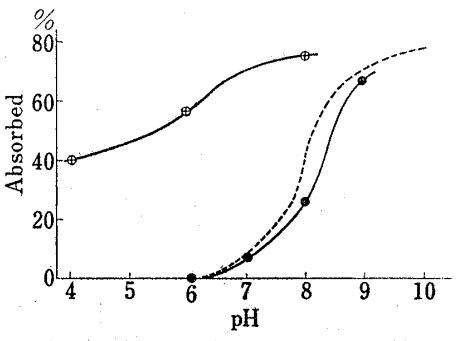

Fig. 10. pH–Profiles of the Absorption Rates from the Small Intestine and the Rectum (D)[a] Hexacol

a) The symbols are same as in Fig. 7.

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

tion sites as the acidic drugs above. From these results, the difference between the small intestine and the rectum is most apparent although the colon is relatively similar to the rectum. The small intestine was found to be specific and potential in respect to the absorption of the ionized drug molecule. These findings are inconsistent with the generalized pH–partition theory of drug absorption, and correlative to the binding process discussed above. Furthermore, in order to approach the problem whether the difference between the small intestine and the rectum was derived from the functional difference of the sites or only of a methodological nature, 5 cm length of the duodenum (from the pylorus) and the ileum (from the cecum) were compared with the equivalent length of the rectum. The results are shown in Fig. 11. The absorbability of the ionized drug and the deviation from the theoretical fraction of the unionized form are apparent especially in the small intestine. The accumulation in the both segments were simultaneously determined, but the amount was negligible. It was concluded that these characteristics are derived from the absorption itself. The comparison of the absorption of xanthine derivatives from the small intestine and the rectum is summarized in Table III.

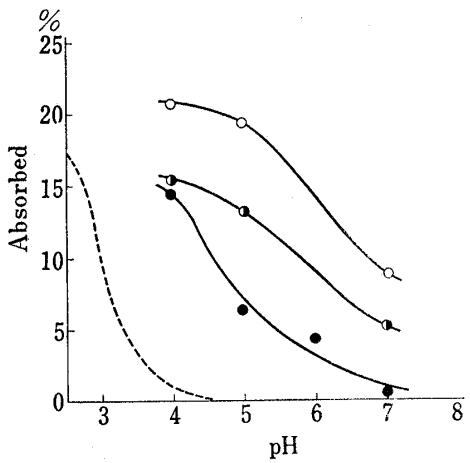

Fig. 11. Site Specificity of the Absorption of Salicylic Acid

—○— absorption rates from the duodenal part of the small intestine (5 cm from the pylorus)
—◑— absorption rates from the ileal part of the small intestine (5 cm from the cecum)
—●— Absorption rates from the rectum, the data are taken from those in Fig. 7.

Table III. Comparison of the Absorption Rates of Xanthines from the Small Intestine and the Rectum

Xanthines	% Absorbed		P.C.
	Small intestine	Rectrum	($CHCl_3$)
Xanthine	61.3	1.3	0.01
Thiobromine	37.6	3.7	0.46
Caffeine	46.6	7.8	20.3

The absorption from the rectum is proportional to the partition coefficients and consistent with the pH-partition theory. The rectal absorption mechanism is simple compared to that from the small intestine. The findings also confirm the results described above with the pH–absorption profiles. The present experiments indicate that the importance of the binding process in the absorption from the small intestine is significant with various types of drugs at both the ionized and unionized states, and the small intestine is specific or imcompatible with the pH–partition theory compared with the other parts of the intestinal tract.

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