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Studies on Absorption and Excretion of Drugs. IX.[1] Relation between Chemical Structure and Absorption Rate. (1). Effects of the Number and the Position of OH-Groups on the Intestinal Absorption Rate of Benzoyl Derivatives

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The aqueous solutions of 24 benzoyl derivatives were perfused through the samll intestine of anesthetized rats.

The absorption rate coefficient calculated from the slope of the straight line which was obtained from the logarithmic plots of the residual ratio *vs.* time was used for comparison of the intestinal absorption rates of these foreign organic compounds.

In each series (benzoic acid–, benzaldehyde–, methyl benzoate–series), the absorption rate decreased as the number of hydroxyl groups increased. When comparing the monohydroxyl derivatives in each series, the *o*–isomer was absorbed faster than *m*– and *p*–isomers, but this was not true of methoxyl derivatives.

From these facts, it is suggested that Danielli's model may be applied to the intestinal absorption of foreign organic compounds.

The intestinal absorption rate is an essential factor for the reasonable design of drugs. In particular, the relationship between chemical structure and the intestinal absorption rate is interesting from the fundamental and the practical points of view.

Studies on the absorption of nutrients from the alimentary tract have been published for a long time; but the absorption of foreign substances is a relatively recent problem subsequent to the discovery of many drugs. There have been some investigations[3–8] on the relationship between chemical structures of foreign organic compounds and their gastrointestinal absorption rates. Brodie, *et al.*[3,4] estimated the virtual pH at the absorption site to be 5.3, which is less than the pH in plasma, 7.4, on the assumption that the intestinal epithelium is completely impermeable to ionized forms, while it is permeable to unionized forms. On the basis of these findings, they proposed the pH–partition hypothesis. Nogami, *et al.*[5,6] investigated the effect of pH in luminal solution on the absorption rate of sulfonamides and interpreted the results on the basis of this hypothesis. Kakemi, *et al.*[7,8] discussed

1) Part VIII: *Chem. Pharm. Bull.* (Tokyo), **15**, 1002 (1967).
2) Location: *a) Hongo, Tokyo; b) Fukushima-ku, Osaka.*
3) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **123**, 81 (1958).
4) C.A.M. Hogben, D.J. Tocco, B.B. Brodie, and L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **125**, 275 (1959).
5) H. Nogami, M. Hanano, and H. Yamada, *Chem. Pharm. Bull.* (Tokyo), **11**, 395 (1963).
6) H. Nogami, M. Hanano, and J. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **12**, 1465 (1964).

the relationship between gastrointestinal absorption rates and oil–water distribution coefficients of the unionized forms of sulfonamides and barbiturates.

In this study an attempt is made in a series of studies to elucidate by the kinetic method the relationship between chemical structure and intestinal absorption rates, especially the effects of substituent groups on intestinal absorption rates. The purposes of this paper are, first, to describe the kinetic method for comparison of intestinal absorption rates varying over a wide range, and, secondly, to discuss the results from perfusion experiments for benzoyl derivatives in terms of microscopic factors such as the intra– and the intermolecular hydrogen bonding capacities. Finally, a model for the intestinal absorption of foreign organic compounds will be discussed.

Experimental

Sample Solution——Twenty–four compounds tested in this study are listed in Tables III to V. The sample solution contained 2 mM of the compound to be tested and 0.9% NaCl. All the sample solutions were adjusted to pH 6.0 by addition of NaOH or HCl.

Experimental Procedure——The recirculating perfusion method based on that of L.S. Schanker, *et al.*[3] was used with a suitable modification. Male rats (body wt. 270±30 g) were fasted for about 24 hr prior to the experiments but water was allowed *ad libitum*. The animals were anesthetized by the subcutaneous injection of 1.25% pentobarbital sodium parenteral solution (0.5 ml/100 g body wt.).

The small intestine was exposed by a midline abdominal incision and cannulated at the immediately distal part and at the 20 cm distal part to the entrance of the bile duct with glass cannulae having inside diameter of 2.5 mm and outside diameter of 3.5 mm. The intestine was replaced in the abdomen, the incision was closed and these cannulae were joined to a perfusion pump.

The small intestine was first cleared of particulate matter by perfusion with 100 ml of 0.9% NaCl solution maintained at 37°. Then the sample solution (50, 100 or 200 ml) maintained at 37° was perfused by recirculation from the proximal to the distal at a rate of 20 ml per min.

After recirculation for the time previously programmed, the sample solution was recovered and the intestinal lumen was thoroughly washed with 100 ml of 0.9% NaCl solution. The remaining drug was completely collected in a measuring flask and measured.

The experiments for each sample solution were repeated three times or more with alteration in the perfusion period.

Analytical Methods

Assay for the sample for which the volume of the perfusion solution was 50 ml was carried out colorimetrically. The sample for which a larger volume of solution (100 ml or 200 ml) was perfused could be assayed by UV absorption, since the blank value was very small.

a) Salicylic acid and protocatechuic acid: The sample solution (50 ml) and washings were transferred into a 250 ml–measuring flask and the volume was made up to 250 ml with distilled water. Ten milliliters of this solution in the case of salicylic acid or 15 ml of the solution in the case of protocatechuic acid were pipetted into a 20 ml–measuring flask and 2 ml of 0.1% Fe $(NO_3)_3$ in 0.07 N HNO_3 was added. The volume was made up to 20 ml with distilled water and the optical density was read on a spectrophotometer (Hitachi Co., Ltd. model EPU–2) at 530 mμ for salicylic acid or at 670 mμ for protocatechuic acid.

b) *m*–Hydroxybenzoic acid, *p*–hydroxybenzoic acid, *β*–resorcylic acid and *α*–resorcylic acid: The method was essentially the same as that described by Pfeifer, *et al.*[9] The sample solution (50 ml) and washings were transferred into a 250 ml–measuring flask and the volume was made up to 250 ml with distilled water. Two milliliters of this solution were pipetted into a 20 ml–measuring flask and 10 ml of M/15 Na_2HPO_4, 4 drops of 2% 4–aminoantipyrine and 2 ml of 1% $K_3Fe(CN)_6$ were added. The volume was made up to 20 ml with M/15 Na_2HPO_4 and the optical density was read on a spectrophotometer at 488 mμ for *m*–hydroxybenzoic acid, at 510 mμ for *p*–hydroxybenzoic acid, at 540 mμ for *β*–resorcylic acid or at 550 mμ for *α*–resorcylic acid.

c) *o*–Pyrocatechuic acid and *γ*–resorcylic acid: The sample solution (50 ml) and washings were transferred into a 250 ml–measuring flask and the volume was made up to 250 ml with distilled water. Two milliliters of this solution were pipetted into a 20 ml–measuring flask and 2 ml N NaOH and 0.7 ml of Folin–Ciocalteu's phenol reagent[10] were added. The volume was made up to 20 ml with distilled water and

7) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964).
8) K. Kakemi, T. Arita, R. Hori, and R. Konishi, The 83rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, November, 1963 (Abstract p. 173).
9) S. Pfeifer and O. Manus, *Pharmazie*, **12**, 402 (1957).
10) O. Folin and V. Ciocalteu, *J. Biol. Chem.*, **73**, 627 (1927).

the optical density was read on a spectrophotometer at 750 mμ for *o*-pyrocatechuic acid or at 745 mμ for *γ*-resorcylic acid.

d) Gentisic acid: The sample solution (50 ml) and washings were transferred into a 250 ml-measuring flask and the volume was made up to 250 ml with distilled water. Two milliliters of this solution were pipetted into a 20 ml-measuring flask and 2 ml N HCl and 0.7 ml of Folin-Ciocalteu's phenol reagent were added.[10] The volume was made up to 20 ml with distilled water. After incubation at 37° for 10 min, the optical density was read on a spectrophotometer at 920 mμ.

e) Other samples: Two hundred milliliters of the sample solution and washings were transferred into a 1 liter-measuring flask and the volume was made up to 1 liter with distilled water. Only for benzoic acid, 100 ml of the sample solution were perfused and collected in a 500 ml-measuring flask. The volume was made up to 500 ml with distilled water.

These recovered sample solutions were diluted with 0.1 N HCl and optical densities measured at 250 mμ for benzaldehyde, at 322 mμ for salicylaldehyde, at 313 mμ for *m*-hydroxybenzaldehyde, at 284 mμ for *p*-hydroxybenzaldehyde, at 310 mμ for protocatechualdehyde, at 278 mμ for *β*-resorcylaldehyde, at 323 mμ for *o*-methoxybenzaldehyde, at 283 mμ for anisaldehyde, at 230 mμ for methyl benzoate, at 302 mμ for methyl salicylate, at 296 mμ for methyl *m*-hydroxybenzoate, at 255 mμ for methyl *p*-hydroxybenzoate, at 230 mμ for benzoic acid, at 295 mμ for *o*-methoxybenzoic acid and at 258 mμ for anisic acid.

Results and Discussion

In our previous paper,[5] the volume change of the sample solution during the perfusion experiment was negligible and the linearity of the logarithmic plot of the residual ratio against perfusion time was found, so that the absorption rates were compared by means of the first-order rate coefficients obtained by using equation (1) in the period during which the drug concentration in the biophase might be negligible.

$$\log\left(\frac{D}{D^0}\right)=-0.434\frac{kt}{V}=-k't \qquad (1)$$

In the equation, t is the perfusion time, D^0 and D are the drug amounts in the solution at $t=0$ and at $t=$t, k and k' are constants and V is the volume of the perfusion solution. Also, in this study, from the results of the preliminary test, it was found that the volume changes during the perfusion experiment were negligible (less than 5%) and that the logarithmic plots of the residual ratio *vs.* time gave straight lines (for example, as shown in Fig. 1), so that the mathematical relation shown in equation (1) could be applied. In equation (1), k' can be obtained as the slope of the straight line from the logarithmic plot of the residual ratio against time and k can be calculated by using equation (2).

$$k=2.303\times V\times k' \qquad (2)$$

The experiments with different volumes (50, 100 and 200 ml) of the perfusion solution were designed in the present study, because the absorption rates of the compounds studied here varied over a wide range. Therefore, a quantity for the comparison of the absorption rates in these experiments with the different volumes of the solution was required. Equation (1) indicates that k may be suitable for this requirement. For the experimental confirmation of this point, by using 50 ml and 200 ml of salicylic acid solution the perfusion experiments were carried out.

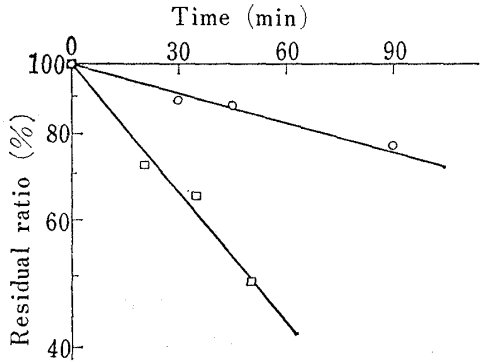


Fig. 1. Logarithmic Plot of Residual Ratio against Time

○ : 200 ml of Salicylic Acid Solution
□ : 50 ml of Salicylic Acid Solution

As shown in Fig. 1, the logarithmic plots of the residual ratio against time gave straight lines. The slopes were obtained by the method of least squares and the values of k were calculated by equation

(2). Also, for *m*-hydroxybenzaldehyde, similar experiments using 100 ml and 200 ml of the perfusion solution were carried out. These values are shown in Table I.

Table I. Independency of *k*-Value on Volume of Perfusion Solution

Compound	Salicylic acid		*m*-Hydroxybenzaldehyde	
V (ml)	50	200	100	200
k'	0.60×10^{-2}	0.14×10^{-2}	0.37×10^{-2}	0.19×10^{-2}
k	0.69	0.63	0.86	0.88

From this table, it appears that k' is dependent and k is independent of the volume of the perfusion solution. These results indicate that k can be used for comparison of the data from the experiments using different volumes.

For each compound tested in this study, the consistency of the ultraviolet absorption curve in the perfusion solution after the experiment with that in the initial solution was experimentally confirmed, so that it was impossible that the compound might have been decomposed in the intestinal lumen. When the sample solutions were perfused with the same apparatus through a 20 cm-glass tube used in place of the intestine, the recoveries were 98% or more. The facts indicate that the drug concentration decreases only in the intestinal part of the perfusion route. Furthermore, for some different types of drugs it was confirmed that the amount of the drug adsorbed on the intestinal mucosal surface, after the washing out of the sample solution with saline at the end of the perfusion experiment, was quite negligible.[11,12] From the results of these preliminary tests, the amount of the drug lost from the sample solution during the perfusion experiment can be regarded as the amount absorbed from the intestine. Therefore, k is called the absorption rate coefficient in this study.

In order to know the variability of the absorption rate coefficient, investigations to obtain a *k*-value from 3 experiments were repeated 4 times in the case of methyl salicylate and 2 times in the cases of β-resorcylaldehyde and methyl *m*-hydroxybenzoate.

Table II. Reproducibility of *k*-Value

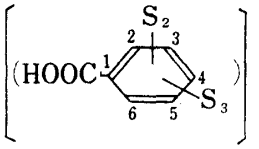
Compound	Methyl salicylate	β-Resorcylaldehyde	Methyl *m*-hydroxybenzoate
Observed value	1.43 1.43	1.06	1.02
	1.40 1.40	1.08	0.80
Mean value	1.42	1.07	0.91
Mean deviation	±0.02	±0.01	±0.11

The results, as shown in Table II, indicate that the reproducibility of the *k*-value is good. The comparative method by means of the *k*-value does not include such complex factors as metabolism and excretion of drugs, so that it may be useful for comparison of the intestinal absorption rate itself. In this case, the term "absorption" means "transfer from the luminal solution into the biophase."

The absorption rate coefficients obtained in this study are shown in Table III to V.

The results for benzoic acid and its hydroxyl derivatives listed in Table III, with the exception of protocatechuic acid, indicate that the absorption rate decreases as the number of hydroxyl groups increases. This exception will be discussed later. According to

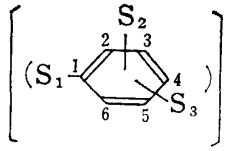
11) H. Yamada and R. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **13**, 1279 (1965).
12) H. Yamada, T. Ichihashi, F. Kogishi, and R. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **14**, 786 (1966).

Table III. Absorption Rate Coefficients 

No.	Compounds	Substituent groups S_2[a]	Substituent groups S_3[a]	pK_a[b]	Absorption rate Coefficients k
1	Benzoic acid			4.2	0.92
2	Salicylic acid	–OH(2)		3.0	0.66[c] {0.69; 0.63[d]}
3	*m*–Hydroxybenzoic acid	–OH(3)		4.1	0.10
4	*p*–Hydroxybenzoic acid	–OH(4)		4.6	0.19
5	*o*–Pyrocatechuic acid	–OH(2)	–OH(3)	2.0	0.09
6	*β*–Resorcylic acid	–OH(2)	–OH(4)	3.2	0.07
7	Gentisic acid	–OH(2)	–OH(5)	3.0	0.01
8	*γ*–Resorcylic acid	–OH(2)	–OH(6)	1.4	0.01
9	Protocatechuic acid	–OH(3)	–OH(4)	4.5	0.29
10	*α*–Resorcylic acid	–OH(3)	–OH(5)	4.0	0.03

a) The position is shown in the parentheses.
b) These values were obtained from Landoldt-Börnstein: "Zahlenwerte und Funktionen aus Physik, Chemie, Astronomie, Geophysik, Technik," Springer Verlag, II Band, 7 Teil, 1960.
c) This is a mean of two values in the brace.
d) Only this value was obtained from the experiments using 200 ml of the perfusion solution and all others were obtained from the experiments using 50 ml.

Schanker,[13] the intestinal absorption rate of weak acids increases as the pK_a; but in Table III salicylic acid, having a hydroxyl group in the *o*–position, is absorbed faster than *m*– and *p*–hydroxybenzoic acids although the pK_a of the *o*–isomer (3.0) is lower than those of the isomers (4.1 for *m*–isomer and 4.6 for *p*–isomer). This fact suggests that the unionized form of the *o*–isomer is absorbed much faster than those of the *m*– and *p*–isomers. Therefore, it seems that the number and the position of the substituent groups are important factors affecting the absorption rate of the organic compounds.

Table IV. Absorption Rate Coefficients 

No.	Compounds	Substituent groups S_1	Substituent groups S_2[a]	Substituent groups S_3[a]	Absorption rate coefficients k
11	Benzaldehyde	–CHO			1.22
12	Salicylaldehyde	–CHO	–OH(2)		1.16
13	*m*–Hydroxybenzaldehyde	–CHO	–OH(3)		0.87[b] {0.88; 0.86[c]}
14	*p*–Hydroxybenzaldehyde	–CHO	–OH(4)		0.94
15	*β*–Resorcylaldehyde	–CHO	–OH(2)	–OH(4)	1.07[b] {1.06; 1.08}
16	Protocatechualdehyde	–CHO	–OH(3)	–OH(4)	0.91
17	Methyl benzoate	$-COOCH_3$			1.43
18	Methyl salicylate	$-COOCH_3$	–OH(2)		1.42[b] {1.43; 1.43; 1.40; 1.40}
19	Methyl *m*–hydroxybenzoate	$-COOCH_3$	–OH(3)		0.91[b] {1.02; 0.80}
20	Methyl *p*–hydroxybenzoate	$-COOCH_3$	–OH(4)		1.03

a) The position is shown in the parentheses.
b) This is a mean of the values in the brace.
c) Only this value was obtained from the experiments using 100 ml of the perfusion solution and all others were obtained from the experiments using 200 ml.

13) L.S. Schanker, *J. Med. Pharmacol. Chem.*, **2**, 343 (1960).

In order to discuss the effects of substituent groups in more detail, benzoyl derivatives without contribution of the ionized form at the virtual pH in the intestinal lumen were used for further experiments. The results are shown in Table IV.

From the estimations of pK_a values for these compounds with a potentiograph (Metrohm A.G. Model-E336), it was confirmed that the contribution of the ionic form of each compound might be negligible at the virtual pH in the absorption site. In each series (aldehyde- or methyl ester series), monohydroxy derivatives are absorbed more slowly than the mother compound; and, when comparing the monohydroxy derivatives in each series, the *o*-isomer is absorbed faster than the *m*- and *p*-isomers.

It is well known that there are large discrepancies in the physical properties between the *o*-hydroxybenzoyl derivative and its *m*- and *p*-isomers in each series.[14] The cause of the phenomena may be that the phenol hydrogens in the *m*- and *p*-isomers give intermolecular interactions, while that in the *o*-isomer may have the tendency toward internal satisfaction of hydrogen bonding capacity, so that the intermolecular interactions may be diminished.[14] It is considered that in an aqueous solution the degree of hydration of the OH group with the intramolecular H-bonding capacity is smaller than that of the OH group without it.[15]

In the field of histology, the structure of the columnar cell, which covers the mucosal surface of the intestine, has been investigated by electron microscopy.[16,17] The luminal border of the cell is lined with microvilli; and the surface of the microvilli are covered by a plasma membrane. It is considered that adjacent epithelial cells cohere tightly. The tight junction between cells appears to function as a diffusion barrier or "seal";[18] thus drug molecules may be absorbed through the plasma membrane. The ultra-structure of biological tissue remains obscure, so that the circumstances surrounding the absorbed drug molecule in the biophase are still unknown. However, it is reasonable to assume that the interaction between the absorbed drug molecule and the surroundings in the biophase may be considerably different from the solute-solvent interaction in the luminal solution. Therefore, it is considered that the interaction of the transferring molecule with water may be broken and a new interaction with the components of the biophase may be developed.

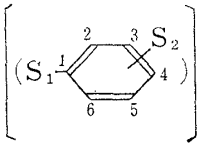
Danielli[19] proposed the theory of penetration of a thin biological membrane; namely, the activated diffusion model. The mechanism on which his theory is based does not require aqueous pores in the membrane, but involves provision of energy sufficient for the diffusing molecule to jump over the energy barrier between the aqueous solution and the membrane.

The experimental results obtained here can be qualitatively interpreted on the basis of Danielli's activated diffusion model as follows: With the number of OH-groups, the power of interaction of the solute molecule with the surrounding water increases, so that the energy sufficient to break this interaction and to transfer into the biophase may increase. Thus, the more OH-groups, the more slowly the drug molecule transfers from the luminal solution to the biophase. The solute-water interaction of the compound has a great tendency to form the intramolecular hydrogen bond and is not so large that the *o*-isomer may be absorbed faster than the *m*- and *p*-isomers. Protocatechuic acid which was previously mentioned has a larger *k*-value than other dihydroxybenzoic acids. It is possible to suppose that not only owing to the higher pK_a-value (4.5) but also owing to the intramolecular interaction between two hydroxyl groups, this compound is absorbed faster than the isomers. Also, for *β*-

14) G.C. Pimentel and A.L. McClellan, "The Hydrogen Bond," W.H. Freeman & Co., San Francisco & London, 1960, p. 167.
15) S. Seki and K. Suzuki, *Bull. Chem. Soc. Japan*, **26**, 63 (1953).
16) T.H. Wilson, "Intestinal Absorption," W.B. Saunders Co., 1962, p. 2.
17) W. Bloom and D.W. Fawcett, "A Textbook of Histology," Chapter 2, W.B. Saunders Co., 1962.
18) M.G. Farquhar and G.E. Palade, *J. Cell. Biol.*, **17**, 375 (1963).
19) H. Davson and J.F. Danielli, "The Permeability of Natural Membranes," Cambridge, 1952, p. 324.

resorcylaldehyde and protocatechualdehyde, the results can be interpreted in terms of intramolecular interaction.

If this consideration is valid, in the case of methoxyl derivatives, which have no intramolecular hydrogen bonding tendency, the *o*-isomer may not be absorbed faster than the *m*- and *p*-isomers. The results of the experiments which were carried out in order to confirm this point are shown in Table V. These results support this view.

Table V. Absorption Rate Coefficients 

No.	Compounds	Substituent groups		pK_a[b]	Absorption rate coefficients k
		S_1	S_2[a]		
21	*o*-Methoxybenzoic acid	-COOH	$-OCH_3$(2)	4.1	0.56
22	Anisic acid	-COOH	$-OCH_3$(4)	4.5	0.78
23	*o*-Methoxybenzaldehyde	-CHO	$-OCH_3$(2)		0.96
24	Anisaldehyde	-CHO	$-OCH_3$(4)		1.22

a) The position is shown in the parentheses.
b) These values were obtained from Landoldt-Börnstein: "Zahlenwerte und Funktionen aus Physik, Chemie, Astronomie, Geophysik, Technik," Springer Verlag, II Band, 7 Teil (1960).

From the above consideration it appears that the activated diffusion model, which was developed for the penetration of single cell membranes, may be applied to the intestinal absorption of foreign organic compounds.

The mathematical relationship between the chemical structure and intestinal absorption rate will be described in the following papers.[20,21]

20) H. Nogami, M. Hanano, and H. Yamada, *Chem. Pharm. Bull.* (Tokyo), in press.
21) H. Nogami, M. Hanano, and H. Yamada, *Chem. Pharm. Bull.* (Tokyo), in press.