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Absorption of benzene derivatives through the body surface and gill membranes of goldfish: substituent and intramolecular interaction effects

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Summary

The substituent effects on the absorption rate constants (k_{fish}) of 35 benzene derivatives at pH 6.0 through the body surface and gill membranes of the goldfish were examined in terms of the substituent constant (K_s) and the intramolecular interaction constant (K_I), and the substituent effects were compared with those reported for the rat intestinal absorption rate constants (k_{rat}) at pH 6.0. The substituent groups giving positive or negative K_s values were the same for the two goldfish membranes and the rat intestine. All the K_I values were positive for the two goldfish membranes as well as for the rat intestine. The logarithmic values of k_{rat} showed good correlations with those of k_{fish} in both body surface ($r = 0.870, n = 34$) and gills ($r = 0.860$, $n = 35$). However, the k_{fish} values of salicylic acid, *p*-hydroxybenzamide, and phenol in the body surface and those of the former two in the gills were significantly outside the critical region ($P < 0.05$). The effects of substituent groups (increase or decrease) and intramolecular interaction (increase) on the k_{fish} values were qualitatively the same as those on the k_{rat} values and quantitatively correlated well with those on the k_{rat} values, except in the cases of salicylic acid, phenol, and *p*-hydroxybenzamide. It was concluded that the substituent effects on the k_{fish} values can be explained in terms of K_s and K_I, as in the case of rat intestine, but the effects on the k_{fish} values of salicylic acid, phenol, and *p*-hydroxybenzamide can not.

Introduction

Many investigators have pointed out that goldfish are useful as test animals for study on drug absorption (Levy et al., 1964a and b, 1965, 1966, 1968), pharmacologic effects of drugs (Gibardi et al., 1968), and drug absorption kinetics (Nightingale et al., 1971; Yalkowsky et al., 1973). Previously, we reported that the drug absorption membranes of goldfish are similar to those of mammalian species, which allow preferential passage of drugs in lipid-soluble, non-ionized form (Sakiya et al., 1974, 1975, 1976), and the aqueous diffusion layers on the gills of the goldfish and the surface of the mammalian gastrointestinal tract show a similar contribution to drug absorption (Sakiya et al., 1979). Nogami et al. (1968a–c) reported that the rat intestinal absorption rate constant (k_{rat}) values of benzene derivatives at pH 6.0 are more affected by the nature and position of the substituent groups than by the lipophilicity of the

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compounds, and can be interpreted in terms of substituent constant (K_s) and intramolecular interaction constant (K_I). However, substituent effects on the absorption rate constant (k_{fish}) values in goldfish have not been clarified yet.

In the present study, we examined the substituent effects on the k_{fish} values of benzene derivatives through the body surface and gill membranes of goldfish and compared the substituent effects on k_{fish} and k_{rat} in terms of the K_s and K_I values calculated by the same method as employed in the case of rat intestine.

Materials and Methods

Materials and sample solutions

35 benzene derivatives used for the absorption study are listed in Table 1. All the compounds were of analytical grade and were obtained com-

TABLE 1

Sample concentrations and apparent partition coefficients between benzene and buffer solution of benzene derivatives at pH 6.0

No.	Compound	Sample solution conc. (mM)	Apparent partition coefficient [a]	Wave length (nm) [b]
1	Benzoic acid	1.0	0.0137	298
2	Salicylic acid	1.0	0.0101	224
3	*o*-Methoxybenzoic acid	0.5	0.0871	296
4	*p*-Methoxybenzoic acid	0.5	0.0435	258
5	Phenol	5.0	2.873	270
6	Pyrocatechol	1.0	0.0621	276
7	Nitrobenzene	1.0	121.4	268
8	*o*-Nitrophenol	1.0	47.31	278
9	*m*-Nitrophenol	1.0	2.739	252
10	*p*-Nitrophenol	1.0	1.344	316
11	*o*-Hydroxybenzamide	1.0	1.119	299
12	*p*-Hydroxybenzamide	1.0	0.152	253
13	Methyl benzoate	1.0	28.64	232
14	Methyl salicylate	0.5	26.94	303
15	Methyl *m*-hydroxybenzoate	1.0	4.941	238
16	Methyl *p*-hydroxybenzoate	1.0	3.087	256
17	Ethyl *p*-hydroxybenzoate	1.0	12.60	257
18	Ethyl protocatechuate	1.0	0.362	293
19	*m*-Ethoxyphenol	1.0	0.155	274
20	Benzaldehyde	1.0	0.654	250
21	Salicylaldehyde	1.0	0.617	257
22	*m*-Hydroxybenzaldehyde	1.0	0.601	254
23	*p*-Hydroxybenzaldehyde	1.0	0.496	284
24	*o*-Anisaldehyde	1.0	11.93	322
25	*p*-Anisaldehyde	1.0	6.890	285
26	2,4-Dihydroxybenzaldehyde	1.0	0.756	278
27	*p*-Acetaminobenzaldehyde	1.0	1.445	295
28	*o*-Nitroaniline	1.0	69.50	283
29	*m*-Nitroaniline	1.0	21.48	258
30	*p*-Nitroaniline	1.0	8.211	252
31	*p*-Nitrobenzamide	1.0	0.348	266
32	2-Nitro-p-cresol	1.0	4.430	286
33	Benzocaine	0.1	8.718	285
34	Phenacetin	0.5	6.667	243
35	*p*-Acetaminophenol	1.0	0.0065	242

[a] At 25 °C. For details, see the text.

[b] The wavelength used for the determination of compounds.

mercially. Benzene was distilled at 80–81° C before use. All other reagents were commercial products of analytical grade. Each compound was dissolved in purified water at the desired concentration (Table 1). The sample solution was adjusted to pH 6.0 by using diluted HCl or diluted NaOH solution.

Animal experiments

Goldfish (*Carrassius auratus*), weighing 13 ± 2 g, were used.

Absorption through the gills only. A single fish with the body enclosed in a rubber bag was placed in a 200 ml flask containing 70 ml of sample solution. The solution was maintained at 25° C in a thermostatic bath during the study and the pH of the solution was also maintained by the addition of diluted HCl or diluted NaOH solution as necessary. A pH meter was used to check the pH of the solution, which was agitated with a stirrer during the study. A time point 10 s after addition of the fish was taken as 0 time, and 1 ml aliquots of the solution in the flask were taken at 0, 2, 4, 6, 8, 10, 20, and 30 min.

Absorption through the body surface only. One end of a piece of polyvinyl tubing was inserted into the mouth of the fish and the tube was tied with a thread. The fish with the tube inserted was suspended from a suitable hanger so that all the body surface (the parts under the gills) was immersed in the sample solution (70 ml in a 200 ml flask), while the gills were not in contact with the solution. The absorption study was then carried out by the same method as in the case of the gills described above.

Analytical methods

All the compounds were determined by measuring the UV absorption of the sample solution diluted with a suitable volume of 0.1 N HCl solution. The wavelength (nm) used for the measurement of each compound is listed in Table 1. The blank reference solution for UV absorption measurement was prepared as follows: a fish with the body surface or gills exposed was placed in purified water of the same volume and pH as the sample solution. Then the water was taken and diluted as described for the absorption study. UV absorption was measured with a Hitachi 200-20 spectrophotometer.

Apparent partition coefficient between benzene and the buffer solution

As the water layer we used a phosphate buffer solution containing the compound at the same concentration and at the same pH as the sample solution of each compound. The buffer solution at pH 6.0 was prepared from 15 M Na_2HPO_4 and 15 M KH_2PO_4. Aliquots of 10 ml of the buffer solution containing the compound and 10 ml of benzene were shaken vigorously together for 24 h at 25° C in a thermostated bath. The concentrations of the compound in the aqueous and benzene layers were determined by UV absorption measurement. The apparent partition coefficient was calculated as the concentration ratio between the two layers (benzene/buffer). In order to examine the dimerization of each compound in benzene, apparent partition coefficients were also determined by using the buffer solution containing the compound at lower concentrations (0.3–0.8 times) than those of each sample solution.

Calculation of the absorption rate constant (k_{fish}) at the body surface and gill membranes

The k_{fish} values for all the compounds were calculated by the same method as reported previously (Sakiya et al., 1974), since the assumption of a first-order absorption rate also appeared to be valid for all the compounds used in this study. All data are given as the means with the standard error (mean ± S.E.M.).

Calculation of K_s and K_I

K_s and K were calculated by the same methods as those used in the rat intestine (Nogami et al., 1968b and c), respectively, i.e.,

$$\log(k_{RS}/k_{RH}) = K_s \tag{1}$$

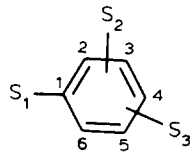
where k_{RS} and k_{RH} are the absorption rate constants of the compound (RS) having the substituent group (S) and the mother compound (RH), respectively.

$$\log(k_{RS(i)\cdots S(j)}/k_{RS(i)S(j)calc}) = K_I \tag{2}$$

where $k_{RS(i)\cdots S(j)}$ and $k_{RS(i)S(j)calc}$) denote the ab-

TABLE 2

The absorption rate constants of benzene derivatives at pH 6.0 and 25 °C through the body surface and gill membranes of goldfish

No. [a]				Absorption rate constant ($min^{-1}\ g^{-1}$) $\times 10^4$			
	Substituent group [b]			Body surface		Gills	
	S_1	S_2	S_3	Mean	S.E.M.	Mean	S.E.M.
1	-COOH			0.515	0.103	2.171	0.360
2	-COOH	-OH(2)		0.135	0.029	0.187	0.019
3	-COOH	$-OCH_3$(2)		0.163	0.033	0.437	0.062
4	-COOH	$-OCH_3$(4)		1.139	0.016	2.255	0.263
5	-OH			1.031	0.049	1.491	0.230
6	-OH	-OH(2)		0.569	0.065	0.584	0.066
7	$-NO_2$			5.897	0.351	7.233	0.750
8	$-NO_2$	-OH(2)		4.393	0.389	8.733	0.991
9	$-NO_2$	-OH(3)		1.581	0.084	2.567	0.494
10	$-NO_2$	-OH(4)		2.602	0.242	2.714	0.491
11	$-CONH_2$	-OH(2)		0.602	0.054	1.028	0.063
12	$-CONH_2$	-OH(4)		- [c]		0.367	0.029
13	$-COOCH_3$			9.394	0.373	9.965	0.548
14	$-COOCH_3$	-OH(2)		9.020	0.244	12.402	0.803
15	$-COOCH_3$	-OH(3)		2.059	0.321	2.799	0.244
16	$-COOCH_3$	-OH(4)		2.659	0.279	3.380	0.430
17	$-COOC_2H_5$	-OH(4)		3.398	0.281	3.724	0.437
18	$-COOC_2H_5$	-OH(3)	-OH(4)	0.883	0.076	1.315	0.198
19	-OH	$-OC_2H_5$(3)		1.713	0.078	2.227	0.142
20	-CHO			11.731	1.531	17.743	0.703
21	-CHO	-OH(2)		6.105	0.613	7.872	0.585
22	-CHO	-OH(3)		1.371	0.263	3.164	0.368
23	-CHO	-OH(4)		1.494	0.174	3.687	0.337
24	-CHO	$-OCH_3$(2)		3.376	0.132	3.889	0.458
25	-CHO	$-OCH_3$(4)		4.200	0.497	6.397	0.560
26	-CHO	-OH(2)	-OH(4)	2.467	0.128	2.622	0.243
27	-CHO	$-NHCOCH_3$(4)		1.871	0.132	3.889	0.458
28	$-NH_2$	$-NO_2$(2)		3.169	0.123	3.416	0.396
29	$-NH_2$	$-NO_2$(3)		1.570	0.286	1.577	0.234
30	$-NH_2$	$-NO_2$(4)		1.592	0.279	2.119	0.289
31	$-CONH_2$	$-NO_2$(4)		1.353	0.136	3.016	0.374
32	-OH	$-NO_2$(2)	$-CH_3$(4)	5.070	0.196	8.958	0.543
33	$-COOC_2H_5$	$-NH_2$(4)		2.432	0.248	2.812	0.302
34	$-NHCOCH_3$	$-OC_2H_5$(4)		0.994	0.066	2.141	0.313
35	-OH	$-NHCOCH_3$(4)		0.125	0.087	0.288	0.022

Number of experiments is 6 or 7 in each case.

[a] Compound numbers are those given in Table 1.

[b] The position is shown in parentheses.

[c] No absorption detectable within 1 h.

sorption rate constants of compounds with and without intramolecular hydrogen bonding, respectively. $k_{RS(i)S(j)calc}$ was estimated from Eqn. 3,

$$\log k_{RS(i)S(j)calc} = \log k_{\phi H} + \sum K_s \quad (3)$$

where $k_{\phi H}$ is the absorption rate constant of benzene estimated from Eqn. 4 and ΣK_s is the sum of the K_s values of the substituents S_i and S_j.

$$\log k_{\phi H} = \log k_{\phi S} - K_s \quad (4)$$

where $k_{\phi S}$ denotes the absorption rate constant of monosubstituted benzene. For the absorption rate constants in Eqns. 1, 2, and 4, the mean values (Table 2) were used. For log $k_{\phi H}$ in Eq. 3, and K_s in Eqns. 3 and 4, the mean values of log $k_{\phi H}$ (Table 4) and K_s (Table 3) were used, respectively.

Results and Discussion

The k_{fish} values

The k_{fish} values of benzene derivatives at pH 6.0 through the body surface and gills of the goldfish are listed in Table 2. The compound numbers correspond to those in Table 1. The k_{fish} values were larger (ca. 1.01–4.2 fold) in the gills than in the body surface for all the compounds tested, supporting our previous suggestion that drugs may be mainly absorbed through the gill membrane (Sakiya et al., 1975). Absorptions of *o*-pyrocatechuic, resorcylic, gentisic, and protocatechuic acids, and also resorcinol and hydroquinone (not listed in Table 1) at pH 6.0 through the body surface and gills were undetectable within 1 h, though these compounds were absorbed from the rat intestinal tract (Nogami et al., 1968a and b).

Relation between the k_{fish} values and the lipophilicity of the compounds

The lipophilicity of the compounds was expressed in terms of the apparent partition coefficient between benzene and the buffer solution (Table 1), because benzene gave suitable apparent partition coefficient values for comparing the lipophilicity of the test compounds, as compared with other organic solvents (e.g., octanol, chloroform, and carbon tetrachloride). No dimerization of any compound in benzene was observed at the concentrations used in this study, i.e., a logarithmic plot of the concentrations in benzene against those in the buffer solution gave a linear relationship with a slope of 1.001–1.02 for each compound. Fig. 1 shows a logarithmic plot of the k_{fish} values in the body surface and gills against the apparent partition coefficient values. Overall, the k_{fish} value appears to increase with increase of the apparent partition coefficient value. However, the correlation was not good for either of the membranes. The correlation was also poor between a logarithmic value of apparent partition coefficient ob-

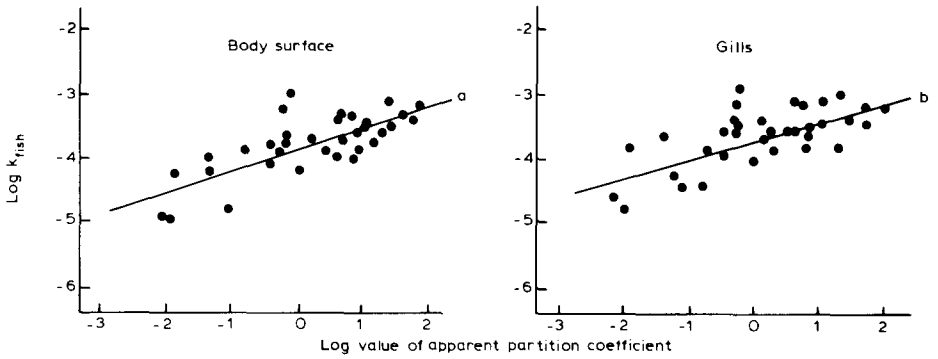


Fig. 1. Logarithmic plot of mean absorption rate constant (k_{fish}) values against apparent partition coefficient between benzene and buffer solution of benzene derivatives for the body surface and gills of goldfish. a and b, the regression lines calculated by the least-squares method (a, $r = 0.735$, $n = 34$; b, $r = 0.660, n = 35$).

TABLE 3

The substituent constant (K_s) values in the body surface (B) and gill (G) membranes of goldfish

Substituent group		No. [a]	No. [a]	$\log(k_{RS}/k_{RH})$ = K_s	Mean	K_s in rat [b]
$-NO_2$	B	**9**	**5**	−0.186	+0.294	+0.22
		10	**5**	+0.402		
	G	**9**	**5**	+0.236	+0.249	
		10	**5**	+0.261		
$-COOCH_3$	B	**15**	**5**	+0.301	+0.357	+0.26
		16	**5**	+0.412		
	G	**15**	**5**	+0.274	+0.315	
		16	**5**	+0.356		
$-CHO$	B	**22**	**5**	+0.124	+0.143	+0.23
		23	**5**	+0.161		
	G	**22**	**5**	+0.327	+0.361	
		23	**5**	+0.394		
$-COOC_2H_5$	B	**17**	**5**	+0.518	+0.355	+0.24
		18	**6**	+0.191		
	G	**17**	**5**	+0.398	+0.374	
		18	**6**	+0.350		
$-OC_2H_5$	B	**19**	**5**	+0.221	+0.221	+0.21
	G	**19**	**5**	+0.175	+0.175	
$-CH_3$	B	**32**	**8**	+0.062	+0.062	+0.12
	G	**32**	**8**	+0.011	+0.011	
$-NH_2$	B	**29**	**7**	−0.575	−0.572	−0.15
		30	**7**	−0.569		
	G	**29**	**7**	−0.663	−0.598	
		30	**7**	−0.533		
$-OCH_3$	B	**25**	**20**	−0.446	−0.446	−0.07
	G	**25**	**20**	−0.443	−0.443	
$-NHCOCH_3$	B	**27**	**20**	−0.797	−0.797	−0.16
	G	**27**	**20**	−0.659	−0.659	
$-OH$[I] [c]	B	**9**	**7**	−0.572	−0.660	−0.16
		10	**7**	−0.356		
		14	**13**	−0.659		
		15	**13**	−0.548		
		22	**20**	−0.932		
		23	**20**	−0.895		
	G	**9**	**7**	−0.450	−0.554	
		10	**7**	−0.425		
		14	**13**	−0.551		
		15	**13**	−0.548		
		22	**20**	−0.749		
		23	**20**	−0.682		
$-OH$[II] [d]	B	**18**	**17**	−0.585	−0.490	−0.05
		26	**21**	−0.394		
	G	**18**	**17**	−0.455	−0.466	
		26	**21**	−0.477		

TABLE 3 (continued)

Substituent group		No. [a]	No. [a]	$\log (k_{RS}/k_{RH})$		K_s in rat [b]
				$= K_s$	Mean	
$-CONH_2$	B	**31**	**7**	−0.640	−0.640	−0.23
	G	**31**	**7**	−0.379	−0.494	
		12	**5**	−0.603		

[a] Compound numbers given in Table 1.
[b] Literature values in the rat intestinal tract (Nogami et al., 1968b and c).
[c] The first OH-group.
[d] The second OH-group.

tained in this study and the reported k_{rat} values (Nogami et al., 1968a–c) ($r = 0.576$, $n = 35$). The k_{fish} values might be more affected by the nature and position of the substituent groups than by the lipophilicity of the compounds, as in the case of k_{rat}. Fig. 2 shows a logarithmic plot of the k_{fish} values against the reported k_{rat} values. Reasonably good, and similar, correlation coefficients were obtained in both body surface and gill membranes. According to the statistical analysis by Hotelling (1940), it was shown that the correlation between the k_{fish} and k_{rat} values was significantly higher ($P < 0.05$) than those between the apparent partition coefficient values and k_{fish} in both goldfish membranes. This suggests a similarity of the substituent effects on k_{fish} and k_{rat}. However, some k_{fish} values deviated markedly from the regression line in each membrane. Therefore, the correlation between the k_{fish} and k_{rat} values was further examined statistically by the method of Hotelling (1940). Compounds **2** and **5** in the body surface and compounds **2** and **12** (which showed undetectable absorption from the body surface within 1 h) in the gills were significantly outside the critical region ($P < 0.05$), although almost all other compounds were within it, suggesting the existence of a broad similarity, with some discrepancies, of the substituent effects on k_{fish} and k_{rat}.

The K_s values

Table 3 summarizes the K_s values in the body surface and gill membranes together with those reported in the rat intestine (Nogami et al., 1968b and c). In order to confirm the reliability of the K_s values, the k_{fish} values of drugs that had been omitted from the K_s calculation were estimated from Eqn. 3, and compared with the observed values. The calculated k_{fish} values of benzocaine, phenacetin, and *p*-acetaminophenol were 2.857, 1.250, and 0.164 ($\times 10^{-4}$) in the body surface, and 3.184, 1.749, and 0.326 ($\times 10^{-4}$) in the gills, respectively. These values agreed well with the observed mean values listed in Table 2, suggesting the reliability of the K_s values. The substituent groups giving positive or negative k_s values were the same in the two goldfish membranes and the rat intestine, indicating that the substituent groups have qualitatively the same effect (increase or decrease) on k_{fish} and k_{rat}. The mean K_s values were almost the same in the two membranes. Further, the mean positive K_s values in the gold-

TABLE 4

Values of log $K_{\phi H}$ [a] in body surface (B) and gill (G) membranes

φ-S		$\log k_{\phi H} = \log k_{\phi s} - K_s$	$\log k_{\phi H}$ in rat [b]
φ-OH	B	−3.327	−0.11
	G	−3.273	
φ-COH	B	−3.074	−0.14
	G	−3.112	
ϕ-NO_2	B	−3.524	−0.11
	G	−3.390	
ϕ-$COOCH_3$	B	−3.384	−0.10
	G	−3.317	
Mean	B	−3.327	−0.12
	G	−3.273	

[a] The absorption rate constant of benzene.
[b] Literature values in the rat intestinal tract (Nogami et al., 1968c).

fish were similar to those in the rat. However, the mean negative K_s values in the goldfish were larger than those in the rat. These results suggest that substituent groups having positive K_s values increase both k_{fish} and k_{rat} values to similar extents, whereas those having negative K_s values have a greater decreasing effect for k_{fish} than for k_{rat}. It was concluded that the substituent effects on k_{fish} are qualitatively the same and correlate well quantitatively with those on k_{rat}, except for the cases of compounds **2**, **5**, and **12**.

Among the compounds having a substituent OH or $CONH_2$ group or both, only the k_{fish} values of compounds 2, 5, and 12 showed no correlation with the k_{rat} values, suggesting that some other factor is important, for example, protein binding in the rat intestine. However, the actual cause remains to be established.

The K_I values

Nogami et al. (1968c) reported that the k_{rat} values for isomers having intramolecular hydrogen bonding decrease in the order of *o*-, *p*-, and *m*-isomers. This order was also observed in the k_{fish} values among compounds **8–10** ($-NO_2 \cdots OH-$), **21–23** ($-CHO \cdots OH-$), and **28–30** ($-NH_2 \cdots NO_2-$) (Table 2). Further, salicylic acid and pyrocatechol were absorbed from both membranes, but their *m*- and *p*-isomers were not absorbed within 1 h, as described above. The k_{fish} values between compounds 3 and 4, and between compounds 11 and 12 were also larger in the *o*-isomer than in the *m*-isomer. These results suggest a similarity in the influence of intramolecular interaction on k_{fish} and k_{rat}. Therefore, intramolecular interaction effects were examined in terms of K_I. Table 4 shows log $k_{\phi H}$ values used for the calculation of K_I in both goldfish membranes together with those in the rat intestinal tract (Nogami et al., 1968c). Log $k_{\phi H}$ was constant, as in the case of rat. Table 5 summarizes the K_I values in the body surface and gills together with those reported in the rat intestine (Nogami et al., 1968c). All the K_I values were positive in both membranes, as those in the rat intestine, indicating that intramolecular hydrogen bonding tends to increase both k_{fish} and k_{rat}. The K_I values were similar in the two goldfish membranes, but were

TABLE 5

The intramolecular interaction constant (K_I) values in the body surface (B) and gill (G) membranes of goldfish

Interaction $-S_i \cdots S_j-$		$-RS_i \cdots S_j-$ No [a]	K_I [b]	K_I in rat [c]
$-CHO \cdots HO-$	B	**21**	+0.630	−0.12
	G	**21**	+0.362	
$-COOCH_3 \cdots HO-$	B	**14**	+0.585	+0.14
	G	**14**	+0.605	
$-NO_2 \cdots HO-$	B	**8**	+0.336	+0.07
		32	+0.336	
		8	+0.519	
	G	**32**	+0.519	
$-OH \cdots HO-$	B	**6**	+0.232	+0.04
	G	**6**	+0.059	
$-CONH_2 \cdots HO-$	B	**11**	+0.407	+0.40
	G	**11**	+0.333	
$-NO_2 \cdots H_2N-$	B	**28**	+0.106	+0.10
	G	**28**	+0.156	

[a] Compound numbers given in Table 1.
[b] $K_I = \log k_{RS(i)\cdots S(j)} / k_{RS(i)S(j)calc}$. For details see text.
[c] Literature values in the rat intestinal tract (Nogami et al., 1968b, c).

larger than those in the rat, though the values for ($-CONH_2 \cdots OH-$) and ($-NO_2 \cdots NH_2-$) in both membranes and for ($-OH \cdots OH-$) in the gills did not differ much from those in the rat. The decreasing order of intramolecular hydrogen bond-forming power has been reported to be as follows: ($-CHO \cdots HO-$), ($-COOCH_3 \cdots HO-$), ($-NO_2 \cdots HO-$), ($-OH \cdots HO-$) (Tsuboi, 1952). This essentially agrees with the order of the K_I values in each goldfish membrane (Table 5), as reported in the case of rat (Nogami et al., 1968c), though the K_I value of ($-CHO \cdots HO-$) in the gills is an exception. The k_{fish} values of all the compounds having intramolecular interaction correlated well with the k_{rat} values (Fig. 2). The results suggest that quantitatively, the increasing effects of intramolecular interaction on k_{fish} and k_{rat} correlate well between the body surface or gills and the rat intestinal tract, though the reliability of the K_I values was not confirmed by a similar method to that used in the case of K_s.

Thus, it was concluded that the substituent effects on k_{fish} in the body surface and gills can be interpreted in terms of K_s and K_I, but those on the k_{fish} values of salicylic acid, phenol, and

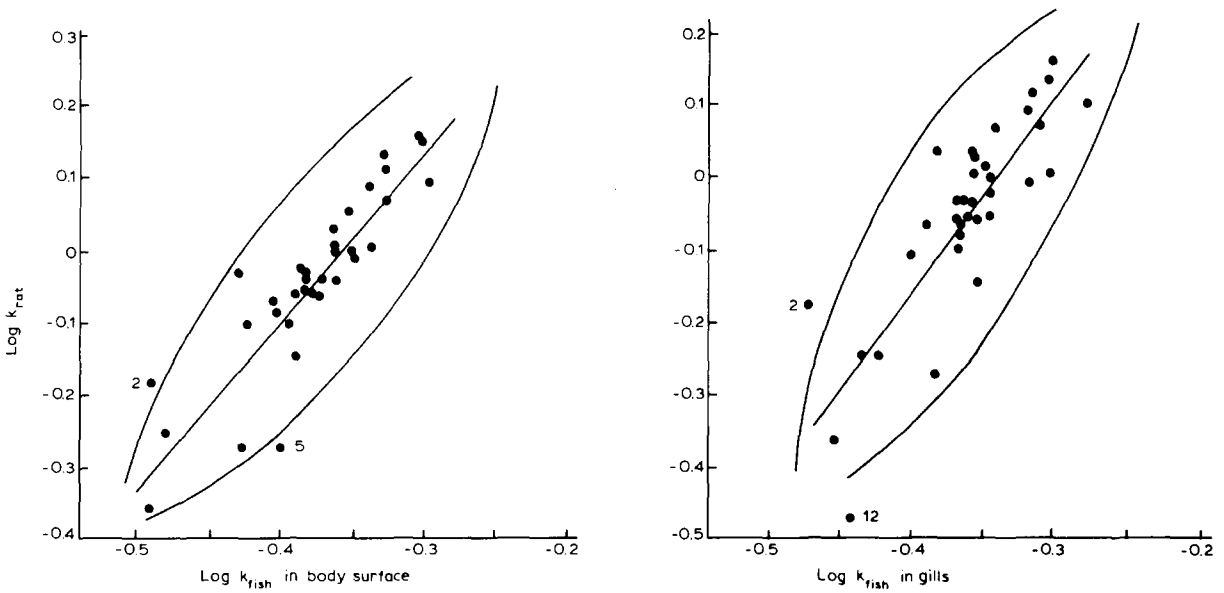


Fig. 2. Logarithmic plot of mean absorption rate constant (k_{fish}) values for the body surface and gills of goldfish against the rat intestinal absorption rate constant (k_{rat}) values of benzene derivatives at pH 6.0. The k_{rat} values were cited from the literatures (Nogami et al., 1968a–c). The straight lines are the regression lines calculated from the least-squares method ($r = 0.870$, $n = 34$ in the body surface; $r = 0.860$, $n = 35$ in the gills). The curved lines indicate the critical region ($P < 0.05$) analyzed by the method of Hotelling (1940). Numbers **2**, **5**, and **12** indicate the compounds in Table 1.

p-hydroxybenzamide cannot, except for phenol from the gills. The qualitative effects fo substituent groups and intramolecular interaction on k_{fish} are the same as those on k_{rat}. Further, the quantitative effects on the k_{fish} values correlate well with those on the k_{rat} values, except the cases of salicylic acid, phenol, and *p*-hydroxybenzamide.

References

Gibaldi, M. and Nightingale, C.H., Bile salt potentiation of pharmacologic effects and drug uptake in goldfish. *J. Pharm. Sci.*, 57 (1968) 1354–1357.

Hotelling, H., *Annual of Mathematical Statistics, Vol. 11*, 1940, pp. 271–283.

Levy, G. and Gucinski, S.P., Studies on biologic membrane permeation kinetics and acute toxicity of drugs by means of goldfish. *J. Pharmacol. Exp. Ther.*, 146 (1964a) 80–86.

Levy, G. and Miller, K.E., Determination of drug absorption rates without chemical assay. *J. Pharm. Sci.*, 53 (1964b) 1301–1305.

Levy, G. and Miller, K.E., Drug absorption and exsorption kinetics in goldfish. *J. Pharm. Sci.*, 54 (1965) 1319–1325.

Levy, G., Miller, K.E. and Reuning, N.J., Effect of complex formation on drug absorption III. *Pharm. Sci.*, 55 (1966) 394–398.

Levy, G. and Anello, J.A., Effect of complex formation on drug absorption. V. Studies on the mechanism of the secobarbital absorption-enhancing effect of polysorbate 80 in goldfish. *J. Pharm. Sci.*, 57 (1968) 1354–1357.

Nightingale, C.H. and Gibardi, M., Kinetics of drug absorption in goldfish. *J. Pharm. Sci.*, 60 (1971) 1360–1363.

Nogami, H., Hanano, M. and Yamada, H., Studies on absorption and excretion of drugs. IX. Relation between chemical structure and absorption rate. (a). *Chem. Pharm. Bull.*, 16 (1968a) 389–395.

Nogami, H., Hanano, M., and Yamada, H., Studies on absorption and excretion of drugs. X. Relation between chemical structure and absorption rate. (b). *Chem. Pharm. Bull.*, 16 (1968b) 580–585.

Nogami, H., Hanano, M., and Yamada, H., Studies on absorption and excretion of drugs. XI. Relation between chemical structure and absorption rate. (c). *Chem. Pharm. Bull.*, 16 (1968c) 586–5891.

Sakiya, Y., Umezawa, N., and Hanano, M., Kinetics on uptake of drugs in goldfish. I. Effect of pH changes in water phase and addition of polyethylene glycol 600 and urea on uptake of salicylic acid. *Yakugaku Zasshi*, 94 (1974) 1123–1130.

Sakiya, Y., Umezawa, N. and Hanano, M., Kinetics on uptake of drugs in goldfish. II. Uptake of benzoic acid, phenol, phenobarbital, and pentobarbital. *Yakugaku Zasshi*, 95 (1975) 402–410.

Sakiya, Y., Umezawa, N. and Hanano, M., Kinetics on uptake of drugs in goldfish. III. Excretion of salicylic acid, benzoic acid, phenol, and phenobarbital from goldfish. *Yakugaku Zasshi*, 96 (1976) 737–741.

Sakiya, Y. and Miyauchi, Y., Kinetics on uptake of drugs in goldfish. IV. Contribution of the stationary layer to drug absorption from the gill. *Chem. Pharm. Bull.*, 27 (1979) 1366–1372.

Tsuboi, M., On the hydrogen-bond-forming powers of atoms or atomic groups. *Bull. Chem. Soc. Jap.*, 25 (1952) 60–66.

Yalkowsky, S.H., Carpenter, O.S., Flynn, G.L., and Slunick, T.G., Drug absorption kinetics in goldfish. *J. Pharm. Sci.*, 62 (1973) 1949–1954.