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Effect of Urea on Intestinal Absorption of Salicylic Acid, Benzoic Acid and Aminopyrine in Rat

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A study was made by the *in situ* recirculating perfusion technique as to the effect of urea (0.1, 0.5, 1.0 M) on the absorption of salicylic acid, benzoic acid and aminopyrine in rat intestinal tract.

The addition of 0.5 or 1.0 M urea resulted in increased drug permeability of the gill of gold fish, while that of 1.0 M urea significantly decreased intestinal drug absorption clearance. With urea added to a perfusate, its osmotic pressure was elevated and the water flux from the intestine to the blood was decreased. A significant correlationship existed with a positive regression coefficient between water flux and clearance. Similarly, both water flux and clearance were reduced by adding NaCl so as to give the same tonicity as 1.0 M urea. This effect was not significantly different from that of adding 1.0 M urea. From these observations it is concluded that urea has no such effect in rat intestines as is observed in gold fish.

Keywords——*in situ* perfusion method; intestinal absorption; water transport; urea effect; salicylic acid; benzoic acid; aminopyrine

In our previous report,[2] it was shown that urea increased the absorption of various drugs into gold fish from the environmental water. The absorption of drugs in gold fish frequently has been compared with that in the mammalian small intestinal tract, and it has been pointed out that gold fish is an experimental animal which is useful in assessing the drug absorption in man.[3]

In this study, the effect of urea on the absorption of salicylic acid, benzoic acid and aminopyrine in rat small intestine as measured by the *in situ* recirculating perfusion method was compared with the previously reported results obtained in gold fish. The addition of urea to the perfusate (in this intestinal drug absorption study) was found to decrease the rate of absorption of each drug in rats, contrary to the results obtained in gold fish.

Drug absorption in the intestinal tract is accelerated by absorption of water, that is, positive water flux through the intestinal tract and decreased by excretion of water, or negative water flux.[4] In gold fish, however, the relationship between the water transport and drug absorption has not yet been established. The addition of urea to the perfusate elevates the osmotic pressure and hence increase excretion of water in the intestine. In estimating the effect of urea on drug absorption in rat intestine and gold fish, therefore, a comparison should be made in terms of the condition on which water flux gives rise to a variation in the rate of absorption.

This study presents a kinetic method for estimating absorption clearance, that is, the ratio of rate of drug absorption to the drug concentration in the intestine, by the *in situ* recirculating perfusion technique in rat small intestine. An estimation was made of the effect

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2) *a)* Y. Sakiya, N. Umezawa, and M. Hanano, *Yakugaku Zasshi*, **94**, 1123 (1974); *b)* Y. Sakiya, N. Umezawa, and M. Hanano, *ibid.*, **95**, 402 (1975).
3) G. Levy and K.E. Miller, *J. Pharm. Sci.*, **53**, 1301 (1964).
4) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 1856 (1975).

of urea added to the perfusate on water flux and on drug absorption. Measurements were also taken of both water flux and absorption clearance between a drug solution in the perfusate with 1 M urea and that with sodium chloride which had an identical osmotic pressure. As a result, it was found that urea exerted an effect on intestinal drug absorption not directly *via* the membrane as in gold fish but simply by way of osmotic pressure.

Experimental

Materials——Salicylic acid (SA, mp 158—159°), benzoic acid (BA, mp 121—122°), aminopyrine (AM, mp 107—109°) and urea (mp 132—133°) were used. Sodium chloride, dibasic sodium phosphate and potassium biphosphate used were of reagent grade and obtained from commercial sources. Ethylene dichloride (EDC) was washed with 1 N NaOH, 1 N HCl and water before used.

Preparation of Drug Solutions——SA, BA and AM were each dissolved in isotonic phosphate buffer (pH 6.4, prepared with 0.123 M Na_2HPO_4 and 0.123 M KH_2PO_4) to obtain a 5 mM solution of SA and BA respectively and a 2 mM solution of AM. Another series of solutions were prepared by adding 0.1, 0.5 and 1.0 M of urea to each of these solutions. Besides, 0.53 M of NaCl was added to each solution without urea in order to make up solutions with the same osmotic pressure as respective drug solutions containing 1.0 M of urea. Each drug solution was adjusted to pH 6.4 by using dilute HCl or a dilute NaOH solution and, on the acidic side, to pH 3.0 for SA and pH 4.0 for BA and AM by using dilute HCl.

Experimental Procedure——The *in situ* recirculating perfusion method based on that of L.S. Schanker, *et al.*[5] was used with a suitable modification. Male Donryu rats, weighing 240—250 g, were fasted for about 24 hr prior to the experiments but water was allowed *ad libitum*. The animals were anesthetized with ether and maintained under anesthesia for the entire course of the experiment. The animals were laparotomized by a midline incision to expose the small intestine, which was then cannulated with polyvinyl tubing at the proximal end of the duodenum and the distal and of the ileum. After closure of the incision, the tubing was connected to the inflow and the outflow cannula, which were placed in a flask containing approximately 100 ml of physiological saline solution at 37°. The small intestine was washed with the saline solution allowed by a perfusion apparatus (Type SJ-1210, Mitsumi Sci. Ind., Inc.) to flow from the duodenum to the ileum at a rate of 4 ml per min and then the intestinal tract was perfused with 110 ml of each drug solution at 37° flowing at a rate of 2.3 ml per min. One ml of the solution was taken out of the flask to sample at zero time and six times at intervals of 10 minutes after zero time. Zero time was set at 10 minutes after removal of the initial 10 ml of the solution.

Phenol red.[5-7] of which intestinal absorption may be considered negligible, was dissolved in the drug solution as a volume indicator. The drug solution in the flask was agitated with a magnetic stirrer throughout the experiment. During the experiment on drug absorption on the acidic side, care was taken to keep the drug solution in the flask at a constant pH by means of dilute HCl.

Analytical Method——i) Phenol Red: To 1 ml samples collected at different times, 2 ml of 1 N NaOH solutions was added in order to measure colorimetrically the absorbance of phenol red at 560 nm. After the measurement, 1 ml of the solution was submitted to drug determination.

ii) Salicylic Acid: One ml of a diluted sample was shaken for 1 hr with 1 ml of conc. HCl and 20 ml of EDC added, and then centrifuged for 15 min at 3000 rpm. The upper layer was removed by aspiration, while 15 ml of EDC in the lower layer, with 5 ml of 0.1 N NaOH solution added, was shaken for 30 min and then centrifuged for 15 min at 3000 rpm before reading the absorbance of the alkaline layer at 298 nm.

iii) Benzoic Acid: To 1 ml of a diluted sample, 2 ml of 6 N HCl and 20 ml of EDC were added. The subsequent procedure was the same as for SA, except that 10 ml of 0.01 N NaOH was used in place of 0.1 N NaOH and the absorbance of the alkaline layer was read at 224 nm.

iv) Aminopyrine: To 1 ml of a diluted sample, 1 ml of 1 N NaOH and 20 ml of EDC were added. The subsequent procedure was the same as for SA, except that 10 ml of 0.1 N HCl was used with 15 ml of EDC and that absorbance was measured of the acid layer at 258 nm. Throughout the experiment, spectrophotometer Model (200-20 (Hitachi) was used for the measurement of absorbance.

v) Urea: A tenth of samples collected at zero time and 60 minutes after measurement of the absorbance of phenol red was submitted to the determination of urea by the direct diacetylmonoxime method.[8]

Apparent Partition Coefficient——To 10 ml of each drug solution prepared in the same manner as those used in the above-referred series of experiment, different organic solvents were added in the same volume and vigorousely shaken at 37° for equilibration. A determination was made of the drug content of the aqueous

5) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exp. Ther.*, **123**, 81 (1958).
6) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **13**, 861 (1965).
7) K. Kakemi, T. Arita, and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1534 (1967).
8) H. Kawauchi, K. Kishinami, F. Haruki, and F. Watanabe (ed.), "Clinical Chemistry Practes," Hirokawa Publishing Co., Tokyo, 1974, p. 84.

phase, from which to calculated the partition coefficient. The organic solvents used here include benzene, chloroform and *n*-heptane.

Results and Discussion

Calculation of Intestinal Absorption Clearance

In estimating the intestinal absorption of a drug by the *in situ* recirculating perfusion technique, it is necessary to employ a parameter standing for the absorbability of the drug which is independent of the volume to be perfused, if varying with water transport. The ability of an organ to dispose of a drug, such as its excretion by the kidney or its metabolism in the liver, is ordinarily expressed in terms of clearance. By the same taken, drug absorption in the intestine can be expressed in terms of absorption clearance (R) as follows:

$$q = RC \tag{1}$$

Where q denotes the rate of absorption of a drug in the intestine, and C the concentration of the drug in the intestine. Since, in experiments by the *in situ* recirculating perfusion technique, the rate of absorption of a drug is determined from the rate at which the amount of the drug contained in the perfusate is decreased, equation (1) may also be expressed as follows:

$$-d(VC)/dt = RC \tag{2}$$

Where V is the volume of the perfusate.

Integrating equation (2) with V and C as function of time, t, on the assumption that the value for R is a constant independent of time, t, then, equation (3) is obtained:

$$\ln (CV/C_0V_0) = -R\int_0^t dt/V \tag{3}$$

Where C_0 and V_0 are the initial value (t=0) for C and V respectively. Equation (3) shows that a straight line running past the origin is obtained by plotting the logarithm of the percentage of a drug remaining in the perfusate against the area lying beneath the reciprocal of volume of perfusate-time curve. R can be calculated from the gradient of the line. In the present study, no direct measurement was made of the volume of the perfusate but, on the assumption of the indicator (phenol red) being nonabsorbable, the area underlying the reciprocal of volume of perfusate-time curve was calculated from the variation in the concentration of the indicator by the following expression:

$$\int_0^t dt/V = \frac{1}{I_0V_0}\int_0^t Idt \tag{4}$$

Where I and I_0 are the concentration at time t and the initial concentration of the indicator respectively.

In the present study, the area lying beneath the concentration of indicator-time curve was calculated by trapezoidal approximation, and the value for R as the regression coefficient of the regression line fixed the intercept to null for the plot obtained from equation (3).

If there is no change with time in water flux or the rate of absorption or excretion of water in the intestinal tract, then, the volume of the perfusate is linearly decreased with time. Hence,

$$V = V_0 - W \cdot t \tag{5}$$

Where W signifies water flux which is defined as positive when in the direction of absorption. Integration of equation (3) after combining it with equation (5), then, equation (6) is obtained.

$$\ln (CI_0/C_0I) = R \ln (1-Wt/V_0)/W \tag{6}$$

Equation (7) also holds good approximately, provided that W/V_0 is well smaller than 1.

$$\ln (CI_0/C_0I) = -(R/V_0)t \qquad (7)$$

The equation corresponds to the one which has been used for calculating the absorption rate constant ($k=R/V_0$) in *in situ* recirculating perfusion experiments.[9]

Figure 1 illustrates the variation with time in the volume of a perfusate of pH 6.4 at the largest positive, the nearest null and the largest negative water flux (W) in an AM absorption experiment.

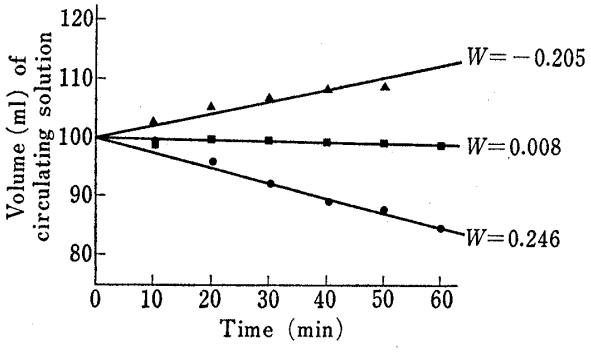

Fig. 1. Time Course of Circulating Volume in Aminopyrine Intestinal Absorption Experiment (pH 6.4) with or without Urea

Upper, middle and lower lines are represented the examples in maximum increase, maximum decreased and minimum change of perfusate volume in the experiments of aminopyrine absorption.
W represents water flux (ml/min per head) across the intestinal wall.
—●—, without urea; —■—, 0.5 M urea; —▲—, 1.0 M urea.

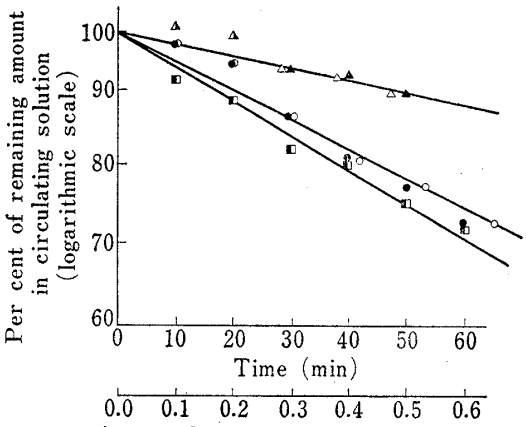

Fig. 2. Examples of Kinetic Plot in Aminopyrine Intestinal Absorption Experiments which Correspond to Fig. 1

R represents absorption clearance (ml/min per head).
Open and closed marks are kinetic plots corrected for volume change (equation 3 in text) and approximated by assumption of minor water flux (equation 7 in text), respectively.
—●— and —○—, without urea; —■— and —□—, 0.5 M urea; —▲— and —△—, 1.0 M urea.

The volume of the perfusate is increased or decreased approximately linearly with time. A similar tendency was observed in all the perfusates used in the present study. The value for W may be considered to remain essentially constant during perfusion of 60 minutes duration.

Figure 2 shows the kinetic plots from equations (3) and (7) in the same experiment as shown in Fig. 1. As is apparent from the figure, approximation of equation (7) tends to give a value for R deviating for the larger when the rate of water absorption is large, *i.e.*, there is a large positive W, and that deviating for the smaller when there is a negative W. If, however, the volume of the perfusate is large enough relative to the value for W, the difference between (3) and (7) is negligibly smaller than any variation in measurements resulting from an error in quantitative determination or a fluctuation in clearance. In fact, the difference between the values for absorption clearance as calculated from the (3) and (7) equations was no more than a few per cent throughout the present study. Although no actual benefit was found in the case of our study, the values for R used in the subsequent discussion were all calculated by means of equation (3) because of only the theoretical accuracy.

9) *a*) H. Nogami, M. Hanano, and H. Yamada, *Chem. Pharm. Bull.* (Tokyo), **11**, 395 (1963); *b*) H. Nogami, M. Hanano, and H. Yamada, *ibid.*, **16**, 389 (1968).

Effect of Urea on Intestinal Drug Absorption and Water Transport

The mean values and standard deviations for the water flux and clearance in perfusates of different compositions are given in Table I.

Table I. Water Flux and Intestinal Absorption Clearance of Salicylic Acid, Benzoic Acid and Aminopyrine at Different pH Values

Drug	pH	Additive	N	Water flux (ml/min) Mean	S.D.[a]	Clearance (ml/min) Mean	S.D.[a]
SA	3.0	None	4	−0.0115	0.0513	0.999	0.190
		U 0.1 M	5	−0.0940	0.0326	1.174	0.146
		U 0.5 M	4	−0.236	0.0458	0.690	0.125
		U 1.0 M	6	−0.349	0.0389	0.792	0.144
		0.53 Eq. NaCl	4	−0.357	0.0713	0.492	0.0928
	6.4	None	5	0.158	0.0520	1.074	0.219
		U 0.1 M	4	0.103	0.0232	0.888	0.0674
		U 0.5 M	4	−0.122	0.0299	0.573	0.0982
		U 1.0 M	4	−0.0655	0.0496	0.373	0.0537
		0.53 Eq. NaCl	4	−0.252	0.0311	0.303	0.0305
BA	4.0	None	4	0.169	0.0372	1.088	0.214
		U 0.1 M	5	0.0251	0.0060	1.147	0.263
		U 0.5 M	4	−0.178	0.0244	0.760	0.0603
		U 1.0 M	6	−0.170	0.0388	0.317	0.188
		0.53 Eq. NaCl	5	−0.270	0.0499	0.333	0.114
	6.4	None	4	0.153	0.0305	0.824	0.883
		U 0.1 M	4	0.0949	0.0503	0.885	0.0855
		U 0.5 M	2	−0.115	0.0262	0.538	0.0198
		U 1.0 M	8	−0.169	0.0479	0.210	0.0811
		0.53 Eq. NaCl	4	−0.373	0.153	0.326	0.0516
AM	4.0	None	9	0.0981	0.0460	0.526	0.165
		U 0.1 M	12	0.0471	0.0270	0.575	0.140
		U 0.5 M	11	−0.185	0.129	0.327	0.103
		U 1.0 M	7	−0.210	0.0315	0.220	0.0525
		0.53 Eq. NaCl	4	−0.289	0.0702	0.142	0.0551
	6.4	None	4	0.164	0.0565	0.493	0.0174
		U 0.1 M	5	0.0509	0.0218	0.5666	0.140
		U 0.5 M	5	−0.0473	0.0481	0.395	0.108
		U 1.0 M	5	−0.180	0.0188	0.156	0.0425
		0.53 Eq. NaCl	4	−0.281	0.0178	0.129	0.0399

Drug	Water flux (ml/min) T. value[b]					Clearance (ml/min) T. value[b]				
SA	11.893[d]	0.232	4.887[d]			1.970	3.653[d]	0.540	5.498[d]	
	6.535[d]	6.371[d]				6.175[d]	0.812			
BA	13.745[d]	3.746[d]	0.665			6.028[d]	0.166	2.281	1.454	
	12.111[d]	3.586[d]				12.033[d]	2.577[c]			
AM	15.122[d]	2.626[c]	2.234[c]			4.693[d]	2.331[c]	0.389	2.242[c]	
	12.942[d]	8.178[c]				14.738[d]	0.972			

a) Standard deviation.
b) Student's t values are calculated by the paired t test between data jointed by line in the table with the assumption of equal deviation.
c) and d) indicate significance at level of p 0.05 and p 0.01, respectively.
N, number of experiments; U, urea; SA, salicylic acid; BA, benzoic acid; AM, aminopyrine.

As seen in Table I, clearance was significantly lower in the perfusates with 1.0 M urea than in perfusates without urea, *i.e.*, isotonic buffer solution. This is contrary to the previous-

ly reported effect of urea, which increased the drug permeability of gold fish gills.[2,10] In the reports, the absorption of drug ions was found to be accelerated about twice in rate with 1.0 M urea added. Addition of urea also reduced water flux in rat intestinal tract. In isotonic buffer solutions, except a SA solution of pH 3.0, water flux was positive and the volume of the perfusate was decreased with time because of absorption of water. With 0.5 M or 1.0 M urea added, water flux became negative and infiltration of water in the perfusate caused a gradual increase in the volume of the perfusate. The difference between the value for W of the perfusate with 1.0 M urea added and that of an isotonic buffer was highly significant for all drugs at all pH values examined. It is known that water flux in the intestine varies with the osmotic pressure of a perfusate,[4] which is undoubtedly elevated by addition of urea. Also, there was no marked fall in the concentration of urea in perfusates during the present study, as shown in Table II. This suggests that urea so added may affect the value for W by way of osmotic pressure.

Table II. Per Cent of Urea Remained in Circulating Solution at 60 Minutes

Drug absorbed	pH	Initial conc. of urea: 0.1 M Mean % of urea remained[a]	N	0.5 M Mean % of urea remained[a]	N	1.0 M Mean % of urea remained[a]	N
SA	6.4	86.5	4	86.3	4	83.5	4
	3.0	91.8	5	85.4	4	95.1	6
BA	6.4	87.2	4	87.0	4	84.8	8
	4.0	92.4	5	84.6	4	91.6	6
AM	6.4	88.6	5	88.0	5	85.6	5
	4.0	89.5	12	86.6	11	86.7	7

a) Mean value of N.
N; number of experiments.
For other keys, see in Table I.

Relationship between pH of Perfusate and Intestinal Drug Absorption

It has been reported that the intestinal absorption of a drug is in parallel with the degree of its hydrophobic property, being correlated with, for example, the partition coefficient between an organic solvent and water.[11] For weakly acidic or weakly basic drugs, more

Table III. Apparent Partition Coefficients of Salicylic Acid, Benzoic Acid and Aminopyrine between Organic Solvents and Isotonic Phosphate Buffer with or without Urea at 37° and at Different pH Values

pH	Benzene/Buffer: SA (5 mM)	SA (5 mM) +U (1.0 M)	CCl_4/Buffer: BA (5 mM)	BA (5 mM) +U (1.0 M)	*n*-Heptane/Buffer: AM (2 mM)	AM (2 mM) +U (1.0 M)
6.4	0.00083[a]	0.00082[a]	0	0	0.0647	0.0554
4.0			0.618	0.514	0.00357[a]	0.00330[a]
3.0	0.884	0.810				

a) These values were calculated by the determination of drug in the concentrated solvent after the evaporation and the accuracy may be doubtful.

10) Y. Sakiya, N. Umezawa, and M. Hanano, *Yakugaku Zasshi*, **96**, 737 (1976).
11) G. Levy and R.H. Reuning, *J. Pharm. Sci.*, **53**, 1471 (1964).

of their nondissociable molecules are absorbed in the intestine than the ionized ones. The intestinal absorption of such a drug is known to depend greatly on the pH of a perfusate.[12]

Table III shows the partition coefficients of each drug between perfusates and organic solvents, the perfusates with 1.0 M urea or without urea that were used in the present study. As seen in Table III, there was a wide variation due to ionization in the partition coefficients of drug in extent of the pH of perfusates used. It is also shown that the partition coefficient was hardly affected by addition of urea. As shown in Table I, on the other hand, no significant difference was present between different pH values as to the clearance from isotonic buffer of any of the drugs tested. Even with 1.0 M urea added, the clearance varied to no marked extent at different pH values, no correlation existed between the variations in the clearance and the partition coefficient. In view of the finding that the pH of a perfusate may be not identical with that on the absorbing surface of the intestinal tract,[13-17] it is likely that in the present study the pH of acidic perfusates was not exactly the same as that on the absorbing surface of the intestine. The water flux from an isotonic perfusate, *i.e.*, a SA solution of pH 3.0 or an AM solution of pH 4.0, was significantly lower than that from an isotonic perfusate of pH 6.4. Accordingly, the possibility of non-physiologic acidic perfusates having affected intestinal function can not be ruled out.

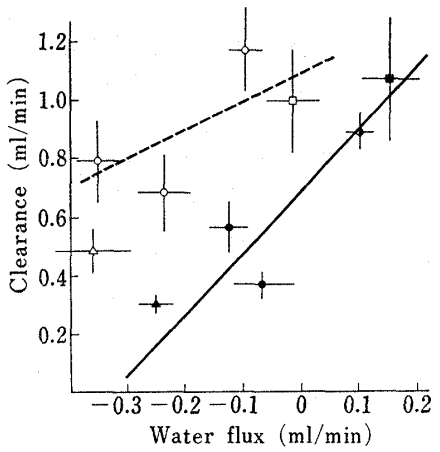

Fig. 3. Relationship between Water Flux and Absorption Clearance of Salicylic Acid

—□— and —■—, control (isosmotic buffer); —○— and —●—, solution with urea (0.1, 0.5, 1.0 M); —△— and —▲—, hyper-osmotic saline. Open and closed marks in above are at pH 3.0 and 6.4, respectively. Cross lines represent standard deviations of water flux and clearance. Solid and dotted lines are the regression lines for data at pH 6.4 (a=0.961, b=1.095, γ=0.576) and 3.0 (a=2.060, b=0.692, γ=0.838) except hyper-osmotic saline, respectively. The regression coefficient (a) and the intercept (b), *i.e.* clearance=aX water flux+b, are calculated by the least square method in which the experimental error for water flux is negrected. The correlation coefficient between clearance and water flux is indicated by γ.

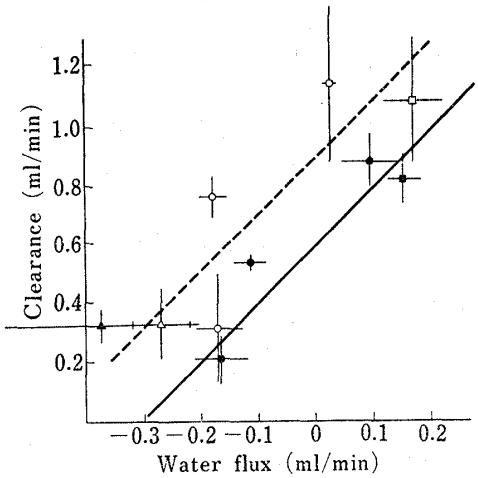

Fig. 4. Relationship between Water Flux and Absorption Clearance of Benzoic Acid

Open and closed marks are at pH 4.0 and 6.4, respectively.
Solid and dotted lines are the regression lines for data at pH 6.4 (a=1.916, b=0.596, γ=0.904) and 4.0 (a=1.903, b=0.885, γ=0.690), respectively. For other keys, see in Fig. 3.

12) J.G. Wagner, *J. Pharm. Sci.*, **50**, 359 (1961).
13) B.B. Brodie and C.A.M. Hogben, *J. Pharm. Pharmacol.*, **9**, 345 (1975).
14) C.A.M. Hogben, D.G. Tocco, B.B. Brodie, and L.S. Schanker, *J. Pharmacol. Exp. Ther.*, **125**, 275 (1959).
15) L.S. Schanker, *J. Med. Pharm. Chem.*, **2**, 343 (1960).
16) L.S. Schanker, *Pharmacol. Rev.*, **14**, 501 (1962).
17) D. Winne, *J. Pharmacokinetics and Biopharm.*, **5**, 1 (1977).

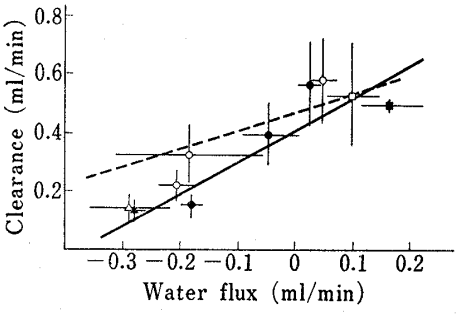

Fig. 5. Relationship between Water Flux and Absorption Clearance of Aminopyrine

Open and closed marks are at pH 4.0 and 6.0, respectively.
Solid and dotted lines are the regression lines for data at pH 6.4 (a=1.100, b=0.411, γ=0.792) and 4.0 (a=0.615, b=0.467, γ=0.600), respectively. For other keys, see in Fig. 3.

Relationship between Water Flux and Drug Absorption

It has been reported that water flux in the intestinal tract affects the rate of drug absorption, and that drug absorption is enhanced with the increase in water absorption but decreased by excretion of water in the intestinal tract.[4] In the present study urea reduced the absorption clearance of drugs and water flux as well.

The relationship between absorption clearance and water flux for each drug is illustrated in Fig. 3 to Fig. 5. Even with urea added to drugs, there absorption clearance is increased with the increase in water flux, although there is a wide variance in both parameters in different individuals. The basic drug AM gave a lower regression coefficient than SA or BA, but no definite satistical evidence in support of the difference could be obtained. Nor was any such definite regression as in the above-referred diagrams observed in the relationship between clearance and water flux under the same experimental condition: urea added in the same concentration of the same drug in a perfusate of the same pH. Consequently, the individual difference in clearance could not be attributed to that in water flux.

It was reported that there was a difference between the rate of absorption of a drug with NaCl added to cause a change in the osmotic pressure and thereby water transport and that of the drug with glucose added.[18] No one can therefore be sure that the rate of drug absorption varies in the same manner and to the same extent following addition of urea as that of NaCl. Nevertheless, a comparative study was made by adding 1.0 M urea and 0.53 Eq. NaCl, which give an identical osmotic pressure. The results obtained are shown in Table I and Fig. 3—5. As seen from Table I, the addition of NaCl gave rise to excretion of water greater than that of 1.0 M urea, except in a SA solution of pH 3.0. On the other hand, the addition of NaCl resulted in significantly lower clearance than that of 1.0 M urea in a SA solution than that of 1.0 M urea in a SA solution of pH 3.0 and significantly higher clearance in a BA solution of pH 6.4 or an AM solution of pH 4.0, although the difference was generally slight. There was no statistical evidence that the results obtained with NaCl added which are marked with triangles in Fig. 3—5, deviated significantly from the regression line made up from the data obtained in perfusates with urea added or in isotonic solutions. In other words, the results obtained with NaCl added were somewhat different in details from those with urea added, but not so if viewed from the aspect of the variation in clearance by way of water flux. The phenomenon of the membrane permeability of drugs being enhanced by urea as in gold fish was not observed in rat intestine, in so far as our present study was concerned. The effect of urea on intestinal drug absorption, which is to reduce the rate of absorption by elevating osmotic pressure with the consequent water excretion in the intestine, is hardly different from the effect of NaCl added which is exhibited by way of elevated osmotic pressure.

Fresh-water fish such as gold fish may well have the function of resisting water flux caused by the intra- and extracorporeal difference in osmotic pressure. In using gold fish as a model for the study of drug absorption, it must be taken into consideration that the variation in drug absorbability due to water flux as an important feature of intestinal absorption will be missed.

18) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **25**, 19 (1977).