[Chem. Pharm. Bull. 23(6)1247—1255(1975)]

UDC 615.31.033.034.076.9

Factors influencing Absorption and Excretion of Drugs. IV.[1] Effect of Hypertonic and Hypotonic Solutions on *in Situ* Rat Intestinal Absorption of Several Drugs

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(Received October 28, 1974)

The effect of the hypertonic and hypotonic solutions on the absorption of acetanilide, salicylamide, sulfisoxazole, and quinine from rat small intestine was studied using the *in situ* perfusion technique. Water net flux into the intestinal lumen occurring in the hypertonic solution diminished the absorption of the drugs. Whereas the enhancement of the absorption of the drugs was observed in the hypotonic solution causing water net flux out of the intestinal lumen. The effect of water net flux on the absorption of the drugs was studied by dividing into the four components. It was found that the solvent drag and rate of blood flow, as the two significant components, played a major role in the absorption of the drugs through the intestinal membrane. The rate of intestinal blood flow played an important role in the absorption of acetanilide and salicylamide, which were lipid soluble and very rapidly absorbed. In the case of the more polar and slowly absorbable quinine, the solvent drag played a significant role. Both blood flow and solvent drag were effective for the absorption of sulfisoxazole, which existed mostly in the ionized form at pH 6.0 and was moderately rapidly absorbed. In addition, it was demonstrated that two other components such as the change of drug concentration in the intestinal lumen and intestinal tissue fluid uptake due to water net flux played a minor role in the *in situ* intestinal absorption of the drugs.

It has been generally known that the gastrointestinal absorption of a drug is influenced by various physiological factors. Some investigators have reported the influence of tonicity on the gastrointestinal absorption of drugs. For example, Mayersohn and Gibaldi[3] reported the influence of hypertonic and hypotonic solutions on the passive transfer of certain drugs across the everted rat intestine. Kitazawa and Ito[4] investigated the effect of tonicity on the absorption of sulfanilamide, sulfisoxazole, and metoclopramide in an *in situ* rat intestine. Ochsenfahrt and Winne[5] studied the absorption of aminopyrine and antipyrine in hypertonic and hypotonic solutions in an *in situ* rat intestine. In addition, Kojima, *et al.*[6] reported the influence of change in tonicity occurring in the intestinal lumenal solutions with different osmolality on the drug absorption process in an *in situ* rat intestinal preparations. The results obtained from the *in situ* experiments described above showed that the drug absorption was enhanced by the hypotonic solution and inhibited by the hypertonic solution.

The present study was carried out to gain further insight into the influence of tonicity on the absorption of several drugs in an *in situ* rat intestine. Drugs used in this study were acetanilide, salicylamide, sulfisoxazole, and quinine. These drugs were selected on the basis of the difference of ionic nature at physiological pH.

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4) S. Kitazawa and H. Ito, "Absorption, Metabolism, and Excretion of Drugs," ed. by K. Kakemi, Hirokawa Publishing Company, Tokyo, 1971, p. 30.
5) H. Ochsenfahrt and D. Winne, *Life Sci.*, **11**, 1115 (1972).
6) S. Kojima, R.B. Smith, W.G. Crouthamel, and J.T. Doluisio, *J. Pharm. Sci.*, **61**, 1061 (1972).

Experimental

Materials and Equipment——Sulfisoxazole and salicylamide were of JP VIII grade. Acetanilide, quinine sulfate, phenol red, and other chemicals were of reagent grade. Vasopressin injection containing 20 units/ml was purchased from Parke, Davis & Co., Detroit, Michigan.

A Shimadzu QV-50 spectrophotometer, a Shimadzu AA-610S atomic absorption spectrophotometer, a Hitachi-Horiba F-5 pH meter, and a Shimadzu freezing-point depression measuring instrument were utilized.

Preparation of Sample Solutions——The components of hypertonic, isotonic, and hypotonic phosphate buffer solutions used as the medium in an *in situ* experiment are listed in Table I. Krebs-Ringer solution, pH 7.0, used in an *in vitro* experiment consisted of 100 ml of 0.9% NaCl, 4 ml of 1.15% KCl, 3 ml of 1.22% $CaCl_2$, 1 ml of 2.11% KH_2PO_4, 1 ml of 3.82% $MgSO_4 \cdot 7H_2O$, and 12 ml of 0.1M phosphate buffer (Na_2HPO_4–HCl, pH 7.4). The solutions, pH 7.0, were also prepared in which the NaCl concentration was reduced to 0.36% (hypotonic solution, 150 milliosmols/kg·water) or increased to 1.25% (hypertonic solution, 360 milliosmols/kg·water).

The initial concentrations of the drugs used were acetanilide (200 μg/ml), salicylamide (1000 μg/ml), sulfisoxazole (250 μg/ml), and quinine (400 μg/ml).

Table I. Preparation of Hypertonic, Isotonic, and Hypotonic Phosphate Buffer Solutions used in the *in Situ* Rat Experiment

	pH	Osmolality (mosm/kg·H_2O)	$NaH_2PO_4 \cdot 2H_2O$ g/liter	NaCl g/liter
Hypertonic solution A	6.0	360	12.880	7.000
Hypertonic solution B	6.0	320	12.880	5.780
Isotonic solution C	6.0	285	12.880	4.500
Hypotonic solution D	6.0	210	12.880	2.000
Hypotonic solution E	6.0	150	12.880	—
Hypotonic solution F	6.0	100	9.190	—

Each buffer solution was adjusted to pH 6.0 with 2N NaOH solution.

Test Animals——Male Wistar rats weighing 180—250 g were used. The rats were fasted 17—20 hr prior to the experiment, but drinking water was allowed *ad libitum*. The rats were kept in cages having wide mesh floors to prevent coprophagy.

***In Situ* Rat Experimental Procedures**——(A) Perfusion Procedure in Anesthetized Rat: Drug absorption was investigated according to a minor modification of the procedure described previously.[7] The rat was anesthetized approximately 30 min prior to surgery with urethan using an intraperitoneal injection of 1.2 g/kg·body weight. The small intestine was exposed by a midline abdominal incision, and two L-shaped glass cannulae were inserted through incisions at the duodenal and ileal ends. The cannulae were secured by ligation with silk suture. The intestine was washed with about 100 ml of perfusion solution[8] warmed at 37° and then rinsed with 30 ml of sample solution. The polyethylene tubings attached to the inflow and outflow glass cannulae were then connected to a flask containing 40 ml of sample solution. The sample solution was continuously perfused at the rate of 5 ml/min through the intestine for 2 hr at 37° using a perfusion pump. A 0.1—0.3 ml aliquot was removed at periodic intervals for assay. The amount of drug remaining in the intestinal lumen was determined as a function of time.

(B) Perfusion Procedure in Unanesthetized Rat: The rat was secured on its back on an animal board. The procedure of absorption experiment from the small intestine of unanesthetized rat was performed according to the procedure in anesthetized rat described above except that the rat was anesthetized lightly with ether only during a surgical operation and subjected to drug absorption experiment in the awakening state.

(C) Perfusion Procedure with Constant Volume in Perfusion Solution: The surgical and perfusion procedures were carried out according to the procedure in anesthetized rat mentioned above. The volume of hypotonic (E) perfusion solution was kept constant throughout the experiment by adding the hypotonic buffer solution E intermittently to the reservoir from a buret to replace fluid loss due to water absorption from the intestine.

Water Net Flux in *in Situ* Rat Intestinal Lumen——To determine water net flux in the *in situ* rat small intestine, phenol red, which was expected as an unabsorbable marker, was dissolved in the perfusion solution.

7) S. Kojima, H. Ichibagase, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **14**, 965 (1966).
8) J.T. Doluisio, N.F. Billups, L.W. Dittert, E.T. Sugita, and J.V. Swintosky, *J. Pharm. Sci.*, **58**, 1196 (1969).

The change in volume of perfusion solution was calculated from the periodical concentration change of phenol red in the perfusion solution. Water net flux was expressed as the percent of volume change.

Determination of Water Content in Rat Small Intestine——The water content in the *in situ* rat small intestine was determined by the procedure reported in the paper from this laboratory.[9]

***In Vitro* Rat Experimental Procedure**——The procedure of drug transfer experiment from the everted rat intestine was the same as that reported in the paper from this laboratory.[10]

Measurement of Portal Blood Flow in Rat——The *in situ* rat intestine preparation was provided by the procedure described previously.[6,8] Ten milliliters of hypertonic, isotonic, or hypotonic phosphate buffer solution was introduced into the intestine preparation. After 1 hr, a L-shaped glass cannula (0.7 mm i.d.) equipped with 30 cm segment of polyethylene tubing (No. 30) containing heparin solution (0.5 mg/ml) was inserted into the portal vein in the usual manner and secured by ligation with silk suture. The blood was collected in a 10 ml graduate cylinder containing 5 ml of 3.8% sodium citrate solution to prevent blood coagulation. The outflow of blood was measured at 1, 2, and 3 min, respectively, after the blood collection was started.

Analytical Procedures——(i) Acetanilide, Salicylamide, Sulfisoxazole, Quinine, and Phenol Red: Acetanilide and salicylamide were analyzed spectrophotometrically as described previously.[9] Sulfisoxazole was analyzed spectrophotometrically using the method of Bratton–Marshall.[11] Quinine was analyzed by the ultraviolet spectrophotometric procedure of Josephson.[12] Phenol red was estimated according to the procedure of Schanker, *et al.*[13]

(ii) Sodium and Potassium: The analyses of sodium and potassium were performed by atomic absorption spectrophotometry. Sample solution was appropriately diluted with 4 mM cesium nitrate solution. The absorbance of the resulting solution was determined at 589 nm for sodium and 766.5 nm for potassium, respectively.

(iii) Measurement of Tonicity: Each sample was centrifuged at 3000 rpm for about 10 min and the osmotic pressure of the supernatant fluid was determined by freezing-point depression using Beckmann's thermometer.[14]

Result and Discussion

Absorption of Drugs in Hypertonic, Isotonic, and Hypotonic Solutions

To understand the influence of tonicity on the intestinal absorption of the drugs such as acetanilide, salicylamide, sulfisoxazole, and quinine which were selected on the basis of the difference of ionic nature at physiological pH, the absorption of those drugs in hypertonic (A and B), isotonic (C), and hypotonic (D, E and F) buffer solutions at the initial pH 6.0 was examined in the *in situ* small intestine preparation of anesthetized rats. The absorption rate constant of each drug was calculated from the slope of the straight line on the semilogarithmic plots of drug concentration in the rat intestinal lumen *vs.* time. Results are summarized in Table II. The hypertonic solutions significantly inhibited the intestinal absorption of all drugs, and the inhibitory effect of the hypertonic solutions on the absorption of those drugs was greater in the hypertonic solution A having higher osmotic pressure. In contrast, the hypotonic solutions significantly enhanced the absorption of the drugs, and the enhancing effect was most marked in the case of the hypotonic solution F having the lowest osmotic pressure.

Changes in Water Net Flux and in Tonicity of Perfusion Solution

The change in osmolality of the perfusion solution was measured using the hypertonic, isotonic, and hypotonic solutions in the *in situ* rat intestine. Results are summarized in Table III. The tonicity of hypertonic solution A decreased and reached the value of about 335 milliosmols/kg·water after 2 hr. The tonicity of hypotonic solution E increased and reached the value of about 280 milliosmols/kg·water after 2 hr. The tonicity of isotonic

9) S. Kojima, T. Tenmizu, T. Shin-o, and M. Cho, *Chem. Pharm. Bull.* (Tokyo), **22**, 952 (1974).
10) S. Kojima and M. Kiyozumi, *Yakugaku Zasshi*, **94**, 695 (1974).
11) A.C. Bratton and E.K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).
12) E.S. Josephson, *J. Biol. Chem.*, **168**, 341 (1947).
13) L.S. Schanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957).
14) J. Samejima, "Butsuri Kagaku Jikkenho," Shoka Bo, Tokyo, 1968, p. 204.

Table II. Influence of Tonicity on Absorption of Several Drugs from the *in Situ* Small Intestine of Anesthetized Rats

Drug	Absorption rate constant[a] (hr^{-1})					
	Hypertonic		Isotonic	Hypotonic		
	A	B	C	D	E	F
Acetanilide	0.58±0.01[b] (−26.6)	0.63±0.01[b] (−20.2)	0.79±0.04	0.99±0.05[b] (+25.2)	1.25±0.04[b] (+58.3)	1.41±0.03[b] (+78.5)
Salicylamide	0.93±0.07[b] (−19.8)	—	1.16±0.06	1.36±0.05[c] (+17.2)	1.73±0.16[b] (+49.1)	—
Sulfisoxazole	0.24±0.01[b] (−38.5)	0.32±0.003[b] (−18.0)	0.39±0.01	0.44±0.02[c] (+12.8)	0.47±0.02[b] (+20.6)	0.69±0.03[b] (+77.0)
Quinine	0.12±0.002[b] (−32.8)	—	0.21±0.01	—	0.38±0.01[b] (+81.0)	—

a) The values represent the mean ± standard deviation of the mean for 3 determinations.
b) significantly different from corresponding value in the isotonic solution C, $p<0.01$
c) significantly different from corresponding value in the isotonic solution C, $p<0.05$
The values in parentheses represent percent inhibition (negative sign) and percent enhancement (positive sign).

solution C also showed a slight increase from 285 to about 310 milliosmols/kg·water after 2 hr. The changes in tonicity of these solutions were in fair agreement with those reported previously.[6] To elucidate whether the net fluxes of the inorganic electrolytes in the intestinal lumen can essentially participate in the change in tonicity of the perfusion solution, the amounts of sodium and potassium in the different tonicity solutions used as the perfusion solutions were determined in the *in situ* rat intestine preparation. Plots of amounts of sodium and potassium in those solutions *vs.* time are shown in Figs. 1 and 2. The amount of sodium slightly decreased in the hypertonic solution A, slightly increased in the isotonic solution C, and scarcely changed in the hypotonic solution E, respectively. On the other hand, in all solutions, the amount of potassium increased gradually, but was only 2 to 4% of the amount of sodium in 2 hr. These results suggest that the net fluxes of sodium and potassium through the intestinal membrane play a minor role in the changes in tonicity of the perfusion solutions.

The water net flux in the *in situ* rat small intestine was determined using phenol red as an unabsorbable marker. The change in volume of the perfusion solution was measured using the phosphate buffer solutions, A, B, C, D, E, and F. Results are summarized in Table III. An increase in volume is due to water net flow into the intestinal lumen, and a decrease in volume refers to water net absorption from the lumen. Accordingly, it was found that the hypotonic solutions produced water net flux out of the intestinal lumen and that the hypertonic solutions produced water stream into the lumen.

Table III. Changes in Volume and in Osmolality of Perfusion Solution in the *in Situ* Rat Intestinal Preparation

	Percent change of volume in 2 hr[a,b]	Osmolality (mosm/kg H_2O)	
		0 hr	2 hr
Hypertonic solution A	+ 6.5± 1.5	360	335±12[a]
Hypertonic solution B	+ 6.4± 2.2	—	—
Isotonic solution C	− 2.0± 1.6	285	307± 4[a]
Hypotonic solution D	−11.5± 8.9	—	—
Hypotonic solution E	−32.0±14.5	150	279±11[a]
Hypotonic solution F	−41.5±11.1	—	—

The data were obtained from the *in situ* absorption experiment of sulfisoxazole.
a) The values represent the mean ± standard deviation of the mean for 3 determinations.
b) positive sign: water net flux into intestinal lumen; negative sign: flux out of intestinal lumen

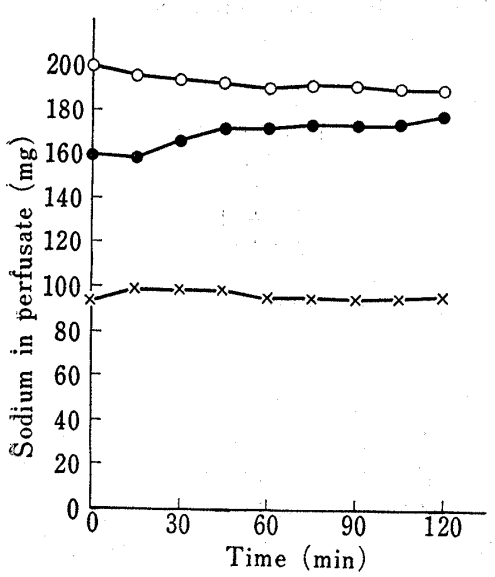

Fig. 1. Amount of Sodium of Hypertonic, Isotonic, and Hypotonic Perfusion Solutions in the *in Situ* Rat Intestinal Preparation

Each value is expressed as the mean of 3 determinations.
—○— : hypertonic (A)
—●— : isotonic (C)
—×— : hypotonic (E)

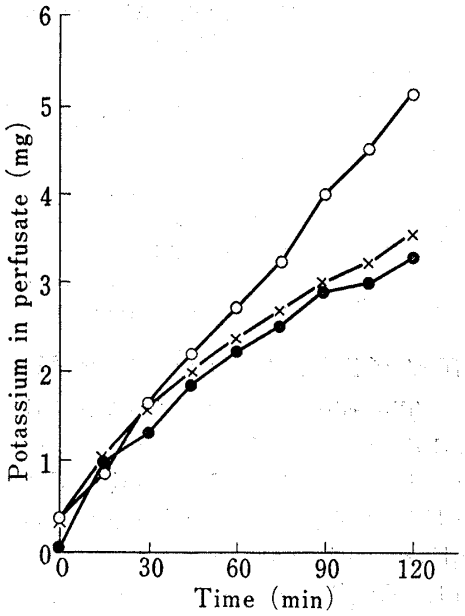

Fig. 2. Amount of Potassium of Hypertonic, Isotonic, and Hypotonic Perfusion Solutions in the *in Situ* Rat Intestinal Preparation

Each value is expressed as the mean of 3 determinations.
—○— : hypertonic (A)
—●— : isotonic (C)
—×— : hypotonic (E)

From the results obtained above, it was elucidated that the changes in tonicity of the hypertonic and hypotonic solutions were predominantly due to water net flux into and out of the intestinal lumen. Thus, it is suggested that the influence of the hypotonic or hypertonic solution on the absorption of the drugs is attributable to water net flux towards the blood or intestinal lumen.

The effect of water net flux on the absorption of the drugs may be divided into the following four components: (a) the change of the drug concentration in the intestinal lumen, (b) the solvent drag in the intestinal membrane,[5] (c) the change of blood flow in the capillaries near the epithelium,[5] and (d) the intestinal tissue fluid uptake.

Effect of Change in Drug Concentration on Drug Absorption

As can be seen from Tables II and III, in both hypertonic and hypotonic solutions, the change of the drug concentration due to the volume change of perfusion solution was considerably smaller than that of the drug absorption. In order to clarify further the influence of the change of the drug concentration on the drug absorption, an investigation was carried out. The absorption of salicylamide and sulfisoxazole was determined by keeping constant the volume of the hypotonic (E) perfusion solution in the *in situ* rat small intestine preparation. Results are shown in Table IV. The absorption rate of salicylamide did not show a conspicuous difference between the experiments with and without the constant volume of the perfusion solution. The absorption rate of sulfisoxazole slightly increased in the experiment with the constant volume of the perfusion solution. These results indicated that an increase in drug concentration due to a decrease in volume of the hypotonic perfusion solution played no role for the enhancement of the drug absorption in the hypotonic solution. Accordingly, it is suggested that the variation of the drug concentration due to the change in volume of the perfusion solution is not a significant component of water net flux influencing the drug absorption.

Table IV. Absorption of Sulfisoxazole and Salicylamide from the *in Situ* Rat Small Intestine with Constant Volume of Hypotonic (E) Perfusion Solution

Drug	Absorption rate constant (hr^{-1})	Osmolality in 2 hr (mosm/kg H_2O)
Sulfisoxazole	0.60±0.02	183±14
Salicylamide	1.60±0.06	188±17

The values represent the mean±standard deviation of the mean for 3 determinations.

Effect of Solvent Drag on Drug Absorption

The phenomenon of solvent drag can be accounted for by the interaction between water and drug molecule during the transfer through water-filled pores or channels in the epithelial membrane. To understand in detail the effect of solvent drag on the absorption of the drugs in this study, an investigation was performed as follows. The absorption of salicylamide and sulfisoxazole was examined using the hypertonic (A), isotonic (C), and hypotonic (D) solutions in the *in situ* small intestine preparation of anesthetized rats treated with antidiuretic hormone, vasopressin, which was expected to inhibit water absorption in the intestinal lumen.[15] The absorption rate constants of those drugs and the changes in volume of the perfusion solutions are summarized in Table V. The changes in volume of the hypotonic and hypertonic perfusion solutions, in other words, water net flux, decreased to greater extent in the rats treated with vasopressin. Consequently, the data suggest that vasopressin considerably diminishes the effect of solvent drag on the absorption of the drugs. The absorption rate of sulfisoxazole in the rats with vasopressin significantly increased in the hypertonic solution and significantly decreased in the hypotonic solution, respectively, compared to that in the rats without vasopressin. Moreover, in the rats treated with vasopressin the absorption rate constant of the drug did not show a conspicuous difference among the hypertonic, isotonic, and hypotonic solutions. On the contrary the absorption rate of salicylamide scarcely changed between the rats with and without vasopressin in all solutions.

Table V. Absorption of Sulfisoxazole and Salicylamide and Changes in Volume of Perfusion Solutions in the *in Situ* Small Intestine of Rats with and without Vasopressin Treatment

	Vasopressin[a]	Absorption rate constant (hr^{-1})[b]	Percent change of volume in 2 hr[b]
Sulfisoxazole			
hypertonic (A)	−	0.24±0.01	+6.5±1.5
	+	0.37±0.02[c]	+2.1±2.0
isotonic (C)	−	0.39±0.01	−2.0±1.6
	+	0.39±0.03[d]	−1.1±2.5
hypotonic (D)	−	0.44±0.02	−11.5±8.9
	+	0.40±0.01[e]	−5.3±3.0
Salicylamide			
hypertonic (A)	−	0.93±0.07	+7.2±2.0
	+	0.88±0.08[d]	+3.5±1.9
isotonic (C)	−	1.16±0.06	−10.6±4.5
	+	1.18±0.05[d]	−7.6±8.2
hypotonic (D)	−	1.36±0.05	−21.1±2.3
	+	1.42±0.02[d]	−6.6±8.1

a) Vasopressin was administered intramuscularly in the following doses: 20 units immediately before the surgical operation and each 5 units at 0, 15, 30, and 45 min after the beginning of perfusion experiment.
b) The values represent the mean±standard deviation of the mean for 3 determinations.
c) significantly different from value without vasopressin, $p < 0.01$
d) not significantly different from corresponding value without vasopressin, $p > 0.05$
e) significant different from corresponding value without vasopressin, $p < 0.05$

Therefore, the results obtained above demonstrate that the solvent drag phenomenon contributes to a great extent towards the intestinal absorption of sulfisoxazole, which exists largely as the ionized form at pH 6.0[9] and is assumed to penetrate mainly through water-filled pores or channels in the epithelial membrane, and thereby that the absorption of the drug is enhanced by the hypotonic solution causing water net loss from the intestinal lumen and inhibited by the hypertonic solution causing water net flux into the lumen. In contrast, the solvent drag may play a minor role in the absorption of salicylamide, which exists mostly as the unionized form at pH 6.0[9] and is assumed to permeate rapidly through the lipid phase of the epithelial membrane.

Effect of Blood Flow on Drug Absorption

Ochsenfahrt and Winne[16] have reported that a decrease in intestinal blood flow can hinder the absorption of certain drugs. More recently, they have also reported[5,17] that water net flux based on the difference of tonicity between the blood and intestinal lumen influences the blood flow in the capillaries near the epithelium, thereby changing the transport capacity of the flowing blood. To elucidate the significance of this blood flow, the blood flow of the *in situ* rat small intestine was measured as portal venous outflow according to the procedure described in the experimental section. Table VI summarizes the results obtained by use of the hypertonic (A), isotonic (C), and hypotonic (E) solutions. The portal venous outflow was significantly enhanced by the hypotonic solution and, in contrast, significantly reduced by the hypertonic solution. Consequently, this result demonstrates that the blood flow changes, which are induced by water net flux occurring in the hypertonic and hypotonic solutions, play an important role in the *in situ* intestinal absorption of the drugs.

Table VI. Blood Flow in the *in Situ* Rat Intestine measured as Portal Venous Outflow

	Blood volume in min[a] (μl/g·body weight)		
	1	2	3
Hypertonic (A)	3.9±0.3	7.0±0.4[b]	9.5±0.3[b]
Isotonic (C)	4.0±0.3	7.6±0.2	10.6±0.1
Hypotonic (E)	6.3±0.1[c]	10.1±0.6[c]	13.1±1.1[c]

a) The values represent the mean ± standard deviation of the mean for 3 determinations.
b) significantly different from corresponding value in the isotonic (C), $p < 0.05$
c) significantly different from corresponding value in the isotonic (C), $p < 0.01$

To examine further the effect of intestinal blood flow on the absorption of salicylamide, sulfisoxazole, and quinine used in this study, the absorption rates of those drugs were determined in the *in situ* small intestine of unanesthetized rats, in which the intestinal blood flow was expected to be faster compared to that in the anesthetized rats. Results are summarized in Table VII. The absorption rates of salicylamide and sulfisoxazole in the unanesthetized rats significantly increased compared to those in the anesthetized rats. In the case of quinine, however, comparison between the unanesthetized and anesthetized rats revealed no difference. Accordingly, it is suggested that the rates of absorption of very rapidly absorbable salicylamide and moderately rapidly absorbable sulfisoxazole are clearly rate-limited by the blood flow and, on the contrary, that the rate of absorption of the more polar and slowly absorbable quinine appears to be independent of the rate of blood flow and diffusion rate-limited.

15) K.H. Soergel, G.E. Whalen, J.A. Harris, and J.E. Geenen, *J. Clin. Invest.*, 47, 1071 (1968).
16) H. Ochsenfahrt and D. Winne, *Life Sci.*, 7, 493 (1968).
17) H. Ochsenfahrt and D. Winne, *Life Sci.*, 11, 1105 (1972).

Table VII. Absorption of Salicylamide, Sulfisoxazole, and Quinine from Isotonic Solution C in the *in Situ* Small Intestine of Unanesthetized Rats

Drug	Absorption rate constant[a] (hr^{-1})	
	Unanesthetized	Anesthetized
Salicylamide	1.59±0.08[b]	1.16±0.06
Sulfisoxazole	0.57±0.02[b]	0.39±0.01
Quinine	0.21±0.01	0.21±0.01

a) The values represent the mean ± standard deviation of the mean for 3 determinations.
b) significantly different from corresponding value in the anesthetized rat, $p < 0.01$

Effect of Intestinal Tissue Fluid Uptake on Drug Absorption

Jackson and Cassidy[18] measured gut fluid uptake using everted sacs of rat small intestine and demonstrated experimentally that this fluid uptake can be accounted for by epithelial cellular swelling. This observation suggests that this increase in cell volume produces an expansion of adjacent cell, resulting in a narrowing of the intercellular aqueous-filled channels and decreasing the effective diameter of these channels.

To examine the effect of tissue fluid uptake on the permeation of the drugs through the intestinal membrane, the water content of rat small intestine used in the *in situ* absorption experiments as described above was measured by the method reported previously.[9] The results obtained are listed in Table VIII. The hypotonic solution E increased the mean water content (%) compared to the value in the isotonic solution. This increase was statistically significant in cases of acetanilide and salicylamide. Whereas the water content of the intestine was slightly decreased by the hypertonic solution A. In the case of the hypotonic solution, it was expected that the intestinal absorption of the ionized drugs such as quinine and sulfisoxazole would be somewhat inhibited by a decrease in the effective diameter of the intercellular channels due to an increase of tissue fluid uptake. Contrary to expectation, however, the absorption of those drugs was significantly enhanced by the hypotonic solution (see Table II).

Table VIII. Water Content (%) of Rat Small Intestine in the Presence of Various Perfusion Solutions

Drug	Water content (%)[a]		
	Hypertonic (A)	Isotonic (C)	Hypotonic (E)
Acetanilide	81.8±0.8[b]	81.7±0.4	83.9±0.1[c]
Salicylamide	74.1±1.3[b]	76.2±1.1	84.7±0.5[c]
Sulfisoxazole	81.5±0.4[c]	83.7±0.8	85.3±0.9[b]
Quinine	78.2±1.1[b]	80.2±0.8	81.4±1.6[b]

a) The values represent the mean±standard deviation of the mean for 3 determinations.
b) not significantly different from corresponding value in the isotonic solution, $p > 0.05$
c) significantly different from corresponding value in the isotonic solution, $p < 0.05$

To investigate further the effect of tissue fluid uptake on the drug absorption, the *in vitro* absorption of sulfisoxazole and salicylamide from hypertonic, isotonic, and hypotonic Krebs-Ringer solutions at pH 7.0 was examined in the everted rat intestine. Results are listed in Table IX. In the case of sulfisoxazole, the hypotonic Krebs-Ringer solution significantly

18) M.J. Jackson and M.M. Cassidy, *Experientia*, **25**, 492 (1969).

inhibited the intestinal transfer and the hypertonic solution had no effect on the intestinal transfer. However, both hypotonic and hypertonic Krebs-Ringer solutions has no effect on the intestinal transfer of salicylamide. Mayersohn and Gibaldi[3] measured intestinal tissue fluid uptake in the hypotonic and hypertonic buffers to test the relationship between gut fluid uptake and inhibition of drug transfer in the everted rat intestine. They showed that tissue fluid uptake was observed in the hypotonic buffer and, in contrast, that a net loss of fluid from the tissue was observed in the case of the hypertonic buffer. From these results, it is assumed that the transfer of sulfisoxazole, which exists mostly in the ionized form,[9] is inhibited by tissue fluid uptake resulting in a decrease of intercellular space and that the transfer of salicylamide, which exists mostly in the unionized form and is lipid soluble,[9] is not influenced by tissue fluid uptake on account of the independence upon use of the intercellular channels and primarily involvement of the movement *via* a lipid route.

Table IX. Influence of Hypotonic and Hypertonic Krebs-Ringer Solutions on Transfer of Sulfisoxazole and Salicylamide across Everted Rat Intestine

Drug	Experiment	Amount transferred in 2 hr	Percent of isotonic experiment
		(μg/g·wet tissue)	
Sulfisoxazole	isotonic	503.6±19.0	—
	hypotonic	428.0±25.7[b]	85.0
	hypertonic	510.3±17.5	101.8
		(mg/g·wet tissue)	
Salicylamide	isotonic	7.93±0.25	—
	hypotonic	8.08±0.09	101.8
	hypertonic	8.03±0.12	101.2

a) The values represent the mean ± standard deviation of the mean for 3 determinations.
b) significantly different from value in the isotonic solution, $p < 0.05$

Therefore, the *in situ* experimental data described above demonstrate that tissue fluid uptake plays a minor role in the absorption of the ionized drugs such as sulfisoxazole and quinine in the *in situ* rat intestine in contrast with the everted intestine which lacks a blood supply and an effective solvent drag and, consequently, that the absorption of those drugs is not inhibited by the hypotonic solution.

From the findings described above, we may conclude as follows; (1) the *in situ* rat small intestinal absorption of acetanilide, salicylamide, sulfisoxazole, and quinine increases in the hypotonic solutions and decreases in the hypertonic solutions; (2) the change of the intestinal absorption of those drugs are due to water net flux occurring in the hypertonic and hypotonic solutions; (3) the solvent drag and the rate of blood flow are the two significant components involved in the effect of water net flux on the absorption of the drugs through the intestinal membrane; the rate of intestinal blood flow plays an important role in the absorption of the lipid soluble and very rapidly absorbable drugs such as acetanilide and salicylamide, the solvent drag plays a significant role in the absorption of the more polar and slowly absorbable quinine, and both blood flow and solvent drag are effective for the absorption of sulfisoxazole, which exists mostly in the ionized form at pH 6.0 and is moderately rapidly absorbed; (4) the change of drug concentration in the intestinal lumen and intestinal tissue fluid uptake based on water net flux play a minor role in the *in situ* intestinal absorption of the drugs.

Acknowledgement The authors wish to thank Miss H. Nishimura for her excellent technical assistance in a part of this work.