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Factors influencing Absorption and Excretion of Drugs. V.[1] Further Study on Effect of Potassium Ion on *in Situ* Rat Intestinal Absorption of Drugs

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The effect of K^+ on the absorption of acetanilide, salicylamide, sulfisoxazole, and quinine from rat small intestine was studied using principally the *in situ* perfusion technique.

1) The intestinal absorption of those drugs was considerably reduced by K^+.

2) The decrease in the blood flow of the small intestine, which is induced by the depression of cardiac action based on the absorption of K^+ from the K or Na–K phosphate buffer solution, markedly inhibits the *in situ* intestinal absorption of the drugs.

3) It is suggested that the decrease in the intestinal tissue respiration in the presence of K^+ produces a depression of functional integrity of the intestinal membrane resulting in the histological changes of the intestinal mucosa, and that a decrease in the absorptive surface area based on the morphological changes in the villi somewhat reduces the absorption of the drugs.

It has been reported that potassium ion (K^+) present in a drug buffer solution can markedly influence the passive transfer of several drugs across the everted rat intestine. For example, the passive transfer of several sulfonamides,[3] riboflavin,[4] salicylate,[4,5] and digoxin and ouabain[6,7] across the everted rat intestine was significantly reduced in the presence of a K^+ buffer. With regard to such a phenomenon, it has been suggested[4,8,9] that the uptake of water by the intestinal membrane increases as a function of the K^+ causing a marked increase in the epithelial cellular swelling and that the increase in cell volume produces an expansion of adjacent cell, resulting in a narrowing of the aqueous-filled channels existing between the adjacent cells and decreasing the effective diameter of these channels.

More recently, Kojima, *et al.*[10] reported that K^+ inhibited the absorption of several drugs in an *in situ* rat small intestine and that such an inhibitory effect of K^+ could be accounted for by a narrowing of the intercellular channels due to tissue fluid uptake in the rat small intestine. The present study was conducted to examine in detail various factors responsible for the inhibitory effect of K^+ on the absorption of several drugs in an *in situ* rat small intestine. The drugs such as acetanilide, salicylamide, sulfisoxazole, and quinine were selected on the basis of the difference of ionic nature at physiological pH.

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2) Location: *5-1 Oehon-machi, Kumamoto, 862, Japan.*
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Experimental

Materials and Equipment——Sulfisoxazole and salicylamide were of JP VIII grade. Acetanilide, quinine sulfate, and other chemicals were of reagent grade.

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

Preparation of Sample Solutions——The components of isotonic Na, Na–K, and K phosphate buffer solutions used as the medium in an *in situ* experiment are listed in Table I.

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 Isotonic Na, Na–K, and K Phosphate Buffer Solutions used in the *in Situ* Rat Experiment

Buffer solution[a)]	pH	$NaH_2PO_4 \cdot 2H_2O$ g/liter	KH_2PO_4 g/liter	NaCl g/liter	KCl g/liter
Isotonic Na^+	6.0	12.880	—	4.500	—
Isotonic Na^+–K^+ (2: 1)	6.0	17.160	—	—	3.930
Isotonic Na^+–K^+ (1: 1)	6.0	12.480	—	—	6.090
Isotonic Na^+–K^+ (1: 1)	6.0	7.800	—	—	8.230
Isotonic K^+	6.0	—	11.200	—	5.230

Each buffer solution was adjusted to pH 6.0 with 2N NaOH or 2N KOH solution.
a) The values in parentheses represent the molar ratio of Na^+ to K^+.

Test Animals——Male Wistar rats weighing 200—250 g were used. The rats were fasted 17—20 hr with drinking water *ad libitum* prior to the experiment. They were housed in cages having wide mesh floors to prevent coprophagy.

***In Situ* Rat Experimental Procedures**——The procedures of *in situ* absorption experiments from the rat small intestine were carried out according to the perfusion method described in the previous paper[1)] and the method of Doluisio, *et al.*[11)]

Measurement of Portal Blood Flow in Rat——The blood flow of the *in situ* rat small intestine was measured as portal venous outflow according to the procedure reported in the previous paper from this laboratory.[1)]

Measurement of Oxygen Consumption of *in Vitro* Rat Small Intestinal Tissue——Ten milliliters of the isotonic Na or Na–K (1: 1) phosphate buffer solution was introduced into the *in situ* rat small intestine preparation provided by the method of Doluisio, *et al.*[11)] After 1 hr, the rat was killed. The jejunal part was immediately removed, chilled in the ice-cold isotonic buffer solution, and cut into slices about 0.1 mm thick with a blade. Five to ten mg (dry weight) of slice was added to Warburg's flasks containing 3.0 ml of Tyrode's solution (pH 7.4) or a modified Tyrode's solution (pH 7.4) in which Na^+ was quantitatively replaced with K^+. A small piece of filter paper imbued with 0.2 ml of 20% KOH was added to the center well and the flasks were shaken in a bath for 1 hr at 37.5°. A Qo_2 was calculated from Eq. (1).

$$Qo_2\,(\mu l/mg/hr) = \frac{\text{oxygen uptake } (\mu l/hr) \times \text{flask constant}}{\text{tissue dry weight (mg)}} \qquad (1)$$

Histological Method——At the end of 2 hr perfusion, the rat jejunal part was taken off, washed with physiologic saline, and immediately fixed in 3% formalin. The jejunal tissue was washed with water, dehydrated in ethanol, embedded in paraffin, serially sectioned at 5 μ thickness, and stained with hematoxylin-eosin.

Analytical Procedures——(i) Acetanilide, salicylamide, sulfisoxazole, and quinine were analyzed spectrophotometrically as described previously.[1)]

(ii) The analyses of Na and K in the perfusion solutions were performed by atomic absorption spectrophotometry according to the procedure reported in the previous paper.[1)]

(iii) Na and K in Rat Serum: After 1 hr recirculation on the isotonic Na or K phosphate buffer solution, the blood was taken by performing a cardio-puncture and the serum was obtained by centrifuging the blood sample at 3000 rpm for 30 min. Na and K in the serum were analyzed by the method described above.

11) J.T. Doluisio, N.F. Billups, L.W. Dittert, E.T. Sugita, and J.V. Swintosky, *J. Pharm. Sci.*, **58**, 1196 (1969).

Result and Discussion

Effect of K^+ on Small Intestinal Absorption of Drugs

In order to understand in advance the effects of the concentrations of both Na^+ and K^+ in the luminal solution on the drug absorption, the absorption of sulfisoxazole was examined in the *in situ* rat small intestine preparation provided by the procedure of Doluisio, *et al.*[11] The absorption rate constants of sulfisoxazole from the isotonic phosphate buffer solutions with various molar ratios of Na^+ to K^+ are summarized in Table II. The absorption of the drug was significantly reduced by replacing Na^+ with K^+ in the media. In cases of the buffer solutions in which Na^+ was replaced by K^+ to give the molar ratio of K^+/Na^+ 1 or above, the extent of the decrease in the drug absorption was approximately the same.

Table II. Effect of K^+ on the Absorption of Sulfisoxazole from the *in Situ* Rat Small Intestine

Buffer solution[a]	Absorption rate constant[b] (hr^{-1})	Percent inhibition of absorption
Isotonic Na^+ buffer	1.29 ± 0.02	—
Isotonic Na^+–K^+ buffer (2:1)	1.16 ± 0.01[c]	−10.1
(1:1)	0.98 ± 0.02[c]	−24.1
(1:2)	0.96 ± 0.01[c]	−25.6
Isotonic K^+ buffer	0.86 ± 0.03[c]	−33.3

a) The values in parentheses represent the molar ratio of Na^+ to K^+.
b) The values represent the mean ± standard deviation for 3 animals.
c) significantly different from the value in the isotonic Na^+ buffer, $p<0.01$

Subsequently, the absorption of acetanilide, salicylamide, sulfisoxazole, and quinine in the isotonic Na–K (1:1) phosphate buffer solution at pH 6.0 was examined by the perfusion method using the *in situ* rat small intestine. As shown in Table III, the results showed that the absorption of those drugs was considerably reduced by K^+ and that the percent inhibition of the absorption of quinine, which exists mostly as the ionized from in the buffer solution at pH 6.0, was smaller than other drugs. In the previous study[10] performed by the method of Doluisio, *et al.*[11] using about 10 ml of luminal solution, however, the absorption of acetanilide and salicylamide was inhibited to less extent in the presence of K^+ buffer. As to such a phenomenon, it was suggested that K^+ caused the swelling of the intestinal tissue, resulting in a

Table III. Effect of K^+ on Absorption of Several Drugs from the *in Situ* Rat Small Intestine by Perfusion Method

Drug	Absorption rate constant[a] (hr^{-1})	
	Isotonic Na^+ buffer	Isotonic Na^+–K^+ (1:1) buffer
Acetanilide	0.79 ± 0.04	0.57 ± 0.03[b] (−27.9)
Salicylamide	1.16 ± 0.06	0.81 ± 0.05[b] (−30.2)
Sulfisoxazole	0.39 ± 0.01	0.28 ± 0.03[b] (−28.2)
Quinine	0.21 ± 0.01	0.17 ± 0.04[b] (−19.1)

a) The values represent the mean ± standard deviation for 3 animals.
b) significantly different from corresponding value in the isotonic Na^+ buffer, $p<0.01$
The values in parentheses represent percent inhibition of absorption.

narrowing of the aqueous-filled channels existing between the adjacent cells and decreasing the effective diameter of these channels. In addition, it was considered that the transfer of the unionized drugs such as acetanilide and salicylamide, which are lipid soluble and probably independent of use of the intercellular channels, was affected to less extent by K^+. However, the present study performed by the perfusion method using about 40 ml of perfusate shows that the absorption of acetanilide and salicylamide is inhibited to greater extent in the presence of K^+ buffer.

In order to elucidate the inhibitory effect of K^+ on the absorption of the drugs, further study was performed by dividing into the following components: (a) the movement of Na^+ and K^+ in the perfusion solutions, (b) the effect of K^+ on the blood flow of the *in situ* rat small intestine, and (c) the effect of K^+-induced histological change on the permeability of small intestinal membrane.

Movement of Na^+ and K^+ in Perfusion Solutions

The movement of Na^+ and K^+ in the perfusion solutions in the *in situ* rat small intestine was measured by use of the isotonic phosphate buffer solutions with various molar ratios of Na^+ to K^+. The results are summarized in Figs. 1 and 2. The absorption of K^+ from the luminal solutions proceeded by the first-order kinetics and 50 to 65% of K^+ in those perfusates was absorbed from the intestine during 2 hr perfusion. In contrast, in all buffer solutions, Na^+ was excreted into the intestinal lumen through the intestinal wall. As shown in Table IV, in cases of all Na^+-K^+ buffers, millimoles of K^+ absorbed from the intestine were approximately the same as those of Na^+ excreted into the intestinal lumen. This result demonstrates the maintenance of total concentrations of Na^+ and K^+ and the isotonicity in each perfusion solution. Thus, it may be not necessary to take into account the effect of water net flux in the intestinal membrane on the absorption of the drugs.

Effect of K^+ on Blood Flow of Small Intestine

As can be seen from Fig. 2 and Table IV, K^+ in the perfusates was considerably absorbed from the small intestine. Thereupon, the serum levels of Na^+ and K^+ were measured in the

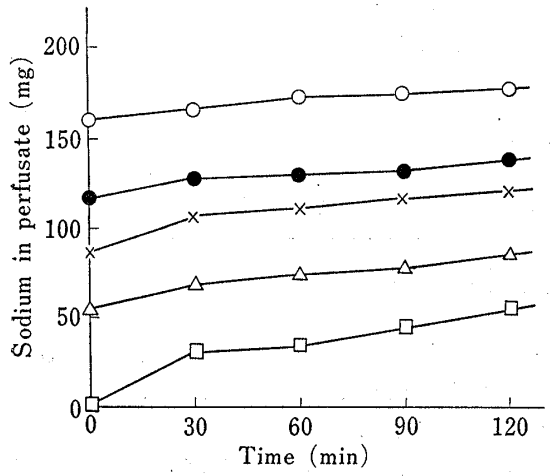

Fig. 1. Amount of Sodium of Isotonic Na, Na–K, and K Phosphate Buffer Solutions in the *in Situ* Rat Intestinal Preparation

Each value is expressed as the mean of 3 animals.
—○—: isotonic Na^+ buffer
—●—: isotonic Na^+–K^+ (2:1) buffer
—×—: isotonic Na^+–K^+ (1:1) buffer
—△—: isotonic Na^+–K^+ (1:2) buffer
—□—: isotonic K^+ buffer

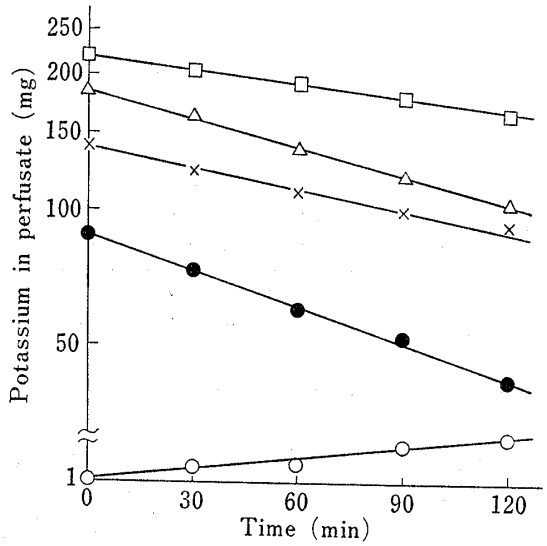

Fig. 2. Amount of Potassium of Isotonic Na, Na–K, and K Phosphate Buffer Solutions in the *in Situ* Rat Intestinal Preparation

Each value is expressed as the mean of 3 animals.
—○—: isotonic Na^+ buffer
—●—: isotonic Na^+–K^+ (2:1) buffer
—×—: isotonic Na^+–K^+ (1:1) buffer
—△—: isotonic Na^+–K^+ (1:2) buffer
—□—: isotonic K^+ buffer

Table IV. Net Flux of Na^+ and K^+ of Isotonic Na–K Phosphate Buffer Solutions in the *in Situ* Rat Small Intestine

Buffer solution[a]	Amount in perfusate[b] (m mole)				Net flux[c] (m mole)	
	Initial		Final			
	Na^+	K^+	Na^+	K^+	Na^+	K^+
Na^+–K^+ buffer (2:1)	5.2	2.1	5.9	1.2	+0.7	−0.9
(1:1)	4.1	3.5	5.2	2.5	+1.1	−1.0
(1:2)	2.6	4.7	3.7	3.1	+1.1	−1.7

a) The values in parentheses represent the molar ratio of Na^+ to K^+ in the buffers.
b) The values represent the mean of 3 animals.
c) positive sign: net flux into intestinal lumen;
negative sign: net flux out of intestinal lumen

rats perfused with the isotonic Na^+ and K^+ buffer solutions. The results are shown in Table V. In the rats treated with Na^+ buffer, the serum levels of Na^+ and K^+ were approximately the same as those of intact rats. However, in the rats treated with K^+ buffer, although the serum level of Na^+ was almost the same as that in the intact rats, the serum level of K^+ showed an abnormally high value, that is about 800 μg/ml (20 mEq/liter). From these results, it is suggested that the serum level of K^+ in the rats perfused with Na^+-K^+ (1:1) buffer is considerably higher than in the intact rats. It has been known[12] that when potassium chloride is administered by slow intravenous infusion to dogs, the changes in electrocardiogram appear at the serum levels of 5 to 8 mEq/liter of K^+, the intraventricular block at 10 mEq/liter, and the cardiac arrest at 14 to 16 mEq/liter. Thus, it is speculated that K^+ absorbed markedly depresses the cardiac action, resulting in a decrease of the intestinal blood flow.

Table V. Serum Levels of Na^+ and K^+ in Rats following Perfusion with Isotonic Na and K Phosphate Buffer Solutions

	Serum level[a] (μg/ml)	
	Na^+	K^+
Intact rat	3025±123	213± 21
Rat with Na^+ buffer	2875±215	188± 32
Rat with K^+ buffer	3067±340	797±115[b]

a) The values represent the mean ± standard deviation for 3 to 5 animals.
b) significantly different from the value in intact rat, $p<0.01$

Moreover, in order to clarify the effect of K^+ on the intestinal blood flow, the blood flow of the *in situ* rat small intestine was measured as portal venous outflow. Table VI shows the results obtained by use of the isotonic Na and K phosphate buffer solutions at pH 6.0. The portal venous outflow was significantly reduced by the K^+ buffer. It has been reported[1,13,14] that a decrease in intestinal blood flow can hinder the absorption of certain drugs.

Thus, these results demonstrate that the decrease in blood flow, which is induced by the depression of cardiac action occurring in the presence of the K^+ buffer, markedly inhibits the

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14) W.G. Crouthamel, L. Diamond, L.W. Dittert, and J.T. Doluisio, *J. Pharm. Sci.*, **64**, 664 (1975).

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

Buffer solution	Blood volume in min[a] (μl/g. body weight)		
	1	2	3
Isotonic Na^+ buffer	4.0±0.3	7.6±0.2	10.6±0.1
Isotonic K^+ buffer	3.2±0.4[b]	5.3±0.2[b]	6.0±0.3[b]

a) The values represent the mean ± standard deviation for 3 animals.
b) significantly different from corresponding value in the isotonic Na^+ buffer, $p<0.01$

intestinal absorption of the drugs. This is also supported by the findings that the absorption of acetanilide, salicylamide, and sulfisoxazole, which appear to be clearly rate-limited by the blood flow,[1] is inhibited to greater extent by K^+ and, on the contrary, that the absorption of quinine, which appears to be diffusion rate-limited,[1] is reduced to less extent by K^+.

Effect of K^+-induced Histological Change on Permeability of Small Intestinal Membrane

To obtain further information on the inhibitory effect of K^+ on the absorption of the drugs, the oxygen consumption of *in vitro* rat small intestinal tissue was measured by Warburg's manometer. Table VII shows the results obtained by use of Tyrode's solution and a modified Tyrode's solution in which Na^+ was quantitatively replaced with K^+. The oxygen consumption of the intestinal tissue was significantly depressed by K^+. This phenomenon demonstrates that K^+ inhibits the basal metabolism in the intestinal tissue, resulting in a depression of functional integrity of the intestinal membrane.

Moreover, the features of small intestinal structure in normal control rat and in K^+-treated one were examined histologically. Fig. 3a—3c show the photomicrographs of jejunal mucosa of the normal control and Na or K phosphate buffer-treated rats. The villous structure of the jejunal mucosa after 2 hr recirculation on the isotonic Na phosphate buffer solution at pH 6.0 was almost the same as that in the normal control. This indicates that the mucosal integrity of the rat treated with the buffer is essentially maintained. In the rat treated with the isotonic K phosphate buffer solution at pH 6.0, however, the morphological changes in the villous structure such as the slight lesions of the villous tips were observed (see Fig. 3c).

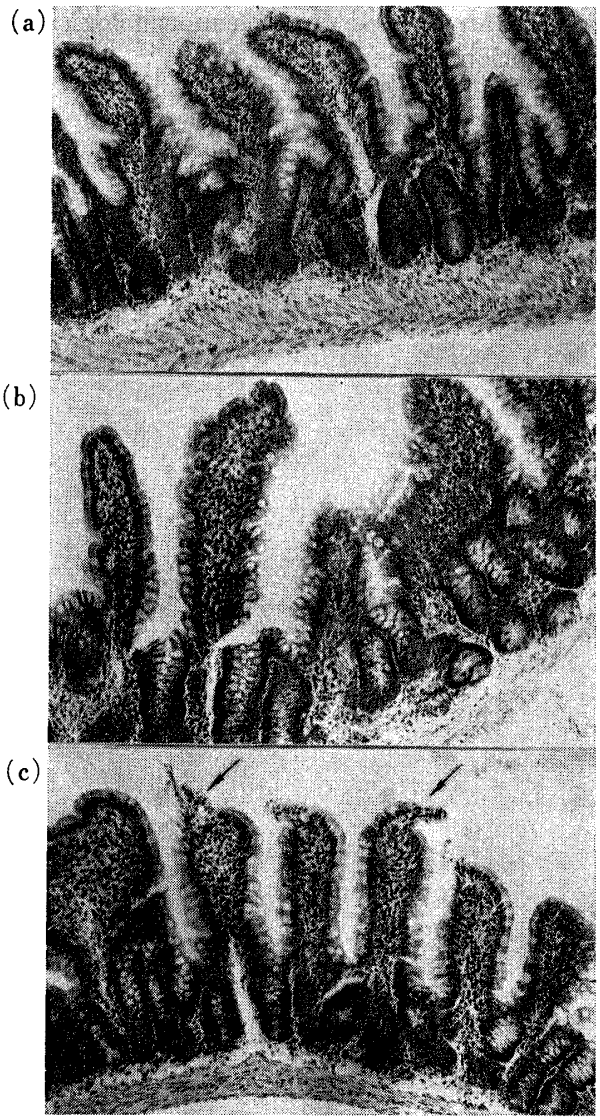

Fig. 3. Photomicrographs of Rat Jejunal Mucosa

(a) intact rat (×100)
(b) rat perfused with isotonic Na phosphate butter (pH 6.0) (×100)
(c) rat perfused with isotonic K phosphate buffer(pH 6.0) (×100)
The arrows indicate the slight lesions of the villous tips of rat jejunum.

TABLE VII. Oxygen Consumption of Rat Small Intestinal Tissue *in Vitro*

Incubation medium	Qo_2[a)]	Percent control
Tyrode's solution	6.4±0.7	—
Modified Tyrode's solution[b)]	2.7±0.7[c)]	42.2

a) The values represent the mean ± standard deviation for 8 or 9 determinations.
b) This solution was prepared by replacement of Na^+ with K^+ in Tyrode's solution.
c) significantly different from the value in Tyrode's solution, $p<0.01$

Thus, these observations suggest that the decrease in the tissue respiration in the presence of K^+ produces a depression of functional integrity of the intestinal membrane, resulting in the histological changes of the intestinal mucosa, and that a decrease in the absorptive surface area based on such morphological changes in the villi may somewhat influence the absorption of the drugs.

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