so far as the fluorometric measurement was used. However, the maximum sensitivity was about a tenth in comparison with the enzyme-isotopic method.[11]

In the dog brain, the highest concentration of histamine was found in the posterior hypothalamus (819 and 641 ng/g, $n=2$). Following values were obtained in other regions: 498 and 424 ng/g in the anterior hypothalamus, 331 and 248 ng/g in the thalamus, 151 and 143 ng/g in the caudate nucleus, 153 and 138 ng/g in the midbrain and 129 and 101 ng/g in the area postrema. The low concentrations (<100 ng/g) were found in the occipital cortex, the cerebellum, the pons, the medulla and the spinal cord. The similar distribution of histamine was reported in the human brain as well as the experimental animal brain.[12] Schwartz, *et al.*[13] found the highest histidine decarboxylase activity in the hypothalamus of the rat brain. Adam[14] has reported the highest concentration of histamine in the area postrema of the dog brain, but our result showed a low value. The reason for this discrepancy is not clear. The present finding and the results reported by previous workers revealed that 1) the hypothalamus contained the highest concentration of histamine in the brain of the experimental animal, 2) histamine in the rat brain was largely found in particles with sedimentation properties in sucrose gradients similar to synaptic vesicles storing noradrenaline and 5-hydroxytryptamine,[15,16] 3) the histamine content in the cat brain was altered after the treatment with drugs,[17] and 4) the formation of histamine from histidine was demonstrated in the cat brain.[18] These facts may indicate non-mast cell site for histamine storage and some role of histamine in the regulation of neuronal activity in the hypothalamus, where mast cells are scarce if present at all.

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

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Some investigators have reported that various monovalent cations such as K^+, NH_4^+, Li^+, and guanidine$^+$ present in a drug-buffer solution can have a marked influence on the passive transfer of several drugs across the everted rat intestine. For example, Nogami, *et al.*[3]

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2) Location: *5-1 Oehon-machi, Kumamoto.*
3) H. Nogami, M. Hanano, and M. Aruga, *Chem. Pharm. Bull.* (Tokyo), **14**, 166 (1966).

have shown that when K^+ replaces Na^+ as the principal cation in the drug solution, there is a decreased transfer rate of several sulfonamides across the everted rat intestine. Mayersohn, *et al.*[4] reported that the presence of K^+ in a drug-buffer solution resulted in a significant reduction in the transfer of riboflavin, salicylate, and sulfanilamide across the everted rat intestine. Caldwell, *et al.*[5,6] showed that the passive transfer of digoxin and ouabain across the everted rat intestine was significantly reduced in the presence of a K^+ buffer. In addition, Benet, *et al.*[7] observed on the influence of K^+ on salicylate transfer.

It has been suggested that K^+ increases intestinal tissue water uptake, resulting in an inhibition of passive intestinal transfer of drug. In order to clarify further this phenomenon, the present study was conducted to examine the effect of K^+ on the absorption of several drugs in an *in situ* rat small intestine and to elucidate the nature of the inhibitory effect of K^+.

Experimental

Materials and Equipment——Sulfisoxazole, salicylic acid, and salicylamide were of JP VIII grade. Sulfanilamide, sulfathiazole, and chloramphenicol were commercial products. Acetanilide, phenol red, and other chemicals were of reagent grade. A Shimadzu QV-50 spectrophotometer and a Hitachi-Horiba F-5 pH meter were utilized.

Preparation of Sample Solutions——The components of isotonic phosphate buffer solutions used as the medium are listed in Table I. The drugs examined and the initial concentrations used were sulfanilamide (200 μg/ml), sulfathiazole (200 μg/ml), sulfisoxazole (500 μg/ml), acetanilide (250 μg/ml), chloramphenicol (400 μg/ml), salicylic acid (1000 μg/ml), and salicylamide (1000 μg/ml).

Table I. Preparation of Isotonic Phosphate Buffer Solutions

	pH	$NaH_2PO_4 \cdot 2H_2O$ g/liter	Na_2HPO_4 g/liter	KH_2PO_4 g/liter	K_2HPO_4 g/liter	NaCl g/liter	KCl g/liter
Isotonic Na-buffer	6.0[a]	12.880	—	—	—	4.500	—
	7.5	2.496	10.590	—	—	1.730	—
Isotonic K-buffer	6.0[b]	—	—	11.200	—	—	5.333
	7.5	—	—	2.177	14.631	—	1.733

a) adjusted to pH 6.0 with 2N NaOH solution *b*) adjusted to pH 6.0 with 2N KOH solution

Test Animals——Male Wistar rats weighing 140—250 g were used. The rats were fasted 17—20 hr prior to use, but drinking water was allowed *ad libitum*. The rats were kept in cages having wide mesh floors to prevent coprophagy.

***In Situ* Rat Experimental Procedure**——The procedure for studying drug absorption in the *in situ* rat gut was carried out according to the method of Doluisio, *et al.*[8]

The extent of pH shift of the sample solution in the intestinal lumen during the experiment was 5.9 to 6.4 or 6.8 to 7.4 at initial sample solution of pH 6.0 or 7.5, respectively.

Determination of Water Content in Rat Small Intestine——At the end of the *in situ* rat gut experiments using the isotonic phosphate buffer solutions or drug solutions, the rat was sacrificed. The small intestine removed was cut into several segments about 5 cm long. The segments were cut open, carefully washed with EtOH and ether successively to remove any excess fluid adhering to the membranes, and then air-dried on a filter paper for about 5 min. The intestinal tissue weight was determined and water content in this tissue was determined by a distillation method.[9] Water content (%) was calculated from Eq. (1).

4) *a*) M. Mayersohn and M. Gibaldi, *J. Pharm. Sci.*, **58**, 1429 (1969); *b*) M. Mayersohn and M. Gibaldi, *Biochim. Biophys. Acta*, **196**, 296 (1970).
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$$\text{water content } (\%) = \frac{\text{water volume (ml)}}{\text{intestinal tissue weight (g)}} \times 100 \qquad \text{(Eq. 1)}$$

Determination of Partition Coefficients——Each sample solution containing sulfanilamide (20 μg/ml), sulfathiazole (20 μg/ml), sulfisoxazole (30 μg/ml), acetanilide (10 μg/ml), chloramphenicol (100 μg/ml), salicylic acid (400 μg/ml), or salicylamide (400 μg/ml) was prepared by dissolving in the Na phosphate buffer solutions of pH 6.0 and 7.5. To 5 ml of sample solution the equal volume of $CHCl_3$ was added. These were kept in a water-bath at 37° with vigorous shaking every 5 min for 1 hr. Then the sample content was determined against aqueous layer, and partition coefficients were calculated by Eq. (2).

$$\text{partition coefficient} = \frac{\text{initial concentration of aqueous layer} - \text{equilibrated concentration of aqueous layer}}{\text{equilibrated concentration of aqueous layer}} \qquad \text{(Eq. 2)}$$

Analytical Procedures——Sulfanilamide, sulfathiazole, and sulfisoxazole were analyzed spectrophotometrically using the method of Bratton and Marshall.[10] Chloramphenicol was analyzed spectrophotometrically by the method Kakemi, *et al.*[11] Acetanilide was extracted from the samples with $CHCl_3$ and the absorbance of the $CHCl_3$ solution was determined at 246 mμ. Salicylic acid and salicylamide were extracted from the samples acidified with 3N HCl with $CHCl_3$. The $CHCl_3$ phase was then extracted with ferric nitrate reagent (1% $Fe(NO_3)_3$ in 0.07N HNO_3). The absorbance of the ferric nitrate reagent phase was determined at 530 mμ.

Result and Discussion

To understand the influence of K^+ on the intestinal absorption of the drugs such as chloramphenicol, acetanilide, sulfanilamide, sulfathiazole, sulfisoxazole, salicylic acid, and salicylamide, the absorption of these drugs in Na and K phosphate buffer solutions at the initial pH 6.0 was examined in the *in situ* rat small intestine preparation. The absorption rate constant of each drug was calculated from the slope of the line on the semilogarithmic plots of drug concentration in the rat intestinal lumen *vs* time. The absorption rate constants of the drugs in the Na^+ and K^+ buffer solutions and the percent inhibition of absorption in the K^+ buffer solution are summarized in Table II. When Na^+ was replaced by K^+ in the buffer solutions, there was a significant decrease in the absorption of all drugs other than acetanilide. Thus, the absorption of sulfisoxazole, salicylic acid, and sulfanilamide were inhibited to greater extent in the presence of the K^+ buffer, whereas the absorption of chloramphenicol,

Table II. *In Situ* Rat Small Intestinal Absorption of Several Drugs from Na^+ and K^+ Buffers at Initial pH 6.0

Drug	Absorption rate constant[a] (min^{-1})		Percent inhibition of absorption in K^+[b]	Level of significance[c] Na^+ *vs.* K^+
	Na^+ buffer	K^+ buffer		
Chloramphenicol	0.0186±0.0006 (3)	0.0155±0.0013 (4)	16.7	$p<0.05$
Acetanilide	0.0383±0.0040 (4)	0.0370±0.0020 (4)	3.4	n.s.
Sulfanilamide	0.0157±0.0009 (3)	0.0107±0.0025 (5)	31.8	$p<0.001$
Sulfathiazole	0.0098±0.0001 (3)	0.0086±0.0001 (3)	12.2	$p<0.001$
Sulfisoxazole	0.0222±0.0007 (3)	0.0148±0.0005 (3)	33.3	$p<0.001$
Salicylamide	0.0522±0.0056 (6)	0.0438±0.0002 (2)	16.0	$p<0.05$
Salicylic acid	0.0760±0.0016 (5)	0.0470±0.0014 (4)	38.2	$p<0.001$

a) The absorption rate constant is expressed as the mean±standard deviation followed by the number of experiments in parentheses.

b) percent inhibition $= \left[1 - \frac{\text{absorption rate constant in } K^+ \text{ buffer}}{\text{absorption rate constant in } Na^+ \text{ buffer}}\right] \times 100$

c) analyzed by Student's t test; n.s.=not significant ($p>0.05$)

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salicylamide, and sulfathiazole were inhibited to less extent. And the absorption of acetanilide was little reduced by K^+.

Also a similar investigation was carried out using Na and K phosphate buffer solutions at the initial pH 7.5. The first-order rate constants for the absorption of the drugs mentioned above from rat small intestine and the percent inhibition of absorption in the K^+ buffer solution are shown in Table III. The results show a significant decrease in the absorption rate of the drugs other than acetanilide in the presence of K^+ buffer. Replacing Na^+ with K^+ in the buffer solution inhibited to greater extent the intestinal absorption of sulfisoxazole, sulfathiazole, salicylic acid, and sulfanilamide. When the pH of the buffer solution was risen from 6.0 to 7.5, the K^+ inhibition of absorption increased markedly in sulfathiazole and sulfisoxazole, and scarcely changed in chloramphenicol, acetanilide, sulfanilamide, salicylamide, and salicylic acid.

Table III. *In Situ* Rat Small Intestinal Absorption of Several Drugs from Na^+ and K^+ Buffers at Initial pH 7.5

Drug	Absorption rate constant[a] (min^{-1})		Percent inhibition of absorption in K^+[b]	Level of significance[c] Na^+ *vs.* K^+
	Na^+ buffer	K^+ buffer		
Chloramphenicol	0.0190±0.0002 (3)	0.0153±0.0006 (3)	19.5	$p<0.05$
Acetanilide	0.0369±0.0016 (3)	0.0353±0.0038 (3)	4.3	n.s.
Sulfanilamide	0.0148±0.0016 (3)	0.0101±0.0013 (3)	31.8	$p<0.05$
Sulfathiazole	0.0089±0.0007 (3)	0.0048±0.0001 (3)	46.1	$p<0.005$
Sulfisoxazole	0.0112±0.0004 (3)	0.0033±0.0002 (3)	70.5	$p<0.001$
Salicylamide	0.0552±0.0031 (3)	0.0448±0.0014 (2)	18.8	$p<0.05$
Salicylic acid	0.0372±0.0027 (4)	0.0240±0.0020 (4)	35.5	$p<0.001$

a) The absorption rate constant is expressed as the mean±standard deviation followed by the number of experiments in parentheses.

b) percent inhibition $= \left[1-\frac{\text{absorption rate constant in } K^+ \text{ buffer}}{\text{absorption rate constant in } Na^+ \text{ buffer}}\right]\times 100$

c) analyzed by Student's t test; n.s.=not significant ($p>0.05$)

The percent of unionized form and the partition coefficient between $CHCl_3$ and the buffer solution at pH 6.0 or 7.5 of each drug used in this experiment are listed in Table IV. From these data, it is understood that the intestinal absorption of sulfisoxazole and salicylic acid, which exist mostly as the ionized forms in both buffer solutions at pH 6.0 and 7.5, is markedly inhibited in the presence of K^+, and that the absorption of chloramphenicol, acetanilide, and salicylamide, which exist mostly as the unionized forms in both buffer solutions, is least affected by K^+. The absorption of sulfathiazole, which exists largely as the unionized form in the buffer

Table IV. Percent of Unionized Forms and Partition Coefficients of Several Drugs

Drug	pK_a	Percent unionized form[a]		Partition coefficient[b] ($CHCl_3$, 37°)	
		pH 6.0	pH 7.5	pH 6.0	pH 7.5
Chloramphenicol	—	100	100	0.27	0.27
Acetanilide	0.3	100	100	5.12	4.37
Sulfanilamide	10.6	100	100	0.04	0.04
Sulfathiazole	7.1	92.6	28.4	0.12	0.05
Sulfisoxazole	5.0	9.1	0.3	0.25	0.05
Salicylamide	8.3	99.5	86.3	2.52	1.88
Salicylic acid	3.0	0.1	0.003	0.02	0.02

a) calculated using the Henderson-Hasselbalch equation

b) values between $CHCl_3$ and Na^+ buffer at pH 6.0 or 7.5

solution at pH 6.0, is also inhibited to less extent by K^+. The percent of ionized forms of sulfathiazole and sulfisoxazole in the buffer solution at pH 7.5 was greater than that in the buffer solution at pH 6.0 and the absorption of these drugs was inhibited to greater extent in the buffer solution at pH 7.5. In addition, although the percent of unionized form of sulfathiazole in the Na buffer solution at pH 6.0 is much greater than that in the buffer solution at pH 7.5, the absorption rate of the drug does not appreciably change between those buffer solutions. This may be due to the somewhat poor absorption characteristic of unionized form of sulfathiazole.

In order to clarify further the inhibitory effect of K^+ on the intestinal absorption of these drugs, the water content of rat small intestine was measured by the method indicated in experimental. The results obtained are listed in Table V. In the K^+ buffer solution at pH 6.0, there was a significant increase in the mean water content (%) compared to the Na^+ buffer solution at pH 6.0. Whereas in the buffer solution at pH 7.5 the water content of rat small intestine increased to a small extent by replacement of Na^+ with K^+. These data have been shown K^+ to increase tissue fluid uptake by the rat small intestine. Some reports demonstrate that K^+ can increase tissue water uptake. For example, Bosackova and Crane[12] and Mayersohn, *et al.*[4,13] pointed out that the uptake of water by the intestinal membrane increased as a function of the K^+ causing a marked increase in the swelling of the tissues. Also, Jackson and Cassidy[14] 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. These observations suggest that this increase in cell volume produces an expansion of adjacent cell, resulting in a narrowing of the aqueous-filled channels existing between the adjacent cells (*i.e.*, the intercellular channels) and decreasing the effective diameter of these channels. Assuming that a lipid-insoluble ionized drug penetrates essentially the rat small intestine by movement along the intercellular channels, the penetration of the drug would be markedly inhibited by materials causing intestinal tissue fluid uptake. Thus, it is demonstrated that the small intestinal absorption of the ionized drugs such as sulfisoxazole, sulfathiazole, and salicylic acid is inhibited to greater extent in the presence of K^+.

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

pH of buffer	Perfused time (min)	Water content (%)[a]		Level of significance[b]
		Na^+ buffer	K^+ buffer	Na^+ *vs.* K^+
6.0	30[c]	75.7±4.65 (2)	83.4±0.93 (4)	$p<0.05$
	60	72.6±0.66 (3)	81.4±1.37 (3)	$p<0.01$
	120[d]	77.8±1.99 (4)	83.1±0.71 (3)	$p<0.02$
7.5	30[c]	80.8±1.02 (3)	83.7±0.77 (4)	$p<0.02$
	60	81.3±0.85 (3)	83.6±0.49 (3)	$p<0.05$

a) expressed as the mean±standard deviation followed by the number of experiments in parentheses
b) analyzed by Student's t test
c) measured using the intestine in the absorption experiment of salicylamide
d) measured using the intestine in the absorption experiment of sulfisoxazole

On the other hand, it is considered that the transfer of the unionized drugs such as chloramphenicol, acetanilide, and salicylamide, which are lipid soluble (see Table IV), is not dependent upon use of the intercellular channels and primarily involves movement *via* a lipid route. Consequently, these drugs are affected the least by K^+ (3.4—19.5%).

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13) M. Mayersohn, M. Gibaldi, and B. Grundhofer, *J. Pharm. Sci.*, **60**, 1813 (1971).
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Moreover, although sulfanilamide exists mostly as the unionized form in both buffer solutions at pH 6.0 and 7.5 and is most likely absorbed as the unionized form in these buffer solutions, the absorption of the drug is significantly reduced in the presence of K^+. This phenomenon may illustrate that the unionized form of sulfanilamide is not sufficiently lipid soluble (see the partition coefficient in Table IV), and hence that significant amounts of unionized form of the drug can penetrate through the intercellular channels. In order to examine the effect of various cations on the gastrointestinal absorption of drugs, further studies are now in progress.

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Coumarins from the Roots of *Angelica morii* Hayata

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Angelica morii Hayata (Japanese name "mori-zengo") (Umbelliferae) is a perenial herb growing in Taiwan, and is called "shan-duhuo (山独活)" which is used as a drug of folk remedy in Taiwan.[2] There is no record of chemical investigation on this plant in literatures. This paper presents the results from an investigation of the coumarins in the plant.

The ether extract of the dried root of this plant upon fractionation over the column of silica gel afforded, in addition to small amount of psoralen, bergapten, umbelliferone and *p*-coumaric acid identified by the comparison with the authentic samples, two crystalline compounds of mp 82—84° (I) and mp 119—120° (II). On the basis of the evidences described below the compounds I and II were identified as pteryxin[3] (3′(*R*), 4′(*R*)-3′-acetoxy-4′-angeloyloxy-3′,4′-dihydroseselin)[4] (Ia) and 3′(*R*),4′(*R*)-3′-acetoxy-4′-senecioyoxy-3′,4′-dihydroseselin[4] (IIa), respectively. Furthermore, the presence of the analogous diester (III) of acetic acid and isovaleric acid was suggested by the nuclear magnetic resonance (NMR) spectrum, but the isolation of III has not so far been successful because III can be hardly freed from the contamination by I. The phenolic fraction of the ether extract afforded a crystaline compound of mp 329—330° (decomp.) (IV), which seems to be a bis-coumarin derivative formed of 2 moles of umbelliferone moieties by the spectral data. It is under investigation on the details.

The compound I was suggested to be O-acetyl-O-angeloylkhellactone by the NMR spectrum, and this was confirmed by the treatment of I with ethanolic sodium hydroxide to give (+)-*cis*-ethylkhellactone (Va), (−)-*trans*-ethylkhellactone (Vb), acetic acid and angelic acid. As the compound of this structure two isomeric diesters, Ia (mp 81.5—82.5°)[3] and isopteryxin

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