Chem. Pharm. Bull. 14(2) 166~173 (1966)

UDC 615.41-03 : 615.778.25-092.25

26. Hisashi Nogami, Manabu Hanano, and Masayoshi Aruga*1 : Studies on Absorption and Excretion of Drugs. VI.*2 Effects of Cations and 2,4-Dinitrophenol on the Transport of Sulfonamides through the Small Intestine of Rat.*3

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Investigations of the intestinal absorption have been conducted on a variety of drugs. Most of investigations, however, deal with blood level, urinary excretion and so on, with the clinical interests. The mechanisms of intestinal absorption of drugs are obscure in many cases. It is considered to be important to investigate the mechanisms in order to design oral pharmaceutical preparations with high availability. Bethesda group[1] and Kakemi, *et al.*[2] have studied quantitatively the intestinal absorption of some sulfonamides. The same subjects have been investigated kinetically in our laboratory.[3] These reports have suggested the mechanism of absorption of sulfonamides would be rather complicate. The effects of cations and 2,4-dinitrophenol on the intestinal absorption and transport of sulfonamides in the rat are reported in the present paper.

The inhibition effect of potassium ion was observed by Budolfsen[4] on the absorption of glucose and sodium chloride from the rat small intestine. Czáky[5] reported the active transports of sugar and amino acid were significantly reduced when lithium or potassium ion was substituted for sodium ion in the medium surrounding both mucosal and serosal surfaces of intestine. The necessity of sodium ion for glucose transport was reported by Rikalis and Quastel,[6] and the direct correlation between the concentration of sodium ion in medium and sugar transport was showed by Crane, *et al.*[7] Although sulfonamides are, of course, considered as the "foreign" compounds, it seems to be possible that sodium ion and potassium ion play some roles in the intestinal absorption of sulfonamides. In the preceding report,*2 the strong inhibitory effect of 2,4-dinitrophenol on the absorption of sulfisomezole was reported. In the present experiments, the effects of sodium and potassium ion have been studied and the effects of 2,4-dinitrophenol on the sulfonamides absorption have also been investigated.

Experimental

Sulfonamides

Three investigated sulfonamides were sulfanilamide (SA), sulfisoxazole (SIX), and sulfisomezole (SIM). The abbreviations in the parentheses represent these sulfonamides, respectively.

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Experimental Procedure

Perfusion Experiments *in vivo*——The recirculating perfusion method and apparatus were same as described in the previous paper[8] of this series. 100 ml. of the solution containing sulfonamide was recirculatingly perfused from duodenum to ileum of male rat (Donryu strain : 300±50 g.). Three kinds of perfusion solutions (pH 6.5~6.6) which contained 1 m*M*/L. of sulfonamide and 3 m*M*/L. of phenol red (as volume indicator) were added 154 m*M*/L. of sodium chloride, 154 m*M*/L. of potassium chloride or 280 m*M*/L. of glucose for giving isotonicity, respectively. The pH-values of the perfusion solution were continuously checked with pH-meter (Toadenppa Co., Ltd. model HM-5A) equipped with microelectrodes during the experiment and kept almost constant (pH 6.5~6.6) by means of the titration of *N* HCl. The volume of the perfusion solution was kept practically constant.

Circulation Experiments *in vitro* Using Everted Intestine——The circulation apparatus and method were almost same as described in the previous paper[9] of this series. In the previous experiments, the normal isolated intestine of rat was used, but in the present experiments, the everted loops of small intestine that were prepared by method of Wilson and Wiseman[10] from male rats (Donryu strain : 270±40 g.) were used. Tyrode's fluid was always used for the circulating solution on the serosal side of the everted loops. The solution on the mucosal side contained sulfonamide (1 m*M*/L.). The volume of the both circulation solutions was 80 ml. The circulation was continued for 2 hr. The pH-value of serosal Tyrode's fluid was maintained between 7.3 and 7.4 during the 2 hr. circulation. The initial pH-value of mucosal circulating solution was adjusted to 6.5 and the final pH-value was between 6.3 and 6.4.

Exchange of Mucosal Circulating Solutions in Experiment *in vitro*——In the circulation experiments *in vitro*, first, the mucosal solution that contained 1 m*M*/L. of sulfonamide and was made isotonic by addition of KCl, was circulated. After 30 min. circulation, the concentration of sulfonamide in serosal Tyrode's fluid was determined at intervals of 10 min. After circulating for 1 hr. from the beginning, the mucosal solution was immediately replaced by the NaCl-isotonic solution containing the same sulfonamide (1 m*M*/L.), but the serosal Tyrode's fluid was not exchanged. Then, the circulation was continued for 40 min., again, with the determination of sulfonamide at intervals of 10 min.

Tissue Incubation Experiments——Agar's tissue incubation method[11] was used to investigate the uptake of sulfonamides by the small segments of the rat intestine. The segments (4 to 6 mm. in length) were cut from everted small intestine of the male rat. These segments (2 to 3 g.) were emersed in the incubation medium that contained 1 m*M*/L. of sulfonamide, and gas bubbles of 5% CO_2 in O_2 passed through the medium during the incubation. The volume of medium was 100 ml. After incubating at 37° for 1 hr., the concentration of sulfonamide in the tissue was determined.

Analytical Method

In the case of perfusion experiments *in vivo* and circulation experiments *in vitro*, the analytical method of sulfonamides in the solutions was the same as described in the previous paper[8] of this series. In the case of incubation experiments, the concentration of sulfonamides in the intestinal tissue was measured as follows. After the deproteinization was carried out with 50% trichloroacetic acid solution, the concentration of sulfonamide in the adequately diluted supernatant solutions was determined according to the method of Tsuda.[12] Optical densities were determined by a spectrophotometer (Hitachi Co., Ltd. model EPU-2) at 553 mμ for sulfonamides and 558 mμ for phenol red.

Results and Discussion

Perfusion Experiments *in vivo*

The per cent of unabsorbed sulfisoxazole in perfusion solution is plotted in the logarithmic scale against time in Fig. 1. Straight lines are obtained as shown in the Fig. 1. The ratios of intestinal absorption rates of SIX in the sodium chloride-, potassium chloride-, and glucose-isotonic perfusion solutions are calculated from the slopes of these lines as the ratio of 1.00, 0.35, and 0.54, respectively. The above results indicate sodium ion and potassium ion can affect the intestinal absorption of sulfisoxazole and potassium chloride-isotonic perfusion solution reduces the absorption.

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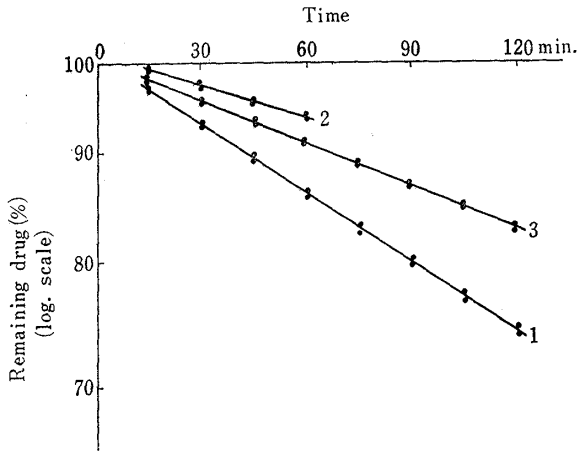


Fig. 1. Absorption of Sulfisoxazole by Rat Intestine

Initial concentration of SIX in perfusion solution; 1 m*M*/L.
(1) NaCl-isotonic perfusion solution
(2) KCl-isotonic
(3) Glucose-isotonic

Effects of Cations on Transport of sulfonamides *in vitro*

The circulation experiments *in vitro* with the isolated small intestine were carried out in order to investigate directly the effects of cations on the transport of sulfonamide.

Five kinds of mucosal circulating solutions were prepared as shown in Table I to each sulfonamide. In order to investigate effects of sodium ion and potassium ion and absence of cation, the solutions were made isotonic by sodium chloride, potassium chloride, and glucose, respctively. Since it has been known that 2,4-dinitrophenol (DNP), metabolic inhibitor, decreases rates of transports of substances, *i.e.*, sodium ion, water etc.,

Table I. Composition of Circulation Solution Containing Sulfonamide (1 m*M*/L)

Principal Solute	Na^+ (meq./L.)	K^+ (meq./L.)	Cl^- (meq./L.)	DNP (m*M*/L.)	PO_4 (m*M*/L.)
(1) NaCl	154	—	150	—	4.2
(2) KCl	—	154	150	—	4.2
(3) NaCl+DNP	154	—	150	1	4.2
(4) NCl+DNP	—	154	150	1	4.2
(5) Glucose ⟶ glucose : 280 m*M*/L., Na^+ : 4.2 meq./L., PO_4 : 4.2 m*M*/L.					

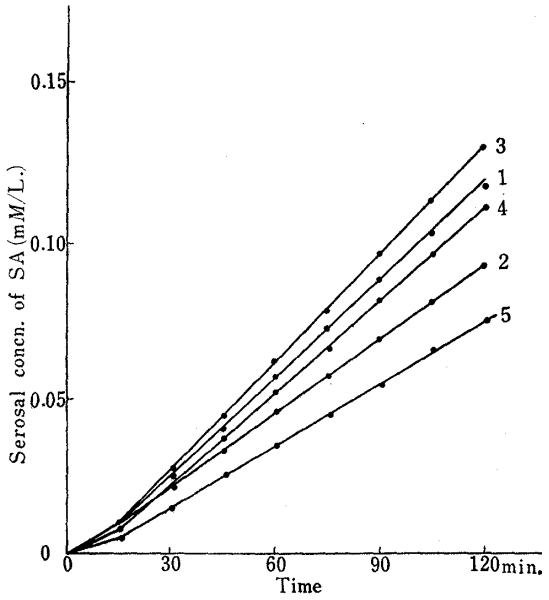
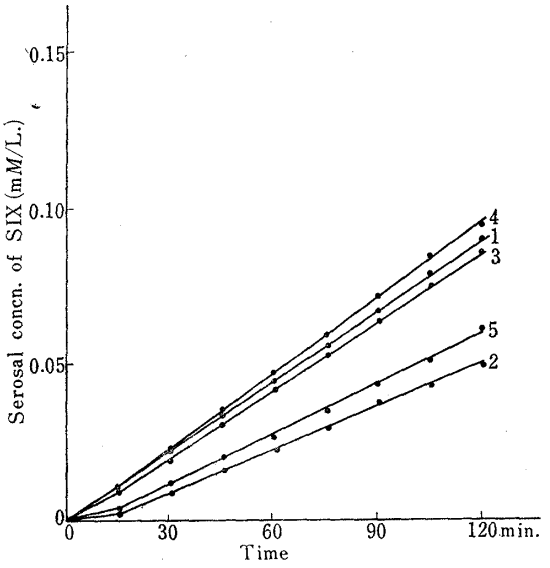


Fig. 2. Intestinal Transport of Sulfanilamide from Mucosal Side to Serosal Side

Initial mucosal concentration of SA; 1 m*M*/L.
(1) NaCl-isotonic
(2) KCl-isotonic
(3) NaCl-isotonic (+DNP)
(4) KCl-isotonic (+DNP)
(5) Glucose-isotonic



Figi. 3. Intestinal Transport of Sulfisoxazole from Mucosal Side to Serosal Side

Initial mucosal concentration of SIX; 1 m*M*/L.
(1), (2), (3), (4), (5); same as Fig. 2.

through rat small intestine, the solutions containing DNP were also prepared. The steady state transport of sulfonamide from mucosal side to serosal side was apparently observed from 15 to 30 minutes of the circulating time and the concentration of sulfonamide in serosal Tyrode's solution increased directly proportionally against time. The experimental results as to sulfanilamide, sulfisoxazole and sulfisomezole are shown in Figs. 2, 3, and 4. In each figure, the average values of repeated experiments (three times) are plotted, and these rates of transport are shown in Tables Ⅱ, Ⅲ, and Ⅳ, respectively. The transport rate is given in the amount of sulfonamide transported through the unit length of small intestine from mucosal to serosal side per minute (10^{-3} μmol./cm. min.). The transport rate of each sulfonamide was reduced significantly in the case of potassium chloride-isotonic solution as compared with the case of sodium chloride-isotonic solution ($p<0.05$). The cations in the circulating solution affected the transport of sulfonamides *in vitro* and the transport was reduced by replacing sodium ion with potassium ion as well as in perfusion experiments of sulfisoxazole *in vivo*. The degree of the decrase of one sulfonamide, however, was different from another sulfonamide, that is, the transport of sulfanilamide was reduced to 78%, sulfisoxazole; 61% and sulfisomezole; 44%.

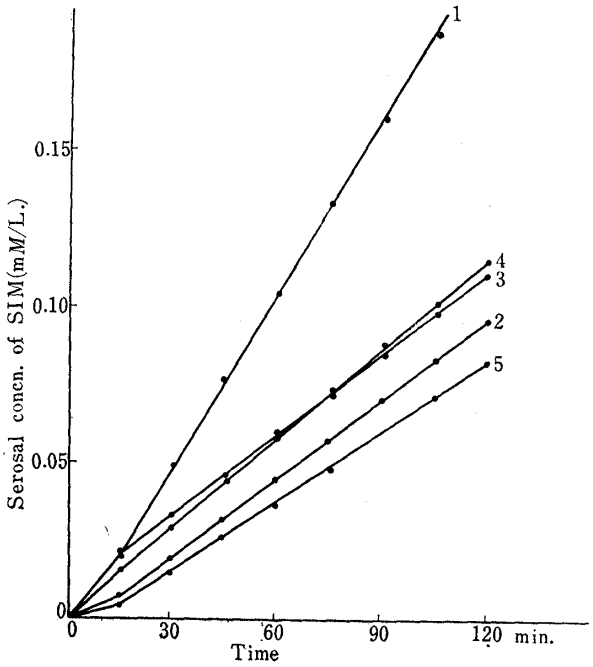


Fig. 4. Intestinal Transport of Sulfisomezole from Mucosal Side to Serosal Side

Initial mucosal concentration of SIM; 1 m*M*/L.
(1), (2), (3), (4), (5); same as Fig. 2.

In the cases of both sulfanilamide and sulfisoxazole, the transport rates were almost not affected by addition of DNP to the mucosal circulating solution of which principal cation was sodium ion. On the other hand, in the case of sulfisomezole, the

Table Ⅱ. Transport Rate of Sulfanilamide

Mucosal circulation fluid	Transport rate (10^{-3} μ*M*/cm./min.)	Average value (10^{-3} μ*M*/cm./min.)	Ratio
(1) NaCl-isotonic	1.96	1.89	1.00
	1.76		
	1.95		
(2) KCl-isotonic	1.35	1.47	0.78
	1.71		
	1.36		
(3) NaCl-isotonic+DNP 1 m*M*/L.	2.12	2.02	1.11
	2.26		
	1.69		
(4) KCl-isotonic+DNP 1 m*M*/L.	1.83	1.76	0.93
	1.85		
	1.65		
(5) Glucose-isotonic	1.43	1.30	0.69
	1.17		

Table III. Transport Rate of Sulfisoxazole

Mucosal circulation fluid	Transport rate ($10^{-3}\mu M$/cm./min.)	Average value ($10^{-3}\mu M$/cm./min.)	Ratio
(1) NaCl-isotonic	1.34	1.40	1.00
	1.26		
	1.58		
(2) KCl-isotonic	0.89	0.85	0.61
	0.77		
	0.91		
(3) NaCl-isotonic+DNP 1 mM/L.	1.35	1.39	0.99
	1.33		
	1.48		
(4) KCl-isotonic+DNP 1 mM/L.	1.35	1.49	1.06
	1.56		
	1.56		
(5) Glucose-isotonic	0.89	0.90	0.64
	0.90		

Table IV. Transport Rate of Sulfisomezole

Mucosal circulation fluid	Transport rate ($10^{-3}\mu M$/cm./min.)	Average value ($10^{-3}\mu M$/cm./min.)	Ratio
(1) NaCl-isotonic	3.68	3.48	1.00
	2.85		
	3.91		
(2) KCl-isotonic	1.58	1.54	0.44
	1.31		
	1.74		
(3) NaCl-isotonic+DNP 1 mM/L.	1.68	1.75	0.47
	2.24		
	1.34		
(4) KCl-isotonic+DNP 1 mM/L.	1.83	1.80	0.52
	1.62		
	1.94		
(5) Glucose-isotonic	1.43	1.37	0.39
	1.33		
	1.34		

transport rate was reduced by addition of DNP, significantly ($p<0.05$). When DNP was added to the mucosal solution of which principal cation was potassium ion, transport of sulfanilamide and sulfisoxazole were apparently not decreased by replacing sodium ion in mucosal solution with potassium ion, but transport of sulfisomezole was still inhibited. As above described, with respect to the effect of DNP, the transport of sulfisomezole was different from those of sulfanilamide and sulfisoxazole. This significance is unknown at present. When mucosal circulating solution was glucose-isotonic, the transport of each sulfonamide decreased significantly in comparison with the case of sodium chloride-isotonic solution, namely, sulfanilamide; 69%, sulfisoxazole; 64%, and sulfisomezole; 39%.

Restoration of Transport Rate by Na^+ *in vitro*

The evidence of the restoratic inhibition of potassium ion was shown as follows. After the transport rate of sulfonamide was reduced by replacing sodium ion with potassium ion in mucosal circulating solution, it was investigated whether the transport rate of sulfonamide was restored by replacing potassium ion with sodium ion

halfway in a circulation experiment *in vitro* or not. The experiments were performed with respect to sulfisoxazole and sulfisomezole of which transport was markedly reduced by replacing sodium ion with potassium ion. The results are shown in Tables V and VI. As shown in Table VI, the transport rates of both sulfonamides that were reduced in potassium chloride-isotonic circulating solution were almost restored by replacing potassium ion with sodium ion.

Table V. Exchange of Circulation Solution (Principal cation : $K^+ \rightarrow Na^+$)

	Time (min.)	Serosal concn. of sulfonamide (10^{-2} mM/L.)			
		Sulfisoxazole		Sulfisomezole	
		A	B	A	B
KCl-isotonic	0	0	0	0	0
	30	1.72	0.98	1.77	2.35
	40	2.18	1.55	2.93	3.62
	50	2.75	2.06	4.00	5.20
	60	3.27	2.75	5.04	6.46
Exchange of circulation fluid		⋮	⋮	⋮	⋮
NaCl-isotonic	70	3.84	3.66	6.43	8.60
	80	4.52	4.81	8.20	11.0
	90	5.32	5.44	9.65	12.9
	100	6.25	6.42	12.1	14.6

Table VI. Transport Rate of Sulfonamides (Exchange of circulation solution)

Mucosal circulation fluid	Transport rate (10^{-3} μM/cm./min.)	
	Sulfisoxazole	Sulfisomezole
KCl-isotonic	A : 0.89	A : 1.87
	B : 0.99	B : 2.28
NaCl-isotonic	A : 1.48	A : 3.42
	B : 1.66	B : 3.62

Effects of Concentration of Sodium Ion and Potassium Ion on Transport Rate *in vitro*

In the expriments mentioned above, the individual effect of sodium ion or potassium ion was investigated. In the following experiments *in vitro*, effects of the

Table VII. Effect of Concentration of Salts on Transport of Sulfonamides

	Concn. of salts (mM/L.)		Transport rate (10^{-3} μM/cm./min.)	
	NaCl	KCl	Sulfisoxazole	Sulfisomezole
(1)	0	150	0.89	1.58
			0.76	1.31
			0.91	1.74
(2)	37	113	1.23	1.99
			1.08	1.97
(3)	75	75	1.56	3.61
			1.35	3.20
(4)	113	37	1.54	3.68
			1.53	2.97
(5)	150	0	1.32	3.68
			1.26	2.85
			1.58	3.91

concentration of both sodium ion and potassium ion in the mucosal solution were studied. The mucosal circulating solutions with 1 m*M*/L. of sulfonamide were added sodium chloride and potassium chloride which total concentration of both salts was kept as 150 m*M*/L. in each case, but the ratio of concentration of both salts was varied. The experimental results are shown in Table Ⅶ. In each case of sulfisoxazole and sulfisomezole, the transport rate did not reduce when the concentration of potassium ion was lower than sodium ion, but it reduced when the concentration of potassium ion was higher than sodium ion.

Effects of Cations on Uptake of sulfonamides by Intestinal Tissue *in vitro*

The effects of cations on the uptake of sulfisoxazole and sulfisomezole by the intestinal tissue were investigated by means of the tissue incubation method. The incubation media contained 1 m*M*/L. of sulfonamides.

The three kinds of incubation media were prepared as shown in Table Ⅷ, respectively. The experimental results are shown in Tables Ⅸ and Ⅹ. The uptake of sulfisoxazole was significantly reduced by replacing sodium ion with potassium ion in media. The uptake in K^+-media was 75% of uptake in Na^+-media. The addition of

Table Ⅷ. Composition of Incubation Media Containing Sulfonamide (1 m*M*/L.)

	Principal cation	Composition of media
(1)	Na^+	Tyrode's fluid
(2)	K^+	Tyrode's fluid with Na^+ replaced by K^+
(3)	Na^+ (+DNP)	Tyrode's fluid +DNP 1 m*M*/L.

Table Ⅸ. Uptake of Sulfisoxazole by Rat Intestine

	Principal cation in media	Amount of uptake (μ*M*/g. tissue)	Average value (μ*M*/g. tissue)	Ratio
(1)	Na^+	0.46	0.47	1.00
		0.49		
		0.46		
(2)	K^+	0.30	0.35	0.73
		0.39		
		0.35		
(3)	Na^+ (+DNP)	0.40	0.42	0.89
		0.45		
		0.42		

Table Ⅹ. Uptake of Sulfisomezole by Rat Intestine

	Principal cation in media	Amount of uptake (μ*M*/g. tissue)	Average value (μ*M*/g. tissue)	Ratio
(1)	Na^+	0.51	0.45	1.00
		0.44		
		0.39		
(2)	K^+	0.28	0.27	0.57
		0.23		
		0.29		
(3)	Na^+ (+DNP)	0.39	0.34	0.76
		0.30		
		0.34		

DNP to Na^+-media did not reduce the uptake of sulfisoxazole significantly. The uptake of sulfisomezole also was significantly reduced by replacing sodium ion with potassium ion, and the uptake in K^+-media was 60% of uptake in Na^+-media. The addition of DNP to Na^+-media, however, reduced uptake of sulfisomezole significantly.

The effects of cations on the transport and the uptake of sulfonamides are mentioned above. At present, the mechanism of the effects is still obscure. A quantitatively similar relation, however, is evidently found on the cations and DNP effect through the transport and uptake of sulfonamides in the rat intestine. The comparison among three sulfonamides seems to be very interesting. The uphill transport of sulfisomezole has been reported in the preceding paper.*[2] In this investigation, sulfisomezole is also distinguished from the others on the inhibitory effects of DNP alone and potassium ion-DNP combination, since these do almost not affect the transport of sulfisoxazole and sulfanilamide. From the physicochemical standpoint of view, sulfisomezole and sulfisoxazole are very similar with the chemical structure, pKa-value and the solubility properties, but sulfanilamide is very different from these sulfonamides. The actual intestinal absorption properties of sulfisoxazole, however, are similar to sulfanilamide other than sulfisomezole. The investigations for these problems may contribute to make clear the mechanism for intestinal absorption of sulfonamides.

Summary

The intestinal absorption of sulfisoxazole was reduced by replacing sodium ion in perfusion solution with potassium ion *in vivo*.

The rates of intestinal transport of sulfonamide, sulfisoxazole and sulfisomezole, also, were decreased by replacing sodium ion in circulating solution with potassium ion *in vitro*. The transport rate and uptake of sulfisomezole in the intestine were decreased by DNP alone, and the combination of the replacing sodium ion with potassium ion and DNP. These of sulfisoxazole and sulfanilamide, however, were not affected by the same treatments.

(Received July 9, 1965)