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UDC 612.386[615.778.25]-084

200. Hisashi Nogami, Manabu Hanano, and Jun Watanabe*1 : Studies on Absorption and Excretion of Drugs. V.*2 On the Mechanism of Penetration of Sulfisomezole through the Intestinal Barrier *in vitro*.*3, *4

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As described in the review of Wagner[1] the pH-partition hypothesis was developed by Brodie, Hogben, Schanker, Schore, and Tocco, and seems well to explain the rate and extent of absorption of a number of acidic and basic drugs from the stomachs and intestines of the rat and human being. The hypothesis gives a considerably clear picture of absorption mechanism of those substance that show high lipid-solubility.

However, probably because of not so high lipid-solubility and of relatively poor water-solubility of sulfonamides, a consistent explanation of the absorption mechanism of all sulfonamides has not been given.[2~5]

In the third paper of this series it was showed that the penetration rate for sulfisomezole was higher than that for other four sulfonamides (sulfamethoxypyridazine, sulfanilamide, sulfathiazole, sulfaguanidine) in the experiment *in vitro*. The simiar tendency was observed by the perfusion method *in vivo*, that was reported in the preceeding paper.*2 But very little is known about the factors involved in the relatively rapid absorption of sulfisomezole, and it is of great interest to find some of these factors.

The present paper describes the observations of the uphill transport of sulfisomezole by means of two experimental techniques, although such phenomenon was not found with sulfathiazole or sulfaguanidine. *In vitro* preparations of rat small intestine were used in the experiments. The uphill transport of sulfisomezole was discussed as compared with the sodium and water transports that were measured simultaneously. The significance of the acid pH at the absorptive site of mucosa on this uphill transport was also described.

Experimental

Animals——Male albino rats (Donryu) weighing 150~320 g. were used. The stock diet was withdrawn from the animals about 20 hr. before the experiment and they were provided with plain water only.

Drugs——Three investigated sulfonamides were sulfathiazole (ST), sulfaguanidine (SG), and sulfisomezole (SI). The abbreviations in the parentheses represent these drugs, respectively. Each sulfonamide drug was dissolved in salt solutions to give the drug concentration of 2 m*M*.

Salt Solutions——Salt solutions used were as follows. Soln. (A), soln. (F), and soln. (G) : Tyrode's fluid.[6] Soln. (B) : one part of veronal buffer*6 was added to three parts of Tyrode's fluid, from which

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*2 Part IV : This Bulletin, **11**, 395 (1963).
*3 Taken in part from the thesis of Jun Watanabe for the degree of Doctor of Pharmaceutical Sciences, University of Tokyo, 1963.
*4 Presented before the Kanto Branch Meeting of Pharmaceutical Society of Japan, Tokyo, March, 1963.
*5 Hongo, Tokyo (野上 寿, 花野 学, 渡辺 淳).
*6 The composition of the veronal buffer : 5.0 ml. *M*/7 barbitone sodium+2.0 ml. *M*/7 sodium acetate +5.5 ml. *N*/10 HCl+12.5 ml. distilled water.

1) J. G. Wagner : J. Pharm. Sci., **50**, 359 (1961).
2) C. A. M. Hogben, *et al.* : J. Pharmacol. & Exper. Therap., **125**, 275 (1959).
3) H. Matsuura : Shikoku Acta Medica, **15**, 738 (1959).
4) H. Nogami, M. Hanano, J. Watanabe : This Bulletin, **10**, 1161 (1962).
5) T. Koizumi, T. Arita, K. Kakemi : *Ibid.*, **12**, 421 (1964).
6) "Biochemists' Handbook" Edited by C. Long, pp. 58 (1961), E. & F. N. Spon Ltd.

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sodium bicarbonate had been excluded. Soln. (C) : 1/2 [Na]-Tyrode's fluid, of which composition resembled almost that of Tyrode's fluid, but sodium chloride concentration was reduced to 0.365% to give one half Na ion concentration of that in Tyrode's fluid. Soln. (D) : 1/10 [Na]-Tyrode's fluid. Sodium chloride was removed entirely from Tyrode's fluid and Na ion concentration was made one tenth of that in Tyrode's fluid. Soln. (E) : $10^{-3}M$-DNP-Tyrode's fluid. 2,4-Dinitrophenol was dissolved in Tyrode's fluid to give the concentration of $10^{-3}M$. Soln. (H) : 2/5 [$NaHCO_3$]-Tyrode's fluid. Only sodium bicarbonate concentration in Tyrode's fluid was reduced to 0.04%. That concentration was two fifths of that in normal Tyrode's fluid. Soln. (I) : 2[$NaHCO_3$]-Tyrode's fluid. Sodium bicarbonate was added to Tyrode's fluid to the concentration of 0.2%.

Apparatus and Experimental Procedure——Two experimental techniques were used. a) The first technique used was that described by Smyth & Taylor[7] and Ross[8] in which three intestinal segments, each about 15 cm. in length, were suspended in air of lower chamber at 37° in thermostatically controlled bath. Various salt solutions containing 2 mM/L. sulfonamides were circulated through the intestinal lumen by means of a 5% CO_2 and 95% O_2 gas lift. Fluid transported to the serosal surface was collected in a tared thin polyethylene sac which was reweighed. Five or six halfhour collections were made and concentrations of sulfonamide and Na ion in the fluid were determined. The sulfonamide concentrations and pH values of mucosal solution were determined at intervals of 30 min. by taking out the samples of 0.5 ml. from the solution. The circulation apparatus described by Wiseman & Smyth and partially modified[9] was used for the experiment.

b) With the second, sacs of everted small intestine, about 25 cm. in length, were formed as described by Wilson & Wiseman.[10] After incubating a everted sac in Tyrode's fluid containing 2 mM/L. SI for 10 min. at 38°, 4~7.5 ml. of another Tyrode's fluid containing 2mM/L. SI was introduced into the sac to form the serosal solution. The mucosal solution was 37 ml. of Tyrode's fluid containing 2 mM/ SI, and was maintained at 38° by a water jacket. The sample of 0.5 ml. was taken out from the mucosal solution at intervals of 30 min. and analyzed. At the end of the experimental period of 90 min. the content of the everted sac was drained into a graduated glass, and the SI concentration and pH value of the serosal solution were measured.

Analytical Method——The concentration of sulfonamides in the adequately diluted samples was determined colorimetrically according to the method of Tsuda[11] with Hitachi Spectrophotometer Model EPU-II. The pH values were measured mostly with Toadenpa pH-meter Model HM-5A and partially with Horiba pH-meter Model P. The concentration of Na ion was determined with Horiba pNa-meter Model SM-4.

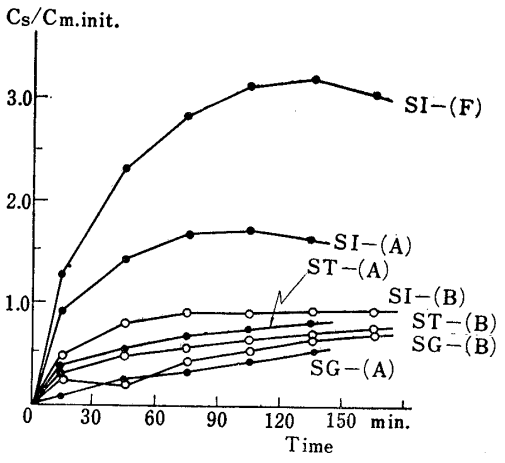

Fig. 1. Concentration of Sulfonamides in the Transported Fluid

SI : Sulfisomezole, ST : Sulfathiazole, SG : Sulfaguanidine. Soln. (A), (B), and (F) : see the text.

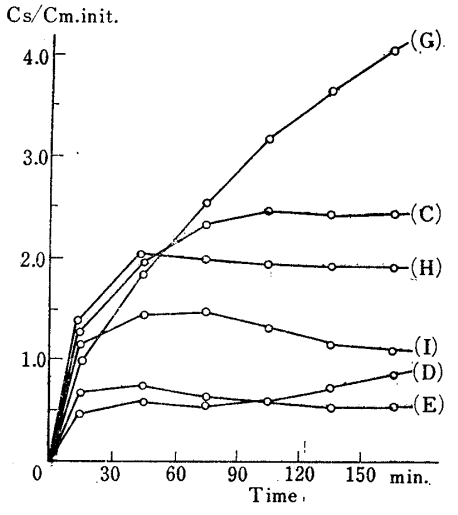

Fig. 2. Concentration of Sulfisomezole in the Transported Fluid

Soln. (C)~(I) : see the text.

7) D. H. Smyth, C. B. Taylor : J. Physiol., **136**, 632 (1957).
8) D. B. Ross : *Ibid.*, **160**, 417 (1962).
9) H. Nogami, T. Matsuzawa : This Bulletin, **9**, 532 (1961).
10) T. H. Wilson, G. Wiseman : J. Physiol., **123**, 116 (1954).
11) K. Tsuda, *et al.* : Yakugaku Zasshi, **62**, 362 (1942).

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Results and Discussion

Obtained values of sulfonamide concentration (Cs) in the fluid transported to the serosal surface are devided by the initial concentration (Cm. init.) of sulfonamide in the mucosal solution and the ratios (Cs/Cm. init.) are plotted *vs.* time as shown in Figs. 1 and 2.

The levels of SI concentration in the transported fluid were higher than those in the mucosal solution, however this phenomenon was not observed with ST or SG. When $10^{-3}M$-DNP-Tyrode's fluid or soln. (B) or soln. (D) was used as mucosal solution and then water transport rate decreased, the ratio (Cs/Cm. init.) for SI decreased below one.

Fig. 3 shows that the level of ST or SG concentration in the transported fluid was maintained approximately constant through the changes of the fluid transport rate.

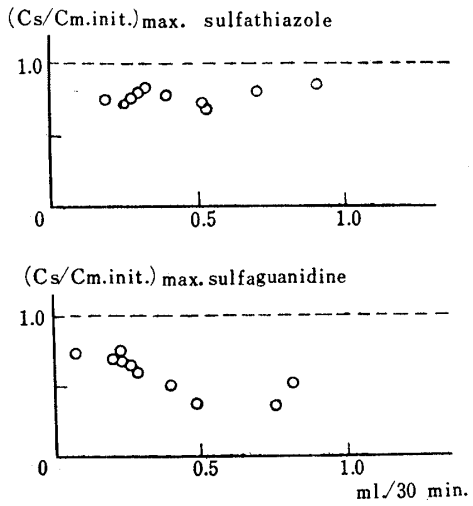

Fig. 3. Maximal Concentration of Sulfonamides and Transport Rate of Fluid

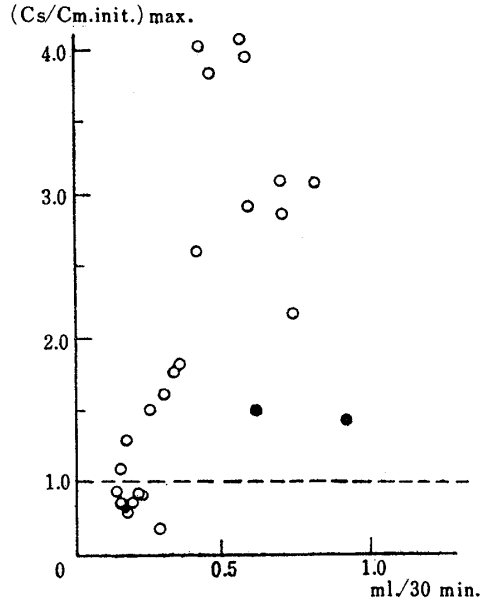

Fig. 4. Maximal Concentration of Sulfisomezole and Transport Rate of Fluid

●: Soln. (I)

On the other hand, the level of SI concentration in the fluid transported to serosal surface was changed with salt solutions, when the fluid transport rate was also changed as shown in Fig. 4. Although the increase of SI concentration in the transported fluid corresponded to the increase of the fluid transport rate, the relationship between them was not linear. When soln. (I), in which sodium bicarbonate concentration was twice as much as in Tyrode's fluid, was made to be the mucosal solution, the level of SI concentration in the transported fluid was relatively low to the high fluid transport rate as shown by black dots in Fig. 4.

Even when SI concentration in the fluid transported to the serosal surface was maximal, the sodium ion concentration in the fluid was approximately equal to that in the mucosal solution as shown in

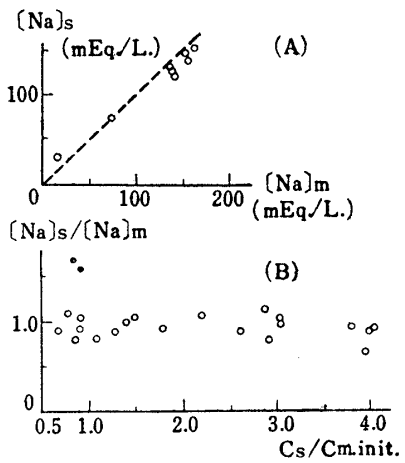

Fig. 5. Concentration of Sodium Ion in the Transported Fluid

●: Soln. (D)

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Fig. 5 (A). Fig. 5 (B) shows that concentration ratio for serosal sodium ion ([Na]s) to mucosal sodium ion ([Na]m) was constant through various levels of SI concentration in the transported fluids. One exception was shown with black dots in Fig. 5 (B), which corresponded to the extremely low concentration of sodium ion in mucosal solution, *i.e.* soln. (D). Then no particular correlation was suggested between the penetration of SI and that of sodium ion.

The fact mentioned above for SI suggests that SI is transported against the concentration gradient under adequate conditions, since the equilibrium for SI concentration is assumed to be established across the intestinal barrier *in vitro* after $t=30$ (min.) and the SI concentration in serosal fluid is higher than in mucosal solution. The uphill transport of SI was observed with a second series of experiments in which the technique of Wilson & Wiseman was used. In these experiments the initial concentration of SI in the serosal solution was equal to that in the mucosal solution. The SI concentration (Cs) in the serosal solution increased and that (Cm) in the mucosal solution decreased at the end of an experimental period of 90 minutes. Table I shows that the ratio, Cs/Cm, increased from 1 to 1.51~2.32. SI in the mucosal solution penetrated to the

Table I. Uphill Transport of Sulfisomezole

Salt soln.	Drug	Cs (mM)		Cm (mM)				Cs/Cm
		0 (min.)	90 (min.)	0 (min.)	30 (min.)	60 (min.)	90 (min.)	90 (min.)
Tyrode's fluid, at 38°	SI_1	1.92	2.40	1.92	1.71	1.63	1.59	1.51
	SI_2	1.86	2.77	1.86	1.79	1.70	1.62	1.71
	SI_3	1.82	3.43	1.82	1.66	1.55	1.48	2.32
Tyrode's fluid+$10^{-3}M$ DNP, at 38°	SI_1	1.82	1.78	1.82	1.82	1.86	1.86	0.96
	SI_2	1.85	1.89	1.85	1.89	1.87	1.89	1.00
	SI_3	1.85	1.79	1.85	1.85	1.85	1.90	0.94

Cs: Concentration of sulfisomezole in the serosal solution.
Cm: Concentration of sulfisomezole in the mucosal solution.

Table II. pH Changes of Mucosal Solution

Drug	Salt soln.	pH_0	pH_{150}	ΔpH	Obsd. maximal Cs/Cm
SI	(C)	7.4	6.7	0.7	3.026
		7.8	6.7	1.1	3.995
	(D)	7.4	7.5	−0.1	0.846
		7.5	7.3	0.2	0.769
	(E)	7.1	7.1	0.0	0.623
		7.5	7.1	0.4	0.855
	(F)	7.3	6.8	0.5	4.274
		7.2	6.8	0.4	4.963
		7.6	6.6	1.0	3.872
		7.9	6.8	1.1	4.189
		7.5	6.6	0.9	5.628
	(G) at 27°	7.4	7.0	0.4	5.448
		7.4	6.7	0.7	5.626
	(H)	6.9	5.8	1.1	3.092
		7.1	5.8	1.3	2.111
	(I)	7.7	7.5	0.2	1.526
		7.8	7.5	0.3	1.593

SI: Sulfisomezole. pH_0: pH at t=0 (min.). pH_{150}: pH at t=150 (min.).
$\Delta pH=pH_0-pH_{150}$ Cs/Cm: see the text.

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serosal side initially when no apparent concentration gradient existed across the intestinal barrier and subsequently penetrated against the concentration gradient. When 2,4-dinitrophenol was added to the mucosal solution, this phenomenon disappeared.

Since it is not assumed that the equilibrium for SI concentration was established across the intestinal barrier *in vitro* in the second series of experiments, an analysis of pH changes of the mucosal solutions in the first series of experiments is attempted to explain the mechanism of the uphill transport SI. Observed pH changes of the mucosal solution are given in Table II. When the decrease of pH value of mucosal solution was very little at the end of an experiment, SI concentration in the fluid transported to the serosal surface was considerably low. On the other hand, SI concentration in the fluid was relatively high and consequently the ratio, Cs/Cm, was higher than 1, when pH value of mucosal solution decreased to some extent. Values of the ratio, Cs/Cm, in Table II were taken from Fig. 6 and Fig. 7 which showed the change of the ratio in the course of time in an experiment. The ratio was calculated with observed Cm values and Cs values which were estimated from Fig. 1 and Fig. 2. Therefore Fig. 6 or Fig. 7 corresponds to Fig. 1 or Fig. 2 respectively. As shown in Table II,

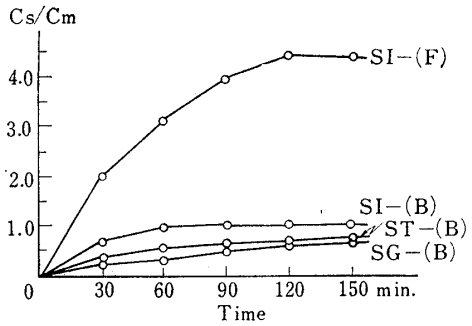

Fig. 6. Concentration Ratio of Sulfonamide on Serosal Side to that on Mucosal Side

Soln. (B) and (F) : see the text.

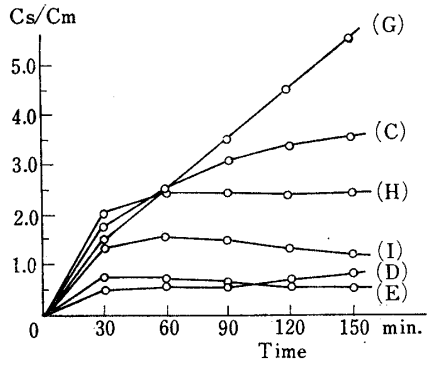

Fig. 7. Concentration Ratio of Sulfisomezole on Serosal Side to that on Mucosal Side

Soln. (C)~(I) : see the text.

Fig. 6 and Fig. 7, the high level of the ratio, Cs/Cm, did not always result from the low value of final pH in the mucosal solution, since initial pH values of various salt solutions were not equal.

It has been suggested in the preceding paper of this series*[2] that SI penetrates through the rat small intestine *in vivo* mainly in undissociated form. Therefore the following equation, derived by Shore, *et al.*,[12] can be applied to the condition of equilibrium for SI concentration across the intestinal barrier,

$$\frac{Cs}{Cm} = \frac{1+10^{(pHs-pKa)}}{1+10^{(pHm-pKa)}} \qquad (1)$$

where pHs or pHm is pH value in the serosal fluid or the mucosal solution respectively. When the mucosal salt solution was Tyrode's fluid, the observed pH value of the fluid transported to the serosal surface was 6.9 and that of the mucosal solution was 6.7~6.8 at the end of an experimental period. The pKa of SI is 5.81. Then the concentration ratio for SI, Cs/Cm, is calculated from Equation (1). But thus calculated ratio of Cs/Cm gives the value of 1.24~1.52, which is considerably lower than the observed value of 3.87~5.63. This suggests that the pH of the absorptive surface is lower than that of the mucosal solution leaving intestine.

12) P. A. Shore, *et al.* : J. Pharmacol. Exptl. Therap., **119**, 361 (1957).

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Robinson, *et al.*[13] described the ability of the intestinal mucosa to establish an acid pH. Hogben, *et al.*,[2] calculated a "virtual" pH at the site of absorption of 5.3 from the results of their experiments in which the intestinal absorption of aniline or aminopyrine was investigated, although the pH of the perfusion fluid leaving the intestine was 6.6. Similarly the pH value at the site of absorption of ST was calculated in the present experiment using the following equation, which had been applied to the condition of equilibrium for ST concentration across the intestinal barrier *in vitro* in the previous paper of this series,[4]

$$\frac{Cs}{Cm} = \frac{P_1(1-\alpha_m)+P_3\alpha_m}{P_1(1-\alpha_s)+P_3\alpha_s} \qquad (2)$$

where the permeability coefficients, P_1 and P_3, are 0.00064 and 0.00132 (ml./cm./min.) respectively. The α_m is the degree of dissociation of ST in the mucosal solution and the αs is that in the serosal fluid. When the mucosal salt solution containing ST was Tyrode's fluid, which was a physiological solution and had little buffering action, the observed pH value of the fluid transported to the serosal surface was 6.9 and, the ratio Cs/Cm for ST was from 0.809 to 0.905. The pKa of ST is 7.12.[14] Then from Equation (2) the calculated pH at the site of absorption gives the value of 6.27~6.64, which is lower than the observed pH value of 6.7~6.8 in the mucosal solution.

When the pH value at the site of absorption is assumed to be in 5.3~6.4, from Equation (1) the calculated ratio, Cs/Cm, for SI gives the value of 10.2~2.5. Therefore, if the pH at the site of absorption is lower than the pH in the mucosal solution leaving intestine and takes the value of about 5.3 (even if the value is not so low as 5.3), the observed ratio, Cs/Cm, for SI, of 3.87~5.63 is explainable from the view-point of pH-partition hypothesis. Such explanation fits also with the fact that when soln. (I), in which sodium bicarbonate concentration was twice as much as in Tyrode's fluid and consequently high pH value was observed, was made to be the mucosal solution, the level of SI concentration in the transported fluid was relatively low to the high fluid transport rate (cf. Fig. 4 and Table II). It does not seem indispensable to consider some specific mechanisms to the uphill transport of SI.

The addition of 2,4-dinitrophenol to the mucosal solution caused not only the inhibition of SI penetration, but also the inhibition of fluid transport and of changes of pH values in the mucosal solution. The transport rate of fluid was decreased by 2,4-dinitrophenol to one third of that for normal Tyrode's fluid. The pH value of mucosal solution containing $10^{-3}M$ 2,4-dinitrophenol was changed scarcely as shown in Table II. Since the relationship between the fluid transport rate and SI penetration is not linear, it is more probable to consider that 2,4-dinitrophenol disturbs the mechanism of physiological pH maintenance at the absorptive surface, causing the decrease of SI penetration rather than to assume that 2,4-dinitrophenol inhibits directly some specific mechanism for the uphill transport of SI. In the strict sense the uphill transport of SI may be an example of active transport, for energy is required to maintain physiological pH at the absorptive surface of mucosa. However, just as described by Hogben, *et al.*,[2] the active transport does not seem synonymous with specific transport which is characterized by chemical specificity, a transport maximum and competitive inhibition.

The authors thank Mr. M. Tadokoro for his technical assistance in the experiment. This work was supported by the Grant-in-Aid for Scientific Research provided by the Ministry of Education, to which they are also grateful.

13) C. S. Robinson, *et al.* : J. Biol. Chem., **155**, 305 (1944).
14) P. H. Bell, R. O. Roblin : J. Am. Chem. Soc., **64**, 2905 (1942).

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Summary

1. The penetration of water and solutes though the intestinal barrier *in vitro* was investigated with the technique described by Smyth & Taylor. Sulfisomezole concentration in the fluid transported to the serosal surface was higher than that in the mucosal solution. This phenomenon was not observed with sulfathiazole or sulfaguanidine.

2. The uphill transport of sulfisomezole was demonstrated with the technique of Wilson & Wiseman employing everted sacs.

3. In experiments with the technique of Smyth & Taylor the level of sulfathiazole or sulfaguanidine concentration in the transported fluid was maintained approximately constant through the changes of the transport rate of fluid. On the other hand the increase of sulfisomezole concentration in the transported fluid corresponded to the increase of the transport rate of fluid, although the relationship between them was not linear.

4. No particular correlation was observed between the penetration of sulfisomezole and that of sodium ion.

5. It was suggested that the uphill transport of sulfisomezole was explainable from the view-point of pH-partition hypothesis and that 2,4-dinitrophenol might decrease the penetration of sulfisomezole after disturbing the mechanism of physiological pH maintenance at the absorptive surface.

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201. Takuzo Nishimura, Bunji Shimizu, and Issei Iwai : Studies on Synthetic Nucleosides. IV.*1 A New Synthetic Method of Pyrimidine and Purine Ribosides.

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In previous papers of this series,*1,1) the authors have synthesized trimethylsilyl derivatives of pyrimidines and purines and introduced a new synthetic method for glucose nucleosides by fusing the silyl compounds with α-acetobromoglucose, followed by removal of protecting groups. The glycosidations, in these cases, have took place at 1- or 9-position of pyrimidine or purine bases, respectively, giving the same type of derivatives as natural nucleosides. Analogously, condensation of trimethylsilylpyrimidines (Ia~c) and -purines (Va, b) with 2,3,5-tri-O-benzoylribosyl chloride (II) in place of α-acetobromoglucose gave benzoylated ribose nucleosides in good yields, which readily furnished the corresponding nucleosides.

Ribofuranosylpyrimidines

Three methods have been previously reported for the synthesis of uridine (IVa).

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*1 Part III. T. Nishimura, B. Shimizu : Agr. Biol. Chem., 28, 224 (1964).

*2 2-58, 1-chome, Hiromachi, Shinagawa-ku, Tokyo (西村卓三, 清水文治, 岩井一成).

1) Part I, II. T. Nishimura, I. Iwai : This Bulletin, **12**, 352, 357 (1964).

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