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Studies on Absorption of Drugs. VI.[1] Effects of Buffer Components on the Absorption of Drugs[2]

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Studies were made of the effects of buffer components (phosphate–citrate isotonic buffer) on absorption of sulfonamides and of a reconfirmation was established of the equation presented previously in reference to an intestinal absorption of drugs.

Absorption of sulfonamides from phosphate–citrate isotonic buffer of pH 5.8 decreased following preperfusion with pH 3.3 or 7.6 isotonic buffer, while it was not decreased when pretreated with Tyrode's solution.

Dilution of phosphate-citrate isotonic buffer with 0.9% NaCl, whose pH was adjusted by a continuous titration of 0.1N HCl or 0.1N NaOH resulted in an enhancement of the increase.

It was concluded that when phosphate or citrate ions were used as perfusion medium possible influences on the intestinal mucous membrane should be considered.

When phosphate–citrate isotonic buffer was used, pH for maximum absorption did not correspond with pH for the maximum fraction of undissociated drug molecules, but when 0.9% NaCl whose pH was adjusted was used as perfusion medium, pH-profile of absorption corresponded with that of the undissociated fraction of drugs.

Regarding such correspondence further study seems necessary in under way.

Various methods have been used in the study of drug absorption. The *in situ* absorption technique has been frequently used because of its simplicity and maintenance of comparatively physiological conditions. It has been studied with mammalian isotonic buffer (phosphate–citrate buffer) which is routinely used as a perfusion medium. The buffer is composed of high concentration of sodium phosphate and citric acid. Effects of phosphate ions and citrate ions on the intestinal mucous membrane have been left uninvestigated. But these ions' chemical characteristics are strongly suggestive of some action such as chelating action.

In the presence of high concentration of phosphate and citrate ions their effects on the absorption of drugs are to be considered.

Presently studies were made on the effects of buffer components on the absorption of drugs as well as the reconfirmation of the equation presented previously[1] in reference to the intestinal absorption of drugs.

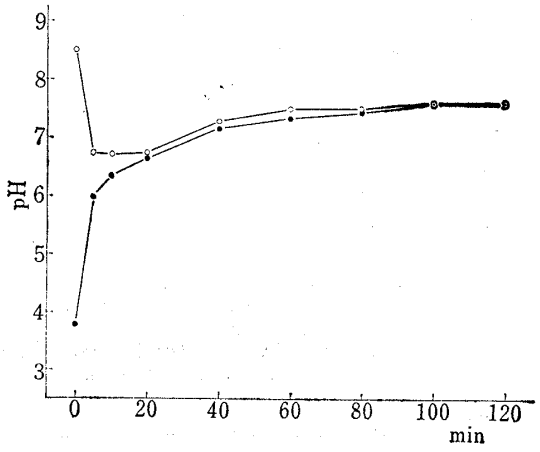

Fig. 1. Chronological Changes of Hydrogen Ion Concentration in Perfusion Solution

Experimental

Procedures were generally similar to those in the previous paper[1] except for the components of perfusion solution. Presently five antibacterial sulfonamides

1) Part V: T. Morishita, N. Yata and A. Kamada, *Yakuzaigaku*, in press.
2) Part of the present report was presented at the 89th Annual Meeting of the Pharmaceutical Society of Japan at Nagoya, April 1969.
3) Location: *Toneyama, Toyonaka, Osaka.*

were examined. A sulfonamide was dissolved in a 0.9% sodium chloride solution whose pH could be adjusted with HCl or NaOH for use. The solution was circulated at a rate of 32 ml/min between the hypoduodenal region and the supraileocecal region. Three to four rats were used at each experiment.

The perfusion solution changed its hydrogen ion concentration rapidly during circulation with its pH reaching almost 7.6 in 60 min (Fig. 1). So it maintained its hydrogen ion concentration by the continuous addition of 0.1N HCl or 0.1N NaOH. A glass electrode of a combined type was immersed in a reservoir and pH of the perfusion solution was continuously checked. A required volume of 0.1N HCl or 0.1N NaOH to maintain a desired pH was at most 3.0 ml for 100 ml of perfusion solution. So, the changes of isotonicity was practically neglected during the absorption. The degree of absorption was calculated from the difference of the amounts of drugs in pre- and post-perfusion. Sulfonamides were analysed routinely by diazotization.

Result and Discussion

Reconfirmation of Equation Presented

In the previous report,[1] the absorption of drugs from a phosphate–citrate isotonic buffer was presented by the following equation:

$$A = R_m M + R_i I \qquad \text{(Eq. 1)}$$

where A is the amount of the drug absorbed in a certain time, M and I are the amounts of the drug initially present in the perfusion solution in undissociated and dissociated forms respectively, R_m and R_i are the absorption ratios of undissociated and dissociated drugs respectively.

Absorption of sulfamethoxazole from physiological saline adjusted at several pH was determined. With M, I and the apparent absorption ratios at several pH, R_m and R_i of sulfamethoxazole were calculated with the least squares analysis and Eq. 2 was obtained.

$$A = 0.709M + 0.533I \qquad \text{(Eq. 2)}$$

With Eq. 2 the absorption of sulfamethoxazole at various pH can be calculated.

The correlation coefficient between experimental and calculated values with Eq. 2 was 0.985 with a standard deviation of 0.014.

Similar equations to Eq. 2 can be obtained on other sulfonamides. R_m and R_i of sulfonamides and correlation coefficients between experimental and calculated values are shown in Table I.

Table I. Correlaion Coefficients between Theoretical and Experimental Absorption of Sulfonamides

Sulfonamides	R_m	R_i	n[a]	r[b]	s[c]
Sulfamethoxazole	0.709	0.533	7	0.985	0.014
Sulfisoxazole	0.470	0.367	5	0.989	0.005
Sulfamerazine	0.402	0.260	5	0.988	0.010
Sulfaphenazole	0.741	0.466	5	0.991	0.016
Sulfanilamide	0.306	[d]	3	[e]	0.018

a) number of experimental pH
b) correlation coefficients between experimental and calculated values
c) standard deviations
d) Sulfanilamide (pK_a 10.45) is present as undissociated form under experimental condition.
e) The theoretical absorption of sulfanilamide remains constant, so correlation coefficient cannot be calculated.

Thus, an Eq. 2 was reasonably applicable to a pH adjusted 0.9% NaCl solution presently used as a perfusion medium.

Effects of pH and Components of Isotonic Buffer Solution on the Absorption of Sulfamethoxazole

With phosphate–citrate isotonic buffer solution, sulfamethoxazole showed its maximum absorption at pH 5—5.8 (Fig. 2).

An attempt was made to confirm whether effects of pH are only due to the changes of

undissociated fraction which have been generally accepted as the advantageous fraction in the intestinal absorption of drugs. Then effects were studied of the isotonic buffer solution following preperfusion. As a tentative standard, the buffer of pH 5.8 was used. The buffer of pH 7.6, 5.8, 3.3 or Tyrode's solution were preperfused respectively for 30 min, followed by washing with a pH 5.8 buffer to keep the intestines free from the preperfused buffer solution, and then perfusion of a pH 5.8 buffer containing sulfamethoxazole (1 mmol/liter) (Table II).

Table II. Effect of Preperfusion with Phosphate-Citrate Isotonic Buffer or Tyrode's Solution on an Absorption of Sulfamethoxazole

Preperfusion medium	Drug solution	% absorbed
——	pH 5.8 buffer	48.1
pH 5.8 buffer	pH 5.8 buffer	48.0
pH 7.6 buffer	pH 5.8 buffer	33.8
pH 3.3 buffer	pH 5.8 buffer	23.3
——	Tyrode's solution	51.7
Tyrode's solution	pH 5.8 buffer	48.1

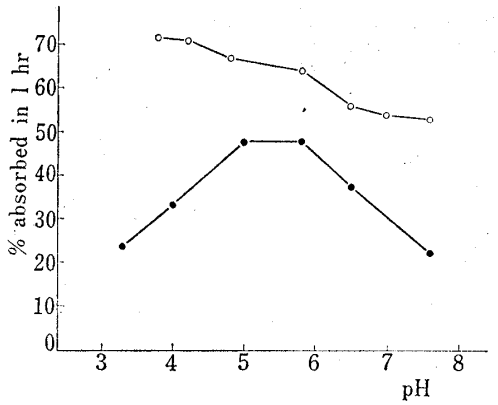

Fig. 2. pH-Profile of the Absorption of Sulfamethoxazole from 0.9% NaCl Solution (○) and Phosphate-Citrate Isotonic Buffer (●)

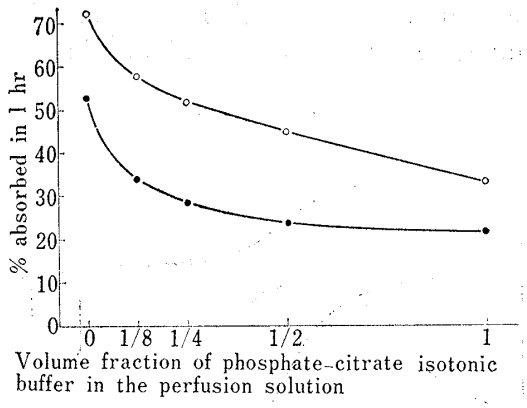

Fig. 3. Effects of Dilution of Phosphate-Citrate Isotonic Buffer with 0.9% NaCl Solution on the Absorption of Sulfamethoxazole

After a preperfusion with pH 7.6 and pH 3.3 isotonic buffers, absorption of sulfamethoxazole from the pH 5.8 isotonic buffer solution decreased from 48.1% to 33.8% and 23.3% respectively. But a preperfusion with Tyrode's solution (pH 7.8) or pH 5.8 buffer did not affect the absorption from pH 5.8 isotonic buffer.

Thus the effects of pH on the absorption of sulfamethoxazole can not be explained only from the degree of dissociation in the perfusion solution. So, the effects of the isotonic buffer solution on the intestinal mucous membrane should be taken into consideration. Considering possible existence of influences already at the preperfusion stage, absorption from a pH 5.8 isotonic buffer solution should be also taken into account.

So, a 0.9% NaCl solution was used as perfusion medium in the following study.

At pH 4.0 and 7.6, sulfamethoxazole was absorbed 33.2% and 23.0% respectively from phosphate-citrate isotonic buffer solution while it was absorbed 53.1% and 71.6% respectively from the 0.9% NaCl solution, whose pH was maintained by a continuous addition of 0.1N HCl or 0.1N NaOH.

The decrease in absorption from the isotonic buffer solution at pH 4.0 and pH 7.6 seemed to be due to the effects of the components of the perfusion medium on the intestinal mucous

membrane.

In order to ascertain the decreasing effects of isotonic buffer on the absorption of sulfamethoxazole, the buffer was diluted with 0.9% NaCl solution. The pH of a diluted buffer was maintained as previously described.

Absorption of sulfamethoxazole at pH 4.0 or 7.6 was enhanced by dilution (Fig. 3).

Thus citric acid and/or sodium phosphate seem to affect the absorption of sulfamethoxazole. Similar results were obtained in the absorption of other sulfonamides.

Correspondence of Maximum Absorption and Undissociated Fraction of Drug Molecules in Intestinal Absorption

It has been generally accepted that a pH-profile of intestinal absorption of drugs is similar to that of the undissociated fraction of drugs in perfusion solution. But it has been also admitted that pH for maximum absorption does not correspond with pH for the maximum fraction of undissociated drugs.

This pH deviation between maximum absorption and undissociated fraction has been explained by the existence of virtual pH on the surface of the intestinal wall, where the virtual pH proves to be 5.3[4] or 6.5.[5]

Presently a similar result was obtained in the absorption of sulfonamides as far as phosphate–citrate isotonic buffer was used as perfusion medium. When 0.9% NaCl solution was used as perfusion medium, pH-profile of the absorption corresponded completely with that of the undissociated fraction of drugs. The pH-profile of sulfamethoxazole is shown in Fig. 4. A similar correspondence was found in other sulfonamides (Fig. 5).

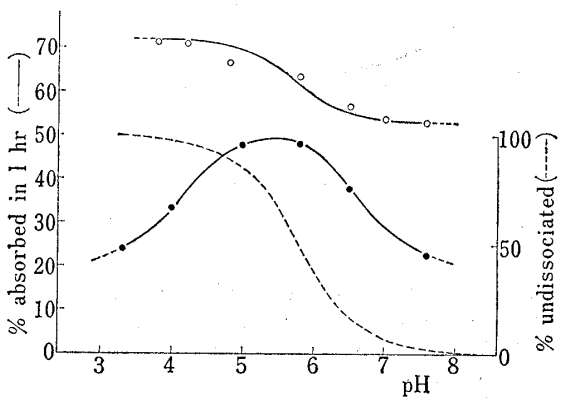

Fig. 4. pH-Profile of the Absorption of Sulfamethoxazole and the Undissociated Fraction

—○—: from 0.9% NaCl solution
—●—: from phosphate–citrate buffer

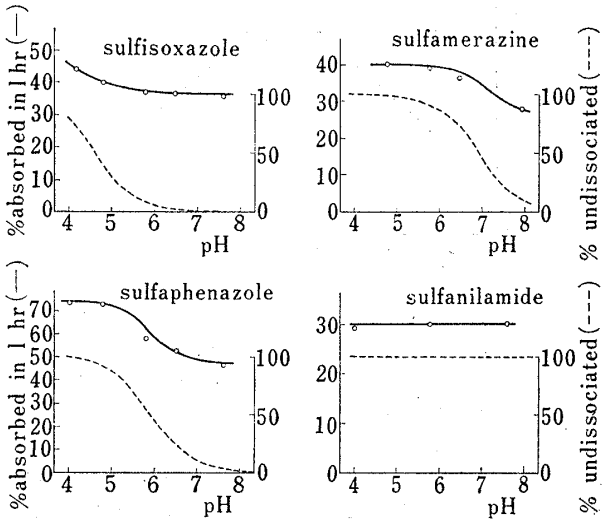

Fig. 5. pH-Profile of the Absorption of Sulfonamides

Although pH of the physiologic saline was checked at only one point, at the reservoir of the perfusion solution, local changes of pH in the intestinal lumen during perfusion could be ignored because the perfusion rate was fairly fast (32 ml/min).

So, discordance of pH-profile of absorption with the undissociated fraction in the isotonic buffer solution is to be attributable to the effects of components of the isotonic buffer. But a role of phosphate or citrate ions in the discordance of pH-profile of absorption with that of the undissociated fraction of drug molecules is left unclarified.

4) C.A.M. Hogben, D.J. Tocco, B.B. Brodie and L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **125**, 275 (1959).
5) T. Koizumi, T. Arita and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).