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Effects of Perfusate Constituents on Transmucosal Fluid Movement and Drug Absorption in Rat Small Intestine

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Effects of various constituents of the perfusates on the sulfanilamide absorption were investigated using *in situ* recirculation perfusion method with the rat small intestine. Constituents employed were salts and chemical which had been used as components in various buffer solutions and these salts were used in a single or in combination with each other.

The results revealed that both of the absorption and the transmucosal fluid movement were considerablly influenced by the constituents of the perfusates. Some salts exhibited lethal effects during the course of the experiment.

These results were not able to rule out by pH of the perfusates. However, it was found out that these results had one regression line which was the same as that obtained from the perfusates containing different concentrations of sodium chloride.

Taking these lines of evidences into accounts, the protocol in the determining the absorption using the *in situ* recirculation perfusion technique was proposed and discussed.

The study of drug absorption from the alimentary tract of animal had been extensively undertaken in fields of clinical medicine and pharmaceutical sciences and many publications appeared in literature in the last two decades. Basic findings presented by these publications have contributed in evaluating efficacy and safety of drug therapy, and also in pursuing more active substance which will be more absorbable. Thus results of the absorption study are evaluated from both of the practical and basic fields of life sciences, therefore the results should be accurate and reproducible. Generally speaking, the results obtained in *in vivo* and *in situ* studies often lack reproducibility. However, this poorness in reproducibility often may be brought about by poor design of the experiment. Especially in drug absorption study, buffer solutions containing phosphate or some other salts and chemicals were employed in *in vivo* or *in situ* experiment, although there have been no demonstrations that the gastrointestinal tract is rich of phosphate or others.[2] Such an experimental design may load unphysiological conditions to the animal through the gastrointestinal tract, however, the condition may be favored only to the subject substance in the study.

Too much attentions have been focused on the substance and not on the physiological conditions of the subjected animal. Based on these attitudes of investigators, the results of animal experiments must be fluctuated and poor reproducibility may be obtained. The drug absorption study must be conducted in certain conditions which are suitable for physiological activities of the intestine, unless otherwise the valuable results might not be obtained.

In the previous report of this series of study,[3] sodium chloride or glucose was employed as a constituent of perfusate in the *in situ* recirculation perfusion experiment. Since the salt is known to exist in abundant in the secretory juice in the gastrointestinal tract of animal

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2) H.W. Davenport, "Physiology of the Digestive Tract," 2nd ed., Year Book Medical Publishers, Chicago, 1966, p. 149.

3) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 1856 (1975).

and man.[4] Moreover, scarce difference in the concentration of the solute in the perfusate brought about considerable difference in the transmucosal fluid movement and also in the drug absorption.

In this report other solutes those of which are used as the buffer components were employed in the perfusate of the *in situ* recirculation perfusion experiment using rat small intestine. Since remarked differences were observed in both of the transmucosal fluid movement and the drug absorption, an appropriate experimental protocol in determining the extent of the absorption of a certain drug was proposed.

Experimental

Animal Procedures——Wistar albino strain of male rat, weighing 150 to 170 g, was fasted for an overnight prior to the experiment, but was allowed free access to tap water. Almost of the animal procedures including operation procedures and perfusion experiments were followed the method described in the previous report.[3] However, attentions were concentrated to the processes affecting a balance of fluid in the animal. Animal was anesthetized by an intraperitoneal injection of 0.5 ml of 1.25% pentobarbital sodium parentheral solution per 100 g body weight. After exposing the small intestine by a midline abdominal incision and cannulating both ends of the proximal duodenum and the distal ileal openings with silicon tubings, the other sides of the openings of the pylorus and the cecum were ligated to prevent loosing body fluid from these openings. The bile duct was also ligated to avoid any inflow of fluid into the intestine during all courses of the experiment. The small intestine was first washed and cleaned from particulates by a single perfusion of exactly 50 ml of 0.9% sodium chloride solution which had been maintained at 37°. This single perfusion was conducted within 5 min.

All the processes of the animal preparation which were similar to those described in the previous report[3] were completed, 30 ml of the perfusate were recirculated in order of duodenum to ileum at a rate of 5 ml per min. After elapsing 10 min, the initial sample was pipetted out and the perfusion experiment was conducted for following one hour and then the final sample was again pipetted out and assay procedures for the transmucosal fluid movement and the drug absorption were followed with these samples.

Test Solutions——All test solutions employed as the perfusate in the perfusion experiment contained one millimole of sulfanilamide and a certain concentration of phenol red which was used as an unabsorbable indica-

Table I. The Salts and Chemical used in the Present Study with Their Isotonic Concentrations and Initial and Final pH of the Perfusate

Name of salts or chemicals	Isotonic concentration (%)	pH of perfusate	
		Initial[a]	Final
Sodium chloride	0.90	5.8	6.4
Sodium sulfate	3.95	6.2	6.4
Disodium hydrogen phosphate	4.45	5.6	6.8
Sodium dihydrogen phosphate	2.18	5.6	6.8
Sodium bicarbonate	1.39	8.6	8.1
Sodium acetate	2.03	7.8	7.4
Trisodium citrate	3.02	7.5	7.1
Potassium chloride	1.19	6.0	6.4
Sodium carbonate	1.32	9.6	9.2
Ammonium chloride	0.80	5.9	6.4
Magnesium sulfate	6.30	6.7	7.1
Potassium dihydrogen phosphate	2.18	4.7	5.2
Sodium borate	4.93	9.3	9.0
Magnesium chloride	2.02	7.3	7.1
Calcium chloride	1.70	7.4	7.0
Taurine	3.58	7.2	6.8

a) The initial pH of the perfusate was measured with the solution containing 1 mM of sulfanilamide and a certain concentration of phenol red.

4) D.S. Dittmer (ed). "Blood and Other Body Fluids, Biological Handbook," Federation of American Societies for Experimental Biology, Washington, 1961, p. 416.

tor. Test solutions contained one of the various solutes which were listed in Table I. Unless otherwise stated, the solute was added to make the test solution isotonic. The isotonicity was checked by Hitachi Perkin-Elmer Molecular Weight Apparatus of Model 115 following method developed by Kitazawa and Komuro.[5] The concentrations of the solutes in the isotonicity were also listed in Table I.

The pH value of these perfusates was measured by pH meter model HM-5A of Toa Dempa Kogyo Co., Ltd, using a flow cell of type 80—54 and the value was recorded continuously by a recorder of model PB-20A during the course of the perfusion experiment. The initial pH of these test solutions indicated their own pH values of the solution, however, pH values were regulated within 10 min of the preperfusion period and thereafter the values were kept almost constant within their respective values during the perfusion. These values were also listed in Table I. Based on these values, sulfanilamide was expected to exist in an unionized form in all of the perfusates during the perfusion experiments.

Analysis Procedures——Analytical procedures for phenol red and sulfanilamide were conducted in the same manner as described in the previous paper.[3] Based on the results obtained the absorption and the transmucosal fluid movement were calculated following the method of Schanker and co-workers,[6] and these results were plotted in illustrations in the same manner as stated in the previous report.[3]

Results and Discussion

Transmucosal Fluid Movement and Sulfanilamide Absorption in the Isotonic Perfusate containing Single Salt

Figure 1 shows the effects of various salts on the transmucosal fluid movement and sulfanilamide absorption. Although tonicities of these perfusates were all set at the isotonic of respective solute, plots scattered in wide range both of the fluid movement and the drug absorption. In the case of magnesium sulfate fluid secretion was apparently observed even in an isotonic perfusate, and the absorption of the drug indicated 47.8%. On the other hand, sodium acetate promoted fluid absorption and the ratio decreased as low as 0.75, and the absorption of the drug increased to approximate 63.7%.

These evidences suggested that the transmucosal fluid movement in the rat small intestine was affected not only by the tonicity of the solute in the perfusate as described in the previous report,[3] but also by the constituents of the solute in the perfusate.

As was apparent from Fig. 1, scattered plots might have a straight regression line. A solid line illustrated in Fig. 1 was a regression line obtained by the perfusion experiments which had been conducted using hypertonic, isotonic and hypotonic perfusates of sodium chloride (n: 30, r: -0.935, equation: $y=-0.0124x+1.598$). Considering from the illustration, it would be possible to estimate that the scattered plots obtained in the present experiment might have the same regression line as that obtained in the previous experiment.[3] As a matter of fact, a regression line obtained from all of the results was expressed as an equation $y=-0.0124x+1.599$ (n: 110, r: -0.906), which was completely the same as that of the regression line of sodium chloride which was presented in the previous report.[3] Based on these evidences, the solid line in Fig. 1 could be acknowledged as the regression line of the plots illustrated in Fig. 1.

As had been observed in the cases mentioned above, some of the salts brought about fluid secretion and some of them brought about reversed flow of fluid in the intestine, and the drug absorption apparently obeyed the rule relating to the relationship between the transmucosal fluid movement and the drug absorption, which was disclosed in the previous report.[3] Moreover, it is of interest that these salts and chemicals indicate to give the same regression line to that obtained in the case of sodium chloride.

Plots of magnesium sulfate, magnesium chloride, calcium chloride and sodium citrate appeared in the region of fluid outflowed. This evidence suggested that these salts might be able to be used as laxatives or purgatives in the field of clinical medicine. Of these salts,

5) S. Kitazawa and T. Komuro, *Hitachi Scientific Intrument News*, **15**, 1143 (1972).
6) C.A.M. Hogben, D.J. Tocco, B.B. Brodie, and L.S. Schanker, *J. Pharmacol. Exper. Therap.*, **125**, 275 (1958).

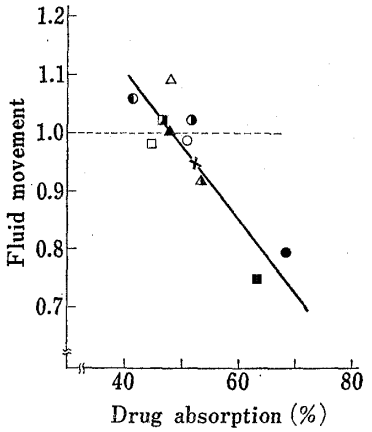

Fig. 1. Relationship between the Transmucosal Fluid Movement and Sulfanilamide Absorption from the Perfusates containing Single Salts and Chemical

A regression line of these plots was calculated following the least square method and found out that the regression line coincided to that obtained with the perfusates containing sodium chloride which had been appeared in the previous report.[3]
key:
△: magnesium sulfate □: taurine
◑: magnesium clhoride
×: sodium dihydrogen phosphate
◐: calcium chloride ▲: sodium bicarbonate
◧: sodium citrate ●: sodium chloride
▲: disodium hydrogen phosphate
■: sodium acetate ○: sodium sulfate

magnesium sulfate was found to be most effective. As a matter of fact, the salt is known as an effective purgative and used practically in clinical medicine. The mechanism of this salt as a purga tive was elucidated experimentally that magnesium sulfate promoted fluid movement from tissues to the lumen of the small intestine.

On the other hand, sodium acetate and sodium chloride did apparently accelerate inflow of fluid from lumen to tissues. Although the acceleration mechanism of sodium chloride had not been elucidated, similar phenomenon was found out in the report of Curran and Solomon,[7] Diamond,[8] and Powell and Malawer.[9] Csaky[10] demonstrated that ethyl acetate stimulated water transport into tissues in an *in vitro* study using an everted sac of the rat small intestine, and after comparative studies using alcohol and esterase inhibitors he concluded that the acetate ion did stimulate the transport of water into tissues. Csaky's findings were also supported in such an experiment of *in situ* recirculation perfusion in the present study. Although mechanism of the stimulation undergoing by acetate ion was not elucidated, the phenomenon might contribute in interpreting the absorption of acetylated compounds from the alimentary tract where esterase activity is abundant. The results related to the intestinal absorption of such compounds will be reported in separate paper from our laboratory in future.[11]

Salts Exhibiting Lethal Effects on Animal

The salts and chemicals employed as the solutes in the present study were selected at random from those of constituents of buffer solutions which had been used in wide fields of medical and pharmaceutical investigations. These salts were employed in the perfusate at the respective isotonic concentration.

However, some salts exhibited lethal effect on animal during the course of the perfusion experiment. For example, in the case of potassium chloride the subjected animal was found to be dead with severe convulsions after approximate 30 min from the beginning of the perfusion. Salts exhibited such an effect were listed with number of experiments and average of survival time in Table II.

As had been listed in Table I, pH of the perfusates containing these salts were not extraordinary far from the virtual pH[12] in the small intestine of this animal, the mechanism involved in exhibiting the lethal effect might not be promoted by pH of the perfusate, but the toxical effect of these respective salts.

Deep considerations concerning toxicities of these salts are not the aims of the present

7) P.F. Curran and A.K. Solomon, *J. Gen. Physiol.*, **41**, 143 (1957).
8) J.M. Diamond, *J. Gen. Physiol.*, **48**, 15 (1965).
9) D.W. Powell and S.J. Malawer, *Am. J. Physiol.*, **215**, 49 (1968).
10) T.Z. Csaky, G. Esposito, A. Faelli, and V. Capraro, *Proc. Soc. Exper. Biol. Med.*, **136**, 242 (1971).
11) S. Kitazawa, I. Johno, and T. Minouchi, in preparation.
12) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).

Table II. The Salts exhibited Lethal Effect during the Perfusion Experiment

Name of salts	No. of experiment[a)]	Average[b)] of survival time (min)
Potassium chloride	8/8	30
Sodium carbonate	8/9	25
Ammonium chloride	7/7	26
Magnesium sulfate	8/16	50
Potassium dihydrogen phosphate	8/8	55
Sodium borate	7/7	55

a) The number in denominator indicates numbers of the experiments and the number in numerator indicates number of animal dead during the experiment.
b) The average was calculated based on the number of animal dead during the perfusion experiment.

study and authors do not intend to seek the reason any more. However, judging from the survival time, potassium chloride, sodium carbonate and ammonium chloride might have severe toxicities for the subjected animal. The concentrations were as high as isotonic of respective salt and these concentrations might correspond to extraordinary high with comparing to the usual dose for man. However, as a matter of fact, potassium chloride is often administered to patients suffered by hypopotassemia[13)] and ammonium chloride is administered as an acidifier of urine.[14)] The present results arouse attentions that the administration should be performed with an extreme carefulness. Same attitude should be required in the case of the administration of magnesium sulfate, since a half of the animal was dead after 50 min in average of the perfusion.

Transmucosal Fluid Movement and Sulfanilamide Absorption in the Isotonic Perfusate containing Two Salts

The data presented so far in the present study were concerned with the isotonic perfusates containing single salt, and the transmucosal fluid movement and the drug absorption brought about with these respective salts were elucidated. However, buffer solutions are usually consist of at least two components of salts.

Our attentions were concentrated to investigate how the results obtained by single salt might be modified when two salts were in the isotonic perfusate.

The first attempt was a comparative study of isotonic perfusates containing equivolume mixtures of the isotonic solutions of sodium sulfate and sodium chloride, and sodium sulfate and sodium acetate. As had been presented in Fig. 1, plots of these three salts were placed on the regression line, and the plots of these mixtures were expected to be on the regression line also and, moreover, at the exact intermediate point between these parent salts. Figure 2 shows the results of these isotonic perfusates. The plots of the mixtures appeared just on the regression line. This evidence suggested that mixing of these salts having known effects concerning the fluid movement and the drug absorption would not change the effect of respective salts in nature.

However, although the perfusates were equivolume mixtures of respective parent solutions, the plots did not appear just on the intermediate but were biased to one of the parent salts. As evident from Fig. 2, the plot of the mixture of sodium sulfate and sodium chloride did appear near the plot of sodium sulfate, and the plot of the mixture of sodium sulfate and sodium acetate was found near the plot of sodium acetate. These evidences suggested that there might be difference in strength of promoting the inflow of fluid in the intestine between these

13) P.F. Gulyassy, C.V.Y. Strihou, and W.B. Schwartz, *J. Clin. Invest.*, **41**, 1850 (1960); C.V.Y. Strihou and J.P. Kassirer, *New Eng. J. Med.*, **279**, 630 (1968).
14) H.B. Kostenbauder, J.B. Portnoff, and J.V. Swintosky, *J. Pharm. Sci.*, **51**, 1084 (1962).

solutes in the perfusate. If the concept of the strength were true, sodium acetate might be strongest in development of its activity in the fluid movement, since sodium acetate could almost cancel the activity of sodium sulfate. Similarilly the activity of sodium chloride was almost contradicted by the addition of sodium sulfate.

These lines of evidences suggested that the transmucosal fluid movement might be more complicated in the isotonic perfusate contained two salts. The ratio of the fluid movement was not followed by an additional mean of those of the parent salts. However, it is of importance that the plot placed just on the regression line indicating the relationship between the fluid movement and the drug absorption obtained with the perfusions having different tonicities of sodium chloride.

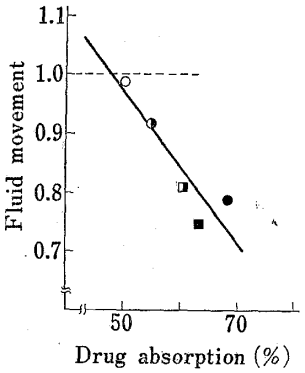

Fig. 2. Relationship between the Transmucosal Fluid Movement and Sulfanilamide Absorption from the Perfusates containing Equivolume of Isotonic Solutions of Sodium Sulfate and Sodium Chloride, and Sodium Sulfate and Sodium Acetate

The solid line in the figure represents the regression line that obtained in Fig. 1.
key:
○: sodium sulfate, isotonic perfusate
◑: equivolume mixture of isotonic solutions of sodium sulfate and sodium chloride
◨: equivolume mixture of isotonic solutions of sodium sulfate and sodium acetate
●: sodium chloride, isotonic perfusate
■: sodium acetate, isotonic perfusate

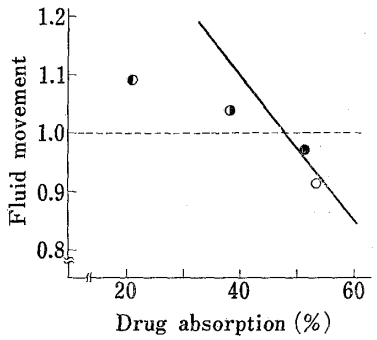

Fig. 3. Relationship between the Transmucosal Fluid Movement and Sulfanilamide Absorption from the Perfusates containing Sodium Carbonate and Sodium Bicarbonate

The solid line in the figure indicates the regression line that obtained in Fig. 1.
key:
○: isotonic perfusate of sodium bicarbonate
●: mixture of isotonic solutions of sodium carbonate and sodium bicarbonate in a ratio of 1/4:3/4
◑: mixture of isotonic solutions of sodium carbonate and sodium bicarbonate in a ratio of 1/2:1/2
◐: mixture of isotonic solutions of sodium carbonate and sodium bicarbonate in a ratio of 3/4:1/4

The second attempt was undertaken with the perfusate containing sodium carbonate. Although sodium carbonate is an indispensable component of Delory and King's carbonate-bicarbonate buffer solution[15] and others which have been used in wide in investigations, the salt exhibited lethal effect on the animal in this experiment. Authors considered that it would be necessary to study more in detail on the effect of this salt.

Concerning toxicity of this salt, it is described in a reference book[16] that ingestion of large quantities of this salt may produce corrision of gastrointestinal tract, vomiting, diarrhea, circulatory collaps and death, and the lethal dose was not mentioned. In the present study the concentration of salts and chemicals used as the solutes in the perfusate was fixed at the isotonic concentration. The dose employed in the case of this salt might be an overdose for the animal and correspond to large quantities described in the reference book.[16] However, judging from the isotonic concentrations listed in Table I, the dose of this salt was not extraordinary high as compared to the other sodium salts.

15) G.E. Delory and E.J. King, *Biochem. J.*, **39**, 245 (1945).
16) The Merck Index, 8th ed., Merck and Co., Rahway, N.J., 1960, p. 948.

The attempt undertaken was a series of perfusion experiments with isotonic solutions having different concentrations of sodium carbonate and sodium bicarbonate. Three perfusion solutions having serial concentration of every a fourth part in volume of the solutions were replaced with isotonic solution of sodium carbonate and sodium bicarbonate in compensatory were perfused in a regular manner. Nine runs of experiments were conducted with respective perfusates and the results were illustrated in Fig. 3.

The results obtained from the perfusate containing a fourth of isotonic solution of sodium carbonate were plotted just on the regression line illustrated in Fig. 1. However, the plot was shifted to the direction indicating decrease in both of the fluid movement and the drug absorption of those of sodium bicarbonate. When the concentration of sodium carbonate was increased, the perfusate containing equivolume of isotonic solutions of sodium carbonate and sodium bicarbonate gave the result detached from the regression line, and the plot appeared in a region of promoting outflow of fluid and the drug absorption was less than that expected from the regression line. This inclination was exaggerated when the concentration of sodium carbonate was increased. Fortunately, we had only one result which the pefusion experiment could be completed with this salt. The result, 1.099 in the ratio of the fluid movement and 9.61% in the drug absorption, was presented on reference to considerations. Taking the result into accounts, a regression line having smooth curvature might be obtained when the results were connected in turn.

Detailed surveys to these results suggested that, although the transmucosal fluid movement might be shifted in proportion to the concentration of sodium carbonate, the drug absorption would not be proportional with the fluid movement, and the absorption were decreased less than that expected. The sudden decreasing in the sulfanilamide absorption was not brought about by pH change due to increasing in concentration of sodium carbonate, since average pH during the pefusion using the perfusate containing isotonic solution of this salt in three fourth parts indicated 9.18 and did not surpass pK_a value of the drug.

Observations on the animal during the course of the perfusion experiments revealed that light convulsions occurred several times especially in the last half of the perfusion period when the prfusate contained more than equivolume of the isotonic solution of sodium carbonate. These observations suggested that something due to sodium carbonate might occur in the physical conditions of the animal and this something might reduce the drug absorption directly and/or indirectly, since such convulsions were never observed in the cases of sodium chloride even in hypertonic perfusates.

These lines of evidences suggested sodium carbonate would bring about something which might influence physical conditions of the subject animal and also absorption of sulfanilamide from the small intestine. However, these influences would not occur when the concentration of this salt was not more than a half of the isotonicity of this salt in the carbonate-bicarbonate buffer solution, and in these cases, the drug absorption and the transmucosal fluid movement would be obeyed the regression line of sodium chloride as illustrated in Fig. 3.

Proposed Protocol in Determining the Absorption of a Certain Substance from Rat Small Intestine with *in Situ* Recirculation Perfusion Experiment

Based on the results presented so far in this report, it might be concluded that the extent of drug absorption would be varied depending on constituents of the perfusate in the *in situ* recirculation perfusion experiments, in other words, effect of the constituents might not be negligible in evaluating the results of the absorption study.

It is well known that, although some exceptions are existed, the pH-partition hypothesis presented by Brodie, Schanker and their co-workers[17] governs the extent of drug absorption

17) P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exper. Therap.*, **119**, 361 (1957); L.S. Schanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *ibid.*, **120**, 528 (1957).

from the gastrointestinal tract. Many results satisfying the hypothesis using the same method as used in the present study have been presented in literature.[18] From the point of our views, the investigators presented such satisfying results were lucky and had an excellent talent in selecting the most appropriate constituents of the perfusate for the study.

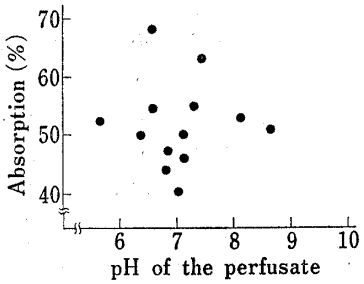

Fig. 4. The Absorption of Sulfanilamide from the Perfusates having Various pH

Sulfanilamide has two pK_a values such as 2.36 and 10.4, and this suggested the absorption of sulfanilamide might not vary in the perfusate having pH between approximate 4 and 9, if the hypothesis were applicable to this substance. All the data obtained in the present study were plotted in a illustration having the absorption on the vertical axis and the final pH of the perfusate on the horizontal axis, and Fig. 4 was depicted.

As was evident from Fig. 4, the extent of sulfanilamide absorption was remarkedly varied indifferent to pH value of the perfusate, and a consistency in the results of absorption was not seemed difficult to obtain. These results drived authors into a maze and an accurate and reproducible extent of the absorption of this substance from rat small intestine would never be obtained.

However, there was a salvation for investigators and a possibility that might solve such a complicated problem. As had been presented in Fig. 1, the absorption of this substance was obeyed to a regression line indifferent to constituents not only sodium salts but also other salts such as magnesium or calcium salts and other chemicals such as taurine. Moreover, the regression line was found to be quite the same as that obtained with perfusates having different tonicities of sodium chloride, which was presented in the previous report.[3] It would be not so difficult to obtain a constant and reproducible result in the extent of the absorption of drug from these scattered plots along a certain regression line. The regression line had an intercept on a line indicating 1.0 in the ratio of the fluid movement and the value of the absorption on the intercept might be the most appropriate value presenting the absorption of a certain drug. Since 1.0 in the ratio of the fluid movement could be regarded as that the absorption might be proceeded in different to the transmucosal fluid movement in the small intestine.

These lines of evidences and considerations encouraged authors to present a protocol in determining the absorption of a certain drug from the rat small intestine using *in situ* recirculation perfusion. The protocol is consist of two essential steps. The first step is concerned to the constituent and its concentration of the perfusate. The most reasonable constituent that presents the absorption of drug in the physiological conditions of the small intestine will be sodium chloride, since the salt was revealed to exist in abundant in the intestine.[4] However, in the case that other salts or chemicals are obliged to select by reasons such as low solubility of the drug or investigating the absorption from solutions having certain constituents, the solutions might be selected along with the lines of purposes of the investigations. The concentration of sodium chloride or other constituents in the perfusate should be set at least three levels, and the most preferable levels will be 1.5 times of the isotonicity, isotonicity and a half of the isotonicity of the constituents of the perfusate.

After proceeded the *in situ* recirculation perfusion experiments, the second step starts with the illustration of each result in the same manner as did in the present study, and after confirming that a straight regression line of the scattered plots may be obtained, an extent

18) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964); K. Kakemi, T. Arita, and S. Muranishi, *ibid.*, **13**, 861 (1965).

of the absorption at an intercept of the regression line and a horizontal line at 1.0 in the ratio of the fluid movement should be obtained. The absorption of sulfanilamide obtained following the protocol revealed 48.2%.

The protocol will not always applicable in all cases, and some further reconsiderations and modifications might be required in drug absorption study. However, the proposed protocol will open the way obtaining more accurate and reproducible results in the absorption investigations.