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# Transmucosal Fluid Movement and Its Effect on Drug Absorption

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The effect of transmucosal fluid movement on drug absorption from rat small intestine was studied, using *in situ* recirculation of perfusion method. Transmucosal fluid movement was obtained by using perfusion solutions of various tonicities of sodium chloride or glucose, and drugs employed were sulfanilamide, sulfisoxazole and metoclopramide.

The results obtained revealed that the drug absorption was increased with increasing the transmucosal fluid movement from lumen to blood, and in the case when the fluid was secreted, that is, counter directional fluid movement was obtained, the absorption was found to be decreased. These relations became apparent from figures in which the absorption on the horizontal axis and the ratio of the fluid movement on the vertical axis.

Using the method of the illustration of figure, the effect of glucose on drug absorption had become occasionally apparent.

Several mechanisms have been implicated in the permeability of substances through biological membranes as well as intestinal mucosa.[2] Despite many lines of evidences related to effects of physico-chemical properties of substances on absorption from the gastrointestinal tract, limited findings have accumulated concerning to the effect of physiological conditions and physiological functions of the tract from which substances transport into another compartment.

When the gastrointestinal tract is free from food stuffs, ratio in the volume of fluid in the tract to total fluid in the whole body is known to be kept at a certain value,[3] and when food is ingested, that is postprandial state, large volume of fluid is secreted into the lumen in forms of digestive fluids or some others, to regulate the osmotic pressure in the lumen, or to make better conditions for digestion and absorption which will follow afterward.[4]

Although the dynamics of this transmucosal fluid movement is not obvious yet, in man about seven or more liters of fluid are secreted into the tract everyday, while only 1.5 liters are ingested orally. As only about 150 ml of fluid are lost in feces, more than eight liters of fluid are secreted and absorbed, in other words, across the intestinal mucosa in a day.[5]

These evidences bring another physiologically important factor, transmucosal fluid movement, into absorption studies. To study the effect of such transmucosal fluid movement on drug absorption will provide many important and practical informations concerning to availability of drugs after oral administration, because many drugs are taken orally in relation to meals.

In this paper, the effect of the transmucosal fluid movement on the absorption of drugs was investigated by the *in situ* recirculating perfusion method using rat small intestine and fluid movement was artificially obtained by using hypertonic, isotonic and hypotonic solutions of sodium chloride or glucose with different concentrations as the perfusate.

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2) T.H. Wilson, "Intestinal Absorption," Saunders Co., 1962; T.B. Binns, "Absorption and Distribution of Drugs," Livingstone Ltd., 1963.

3) F. Gotch, J. Nadell, and I.S. Edelman, *J. Clin. Invest.*, **36**, 289 (1957).

4) J.S. Fordtran and T.W. Locklear, *Am. J. Digest. Diseas.*, **11**, 503 (1966).

5) C.W. Carter, "Biochemistry in Relation to Medicine," London, Longmans Green & Co., 1959, p. 246.

## Experimental

**Animal Procedures**——According to the recirculating perfusion method which was devised by Schanker, *et al.*,[6] amount of the drug disappeared in the perfusate was regarded as the amount absorbed from the site of the lumen which was exposed to the perfusate. This method was employed in this study throughout.

Wistar albino strain male rat, weighing 150 to 170 g, was fasted for an overnight prior to the experiment, but was allowed free access to water. Animal was anesthetized by an intraperitoneal injection of 0.5 ml of 1.25% pentobarbital sodium parenteral solution per 100 g body weight.

The small intestine was exposed by a midline abdominal incision and cannulated at the both ends of the proximal duodenal and distal ileal openings with silicone tubings having inside diameter of 2.2 mm and outside diameter of 3.0 mm. These cannulae were fastened with strings and openings of the site of stomach and cecum were also ligated. The bile duct was also ligated in all experiments to protect any inflow of fluid into the small intestine during the experiment. These ligatures were made with cautions not to occlude any blood vessels.

The small intestine was first washed and cleaned from particulates by a single perfusion of 50 ml of 0.9% sodium chloride solution which had been maintained at 37°. The intestine was stored in the adbomen and incision was closed with metal clips and the cannulae were joined to a glass tubings which were connected to a perfusion pump (CV-I type, Tokyo Kagaku Seiki Co.).

The closed circuit of the recirculating system used in the experiment was consisted of the small intestine, silicone tubings, glass tubings, the pump and a reservoir with a volume of 50 ml. The reservoir was immersed in a water bath of a temperature of 37° to keep temperature of the perfusate constant during the experiment. To prevent evaporation and decreasing the volume of the perfusate, the reservoir was stoppered with a cork which had three openings, two of which were used to inlet and outlet glass tubings respectively and the rest, which was as small as possible, was used to pipette out samples from the perfusate.

Thirty milliliters of the perfusate were recirculated in order of duodenum to ileum at a rate of 5 ml per minute. Ten minutes after the beginning of the perfusion, an initial sample was pipetted out from the reservoir. This period was estimated enough to mix the perfusate with a small amount of the washings which had been left in the tract and the sample was able to regard as the starting condition concerning to both concentrations of drug and phenol red which was used as a nonabsorbable indicator. The perfusion was followed for one hour and then a final sample was pipetted out and assay procedures were followed.

In the case of an investigation of the effect of vasopressin, the hormone (obtained from Parke Davis & Co., Detroit, U.S.A.) was injected intraperitoneally one half hour prior to and ten minutes after the beginning of the perfusion, and the dose was 4 pressor unit per body for each administration.

Blood samples were collected, if necessary, into a small heparinized beaker by cutting off the end of the tail of the animal at a given interval.[7]

**Test Solutions**——Considering the purpose of the experiment, test solutions should contain the simplest components capable at least to occur bidirectional fluid movement across the epithelial layer of the small intestine. Unless otherwise stated, test solutions employed in the experiments contained one millimole of drug, an adequate concentration of a nonabsorbable marker and sodium chloride or glucose. Osmotic pressures of the test solutions were adjusted by increasing or decreasing in the concentration of sodium chloride or glucose to obtain hypertonic, isotonic and hypotonic solutions.[8] However, as mentioned in Results and Discussion, upper limit of hypertonicity was set at twofold of an isotonic and that of hypotonicity was a half of an isotonic concentration for each sodium chloride and glucose. As the result of these, the concentrations of the salt and glucose added to test solutions were determined in a range of 0.45 to 1.8%, and 2.5 to 10.0% respectively.

In the experiment designed to reveal a relationship of the fluid movement to the concentration of the salt or glucose, the perfusate having different tonicities was used without any drug.

To compare the results obtained with the perfusate mentioned above to that with a perfusate which had been widely used in the absorption studies, phosphate buffer of pH 6.5 with 1 mM of drug was employed as the perfusate. The component of the buffer was $Na_2HPO_4 \cdot 12\ H_2O$ 7.2 g, $KH_2PO_4$ 6.4 g, and NaCl 5.9g in distilled water to a volume of 1000 ml.

As an example of the perfusate having the least osmotic pressure, distilled water containing 1 mM of drug and not the salt nor glucose was perfused through the rat small intestine.

6) C.A.M. Hogben, D.J. Tocco, B.B. Brodie, and L.S. Schanker, *J. Pharmacol. Exper. Therap.*, **125**, 275 (1958).

7) H.C. Grice, *Lab. Animal Care*, **14**, 483 (1964).

8) Physiological isotonic concentrations of sodium chloride and glucose are 0.9% and 5% respectively. Perfusion solutions having various concentrations more than these isotonic concentrations of the solute are nominated "hypertonic solution," and solutions having less than the isotonic concentration are nominated as "hypotonic solution" in this report.

Phenol red, which had been used as a nonabsorbable marker, was included in all perfusates so that the degree of dilution or concentration of the test solution druing the perfusion study could be analysed and the transmucosal fluid movement was able to estimate from changes in the concentration of the marker.[6]

**Drugs**——Drugs used in these experiments were sulfanilamide, sulfisoxazole and metoclopramide, their molecular weights and p$K_a$ values are listed in Table I.

TABLE I. Molecular Weight and p$K_a$ of the Drug used in the Study

| Drug | Molecular weight | p$K_a$ |
|---|---|---|
| Sulfanilamide | 172.21 | 10.4 |
| | | 2.36 |
| Sulfisoxazole | 267.33 | 5.0 |
| | | 1.55 |
| Metoclopramide | 299.81 | 8.97 |

As could be understood, the test solution used in the study did not contain substances which had functions to keep pH of the perfusate except the case using pAosphate buffer solution, however, the pH of the perfusate was found to be kept almost constant at about 6.4 during the experiment. This constancy in pH of the perfusate suggested that sulfanilamide existed as an unionized form, and sulfisoxazole in the form of anion and metoclopramide in the cationic form in the test solution during all over the period of the perfusion study.

**Analysis of Samples**——Phenol Red: Five tenth milliliters of the pertusate were pipetted out and analyzed spectrophotometrically at a wave length of 550 mμ, immediately after alkalization by adding 5 ml of 1N sodium hydroxide solution.

Drugs: As all the drugs employed in the experiment had an aromatic amino group, so the method of diazo-coupling using Tsuda's reagent was applicable in all cases. Drugs in the perfusate and in the blood[9] were diazotized with the regular manner and coupled with 2-diethylaminoethyl-1-naphthylamine (Tsuda's reagent) and after developing color, their optical densities were determined spectrophotometrically at a wave length of 550 mμ using Hitachi spectrophotometer of model 139.

In analyzing the perfusate, drugs and phenol red were coexisted, so careful investigations were made concerning to their interferences to each other, and enough validities were found in each case of the drugs. These analytical procedures were carried out to all the initial and the final samples and absorption of the drugs and the transmucosal fluid movement were calculated following the method of Schanker, *et al.*[6]

## Results and Discussion

### Relationship of Transmucosal Fluid Movement to Concentrations of Sodium Chloride or Glucose in the Perfusate

Although many literatures had cited that bulk flow of fluid across the epithelial membrane of the small intestine of man and of some animals was observed using perfusates of different concentrations of solutes, they did not mention about a quantitative relationship between the volume of fluid transferred and concentrations of solutes. Especially in such an experiment of *in situ* recirculating perfusion of rat small intestine, little information concerning to the relationship could be obtained from literatures.

To reveal a relationship between drug absorption and fluid movement, it was thought to be necessary to find out a relation of the fluid movement to tonicities of the perfusate which would be determined by the concentrations of the solutes, and an experiment was designed to obtain such a rudimental relationship. A series of perfusates having different concentrations of sodium chloride were perfused through the rat small intestine for one hour. The result is shown in Fig. 1, in which the transmucosal fluid movement which is represented by the ratio of the initial concentration over the final concentration of the nonabsorbable indicator is plotted on the vertical axis and the concentration of sodium chloride is on the horizontal axis. Values on the vertical axis express ratios of the volume of the fluid which moved across the epithelial surface of the rat gut during the experiment, and as the result of course, when the ratio is smaller than 1.0, it is able to regard that the fluid in the lumen is moved into epi-

9) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).

thelial tissues, that is, absorption of the fluid is found to be dominant, and reversely, when the ratio is larger than 1.0, fluid comes out from tissues, that is, secretion of fluid is observed to be dominant during the course of the perfusion study. The volumes of these inflow and/or outflow of the fluid are recognized larger when the differences in the ratio from 1.0 are increased.

As it became clear from Fig. 1 that when the concentration of sodium chloride in the perfusate was as low as 0.3%, large volume of the perfusate was observed to be absorbed, and this absorbable volume of the fluid gradually decreased with increasing the concentration of the salt, and when the concentration of the salt reached to 1.2%, the inflow and outflow of the fluid was found to be balanced and an apparent transmucosal fluid movement was not observed. Over the concentration of the salt of 1.2%, the volume of fluid outflowed increased with increasing the concentration of the salt up to 1.8%.

Over the concentration of 1.8% of sodium chloride, large amount of mucous and other epithelial substances were peeled off and the recirculation was found to be difficult to complete. From this evidence, it was concluded that the upper limit of the concentration of the salt should be about 1.8% and that an unphysiological condition for the rat gut would be brought when the concentration was over this limitation. On the other hand, when the concentration of the salt was lower than 0.3%, almost of the perfusate was absorbed and as the result air bubbles were observed in the perfusate at the last period of the experiment. To exclude the effect of the air bubbles, it was considered to be preferable to set this concentration the lowest limitation in the perfusion study.

Taking these lines of evidences into consideration, the concentration of the salt was limited from 0.45%, which corresponded a half of the isotonicity of this salt, to 1.8%, which corresponded to twofold of that value, and so the results obtained would be able to recognize to be free from these unphysiological effects.

Figure 1 supports the findings of Curran and Solomon,[10] and Diamond,[11] and Powell and Malawer[12] that even from the isotonic perfusate of 0.9% of sodium chloride, the inflow

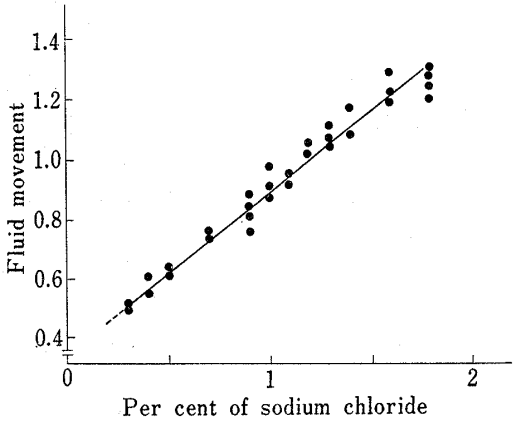


Fig. 1. Effect of Concentration of Sodium Chloride on Fluid Movement

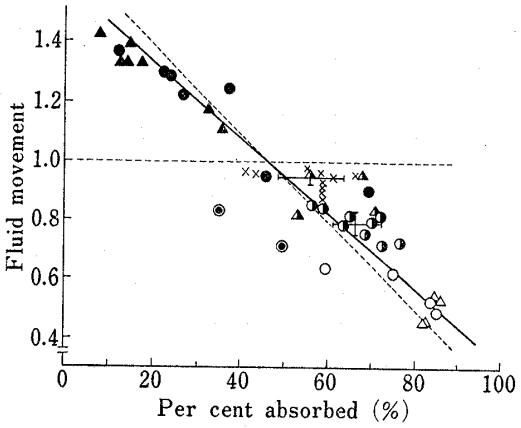


Fig. 2. Relationship between Per Cent Absorbed of Sulfanilamide and Fluid Movement

▲ : glucose hypertonic
▲ : glucose isotonic
△ : glucose hypotonic
● : sodium chloride hypertonic
◑ : sodium chloride isotonic
○ : sodium chloride hypotonic
× : pretreated with vasopressin (2×4 units per body)
◉ : distilled water

10) P.F. Curran and A.K. Solomon, *J. Gen. Physiol.*, **41**, 143 (1957).
11) J.M. Diamond, *J. Gen. Physiol.*, **48**, 15 (1965).
12) D.W. Powell and S.J. Malawer, *Am. J. Physiol.*, **215**, 49 (1968).

of the fluid is observed. Figure 1 also demonstrates that the volume of fluid inflowed and outflowed is susceptively influenced by the concentration of the salt in the perfusate, for the difference of 0.1% in the concentration corresponded to 0.1 on the vertical axis.

Almost the same results were also obtained in the case where glucose was used in the place of sodium chloride. As the concentration of isotonic solution of glucose is 5%, the range in the concentration used was from 2.5 to 10.0%, which corresponded to a half of and twofold of the isotonic concentration of this solute. These findings supported that both of sodium chloride and glucose were undoubtedly the solute that were able to move the fluid across the epithelial membrane of the rat small intestine.

**Fluid Movement and Sulfanilamide Absorption**

All the datum obtained from the experiment concerning to absorption of sulfanilamide from rat small intestine were plotted in a graph, in which the ratio of fluid movement is on the vertical axis, as in Figure 1, and in this case absorption of the drug is on the horizontal axis. Thus Figure 2 is obtained and shows a relationship between fluid movement and absorption of the drug clearly.

Ratios of fluid movement obtained from the perfusates of different tonicities of sodium chloride and glucose were found almost the same as that obtained without the drug which is illustrated in Figure 1. So the effect of the drug on the fluid movement was recognized negligible in these studies. As shown in Figure 2, plots obtained from hypotonic, isotonic and hypertonic perfusates of sodium chloride made a regression line ($n$: 30, $r$: $-0.935$) and the line was found straight for a wide range of the percentage of the absorption of the drug. The absorption of the drug changed from 10%, when tonicity of the perfusate was adjusted to twofold of a isotonic, to about 85% when the tonicity was a half of the isotonicity. That such a slight change in the concentration of the salt in the perfusate, that is from 1.8 to 0.45%, made a considerable change in the absorption of the drug from the rat small intestine was now understood. In other words, the absorption of sulfanilamide remarkably affected by tonicity of the perfusate used in the experiment.

It was clearly demonstrated that when the fluid of the perfusate was inflowed, the absorption of the drug appeared increase, and when fluid was outflowed into the perfusate the absorption of the drug observed decrease, in other words, the drug could transport along a current of the fluid but could not transport against a current of the fluid.

Concerning to the tonicity of the perfusate, a test solution without sodium chloride or glucose should have the lowest tonicity in these experiments. The result of the experiment using "distilled water" as the perfusate was anticipated on the line extrapolated to the right hand side of the regression line. Contrary to the expectation, the plots were not on the line and shifted to a region that the absorption was decreased and, moreover, the ratio of the fluid movement seemed to be much higher than that of the hypotonic perfusate. Dennis[13] demonstrated that distilled water damaged the epithelial surface of the rat small intestine anatomically, and this damage might excrete fluid into the lumen. These findings were convenient to explain well the result, as that, distilled water damaged the surface of the lumen, this would bring decrease the absorption of the drug, and as the result of fluid secretion from the damaged part of the tract, apparent absorption of the perfusate thus decreased.

One of the physiological functions of vasopressin is to inhibit the absorption of fluid from the large intestine.[14] It seemed of interest to use the hormone when the isotonic perfusate was used in the experiment. Vasopressin was injected intraperitoneally before and during the perfusion experiment to maintain the effect of the hormone. The result revealed that the fluid absorption was apparently inhibited and the plot shifted along the regression line

13) C. Dennis, *Am. J. Physiol.*, 129, 171 (1940).
14) K.H. Soergel, G.E. Whalen, J.A. Harris, and J.E. Greenen, *J. Clin. Invest.*, 47, 1071 (1968).

to the direction of decreasing the inflow of fluid from the lumen and, as the result the absorption of the drug was found also decreased.

To promote the absorption study using the method of perfusion in advance, a few points should be considered and made sure in this stage of the study.

The first thing to be made sure is blood level of the drug during and after the experiment. The defect of the method used in this study is the amount of the drug that is bound or stored in the surface of the gastrointestinal tract is also accounted as the amount of the drug absorbed. In general, intestinal drug absorption consists of two main steps, binding of drug to surface of the intestinal tract and diffusion of the drug molecule to circulatory systems. The increase in drug absorption with increasing of inflow of the fluid, as illustrated in Fig. 2, might be misunderstood that increasing of the inflow affected only the binding process and not the whole processes of the absorption. From this point of view, it is important to measure blood levels of the drug during and after the absorption experiments. The results obtained are shown in Fig. 3.

As illustrated in Fig. 3, the blood levels of the drug apparently indicate differences between the cases when the hypertonic solution and the hypotonic perfusate were used in the perfusion. From these results, it might be able to conclude that the increase of fluid inflow brought increase in the absorption.

These data were obtained from 0.1 ml of the blood of the animal used in the studies. In the experiment with hypotonic perfusate, the blood of the rat would be diluted and in the case of hypertonic perfusate, the blood would be concentrated, because the fluid movement might affect on the volume of plasma water in the blood. The results in Fig. 3 expressed simply the concentrations of the drug in each volume of the blood without taking these estimations into consideration, and if these were taken into account, the differences of these curves would be more exaggerated.

The second thing to be made sure is the absorption of phenol red, which had been considered not to be absorbed in the gastrointestinal tract from isotonic perfusates and had been widely used as a nonabsorbable marker in the absorption studies. Careful experiments were conducted to know whether the marker was absorbed in both cases of hypotonic and hypertonic perfusates. The results revealed that the average of the absorption of the marker was almost zero percent with meaningless variations, so it was possible to conclude that the marker was not absorbed even from the hypotonic and also from the hypertonic perfusates of both sodium chloride and glucose. Moreover, if the marker was absorbed, the slope of the regression line in Fig. 2 would become more gentle so that the effect of the fluid movement on the absorption of the drug would be more exaggerated.

The third thing to be made sure is the effect of volume of perfusates on the absorption of the drug. Yamada, *et al.*[15] found that the absorption of drug was inversely proportional to the volume of the perfusate in the *in situ* recirculation experiment which was employed in this study. In these experiments, in which the plot was below 1.0 on the vertical axis, the volume of the perfusate in the reservoir was observed gradually decrease during the perfusion, of course, this was brought by the absorption of the fluid, and on the contrary, the volume increased gradually when the plot was above 1.0 line of the vertical axis.

The absorption of the drug was calculated following Yamada's equation using the volume of a half of the initial which corresponded to the condition that the initial volume of the perfusate was decreased to a half of that during the experiment, that is, the results when the plot obtained 0.5 of the ratio of the fluid movement. This calculation was also made in the case of the hypertonic perfusate using values obtained the ratio of 1.2. The results revealed that the regression line should be corrected to the dotted line which is illustrated in Fig. 2. The slope of the dotted line is shifted a little bit steeper, but the difference between two lines is

15) H. Nogami, M. Hanano, and H. Yamada, *Chem. Pharm. Bull.* (Tokyo), **11**, 395 (1963).

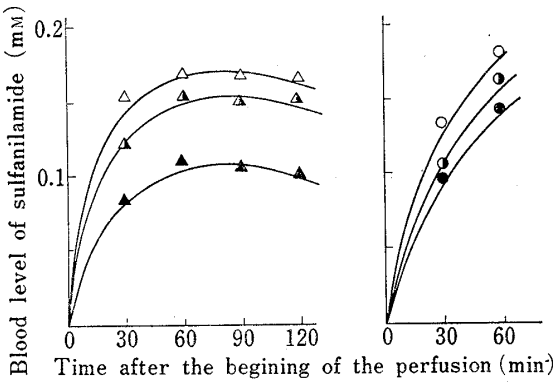


Fig. 3. The Difference in the Blood Levels of the Animal after the Perfusion of Different Tonicities of the Solute in the Perfusate

▲:glucose hypertonic ●:sodium chloride hypertonic
▲:glucose isotonic ◑:sodium chloride isotonic
△:glucose hypotonic ○:sodium chloride hypotonic

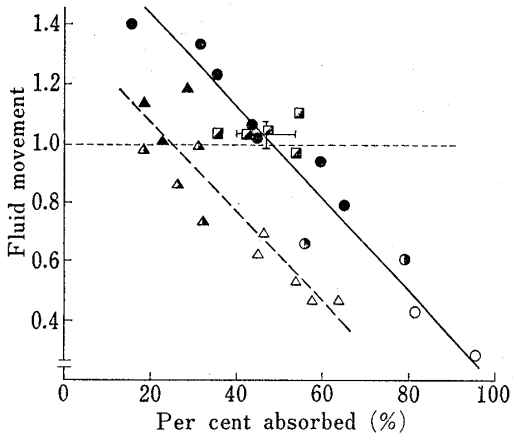


Fig. 4. Relationship between Per Cent Absorbed of Sulfisoxazole and Fluid Movement

▲:glucose hypertonic ●:sodium chloride hypertonic
▲:glucose isotonic ◑:sodium chloride isotonic
△:glucose hypotonic ○:sodium chloride hypotonic
◪:phosphate buffer (isotonic, pH 6.5)

not still so remarkable. Upon these considerations, it was possible to conclude that the effect of the volume change during the experiment on the absorption of the drug was negligible.

**Sulfisoxazole Absorption**

Sulfanilamide which exists in the form of an unionized molecule in the perfusate during the experiment has been the subject of this study, and the intestinal absorption of this form of the drug was found to be sensitively affected by the transmucosal fluid movement. Then another question arises whether the absorption of drug which exists in the form of an ionized molecule is also affected by the fluid movement. Sulfisoxazole was picked up to be investigated. The drug exists in an anion in the perfusate of the experimental condition. The results are depicted in Fig. 4.

The absorption of the anion was also affected by the transmucosal fluid movement which was caused by different concentrations of sodium chloride or glucose in the perfusate, and the relationship between the absorption and the fluid movement was expressed by straight regression lines with almost the same slope as that obtained when sulfanilamide was a subject of the experiment.

In the case of sulfanilamide, the regression lines of sodium chloride and glucose were overlapped over a wide range of the fluid movement. Contrary to the results shown in Fig. 2, two regression lines were observed, one was of sodium chloride ($n$: 11, $r$: −0.978), which is depicted by a solid line in Fig. 4, and the other was of glucose ($n$: 12, $r$: −0.912) in the perfusate, which is illustrated by a broken line. These two regression lines were found to be parallel to each other. Because of this parallel relationship, the reason of this separation could not be explained by the transmucosal fluid movement in the rat small intestine.

The broken line of glucose was shifted to the region where the absorption of the drug was decrease. The difference of these in the absorption at 1.0 on the vertical axis which is expressed as intercepts of the regression lines and a dotted line in Fig. 4 is found about 20%. This suggests that the absorption of the drug from the rat small intestine in the perfusate which contains glucose will be given always less than that of sodium chloride by about 20%.

In such an experiment of *in situ* perfusion study of rat small intestine, buffer solution were usually used to keep the luminal pH at a constant value so that the drug in the perfusate existed in one form of the molecule during the course of the experiment. To obtain the absorption of the drug from one of the most usually used buffer solution and to compare

the result to that obtained in this study, an isotonic phosphate buffer of pH 6.5 which was considered as physiological pH of the small intestine of this animal was employed as a perfusate. The plots of the result which are shown in Fig. 4 as half solid squares, appeared just on the regression line of sodium chloride and, moreover, the fluid movement was observed decrease. As the result of course, the absorption of the drug was also decreased. This result suggested that despite of using an isotonic perfusate in the experiment, different results both in the fluid movement and in the drug absorption might be obtained when the solutes which were added to adjust the tonicity of the solution were different.

**Metoclopramide Absorption**

Two former substances are belonged to a group of sulfonamides, and their absorptions were found to be susceptively affected by the movement of fluid through the surface of the rat small intestine. Another substance, which exists in a form of cation in the perfusate should be followed in turn. Metoclopramide, which is not sulfonamide and is in a cationic form in the physiological pH of the rat small intestine, was chosen for a comparison to sulfisoxazole which gave an anionic form in the perfusate. The results are represented in Fig. 5.

The absorption of this drug was also found to be affected by the fluid movement which was caused by different concentrations of sodium chloride or glucose in the perfusate. As observed in the case of sulfisoxazole, two separate regression lines of sodium chloride and glucose were obtained and they were still parallel relationship to each other. Surprising difference was observed. Contrary to the case of sulfisoxazole where the regression line of glucose was on the region of the left hand side of that of sodium chloride, in this case of metoclopramide, the regression line of glucose appeared in the right hand side of that of sodium chloride, that was, the region where the absorption of the drug is increase. The difference in the absorption was found to be about 20%, which was almost the same difference as observed in the case of sulfisoxazole.

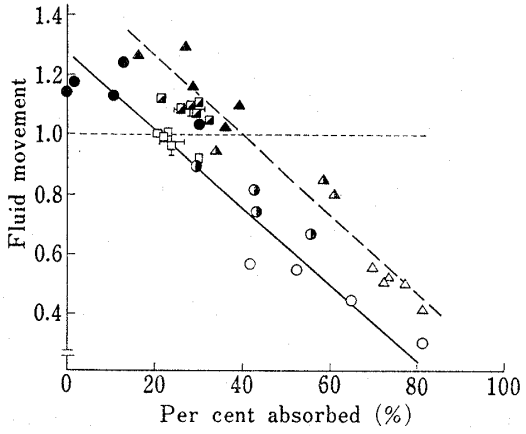


Fig. 5. Relationship between Per Cent Absorbed of Metoclopramide and Fluid Movement

▲:glucose hypertonic ●:sodium chloride hypertonic
▲:glucose isotonic ◑:sodium chloride isotonic
△:glucose hypotonic ○:sodium chloride hypotonic
◪:phosphate buffer (isotonic, pH 6.5)
□:phosphate buffer with Saline (3: 1)

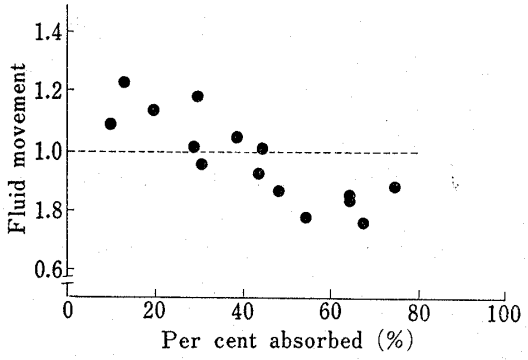


Fig. 6. Relationship between Per Cent Absorbed and Fluid Movement of Hydrocortisone in Man, Using Glucose-Ringer Solution with Various Tonicities as the Perfusate

The site of the study was 100—200 cm from nares. The data are quoted from the study of Schedle and Clifton[16]

**Transmucosal Fluid Movement and Drug Absorption**

In the course of this study, authors encountered a literature by Schedle and Clifton[16] concerning to the absorption of hydrocortisone from the gastrointestinal tract of man, with

16) H.P. Schedle and J.A. Clifton, *Gatroenterol.*, 44, 134 (1963).

various tonicities of glucose-Ringer solutions as the perfusate. Based on the results of the absorption from the site of the tract of 100 to 200 cm from the nares, which were in a Table of the literature,[16] plotting was tried on the figure in the same manner as employed in Fig. 2, and Fig. 6 was obtained.

Figure 6 indicates that the absorption of hydrocortisone from the gastrointestinal tract of man is also affected by the transmucosal fluid movement. Fordtran, *et al.*[17] presented the data of urea absorption from human alimentary tract and suggested that the absorption was influenced by the current of the fluid across the epithelial layer of the small intestine.

These lines of evidences suggest possibilities that the conclusions drawn in the present study using rat as the experimental animal might be dilated in the absorption in man, and that the size of the molecule of the drugs might not affect these phenomena, because the molecular weight of hydrocortisone and urea are 362.5 and 60.1 respectively and that of the drugs used in this study are between them.

Intestinal drug absorption may be viewed as the movement of drugs from the lumen to circulatory system. Drugs should penetrate numbers of barriers. These barriers include unstirred layer of fluid,[18] the fuzzy coat of microvilli,[19] the apical cell wall, cytoplasma, the basal and lateral cell wall, basement membrane and capillary endothelium. As all of these membranes and walls have characteristics of semipermeable, they may offer some resistances to penetration of drugs. However, our findings demonstrated that these penetrations seemed not to be so much disturbed from the fact that the differences in blood levels of the drug were observed without delay as shown in Fig. 3. This evidence might indicate that the drugs are not dominantly absorbed through the pathway of such apical cell wall or cytoplasm and so on, especially in the case when the fluid is moved bidirectionally through the epithelial layer of the tract.

It is well known that the epithelial cell systems are perforated by water-filled pores with a certain width of radius and diffusion and filtration of fluid are mainly proceeded through these pores or channels. Solomon[20] estimated the radius was about 4Å, and Renkin[21] yielded 7.9Å in the cell system of human jejunum and also Soergel[22] and his coworkers assumed the radius of the pores about 30Å in the human intestinal mucosa. Assuming the radius is wide enough to 30Å, it should support our findings of irrelevancy to molecular weight in the transepithelial movement of these drugs employed in this study.

A question remains why phenol red was not absorbed through the wide radius of the pores during the course of the perfusion experiment. The molecular weight of this substance is 354.4 and is not extraordinarily large compared to drugs employed in these experiments. From these facts, it is obvious that only phenol red was offered resistance to the penetration and/or filtration through the mucosal pores or channels, although almost the same molecular weight as other drugs. This evidence suggested that discrimination of substances which is to be filtered is functioning even in such a condition of bulk flow of fluid through wide radius of pores or channels.

Ussing observed small electrolytes of sodium and chloride were absorbed along the current of fluid which moved transepithelialy using frog skin.[23] Based on these findings, the concept of solvent drag was established.

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Evidences presented in this report suggest that solvent drag hypothesis is applicable not only to substances of small molecule of electrolytes, but also those of comparatively large and organic substances in the condition occurring bidirectional fluid movement across the epithelial tissue of the small intestine. Taking these evidences into account, it should be emphasized in this report that the tonicity of the perfusate should be adjusted with utmost carefulness in the absorption studies. On the other hand, this report also suggest that solvent is not able to drag all kinds of organic substances but there is some restrictions which have been observed in the case of phenol red.

**Effect of Glucose on the Intestinal Drug Absorption**

Several reports in the literature suggested that glucose may alter intestinal absorption of drugs and many *in vitro* and *in vivo* studies had been undertaken to elucidate the effect. Mayersohn and Gibaldi gave evidences that glucose significantly decreased transfer of riboflavin, salicylate and sulfanilamide through the everted rat intestine,[24] and Nogami, *et al.* found out decreasing of the intestinal absorption of sulfonamides in the presence of the hexose.[25]

Supposing from these lines of evidences, studying the effect of glucose on the intestinal absorption of drugs is still on the way of argumentations. The results obtained in this report present another evidence that the effect of glucose might differ in relation to the charge of the drug molecule existed in the luminal fluid. Although it is impossible to draw out conclusions concerning to the effect of glucose at the present stage of this study and, of course, more efforts may be required, the method presented in this report of illustration of the relationship between drug absorption and ratio of the transmucosal fluid movement, which has been employed in illustrations of Figures 2, 4, 5 and 6, may open the way to elucidate further the nature and mechanism of the effect of the hexose on the intestinal drug absorption.

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