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Segmental Difference in Transmucosal Fluid Movement and Glucose Effect on Drug Absorption from Rat Gastrointestinal Tract

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Segmental difference in the transmucosal fluid movement and its effect on the drug absorption was studied with sulfanilamide, sulfisoxazole and metoclopramide. The segments investigated were stomach, duodenum, jejunum, ileum and colon, and the ligation method was employed in the experiment of the stomach, and the rest of the segments were investigated by the simultaneous four *in situ* recirculation perfusions method which was devised originally in our laboratories.

The results revealed that the absorption of the drugs in the stomach was not affected by the transmucosal fluid movement, while large volume of fluid secretion was observed. However, the drug absorption in the rest of the segments was affected in some extent by the fluid movement. Segmental differences in the absorption of these three drugs were also elucidated. Concerning the glucose effect, it was revealed that the effect was localized only in the upper part of the small intestine.

Keywords——transmucosal fluid movement and drug absorption; stomach; duodenum; jejunum; ileum; colon; simultaneous four recirculating perfusions technique; sulfanilamide; sulfisoxazole; metoclopramide

In the previous reports from our laboratories, it was reported that the transmucosal fluid movement affected drug absorption in considerable extent from the small intestine of normal[2] and abnormal[3] physiological condition of rat. Since the affection was unnegligible extent, it was suggested that no one could interpret results obtained by such an experiment as *in situ* recirculating perfusion which had been employed extensively in the study of drug absorption without taking the effect of the transmucosal fluid movement into account. During the course of the previous study,[2] glucose in perfusion solutions affected peculiar effect on the absorption of an ionized form of drug. This glucose effect was verified also *in vivo* study.[4] These evidences concerning the glucose effect had brought another problem in the study of this field.

To elucidate these effects more in detail, our attentions were concentrated to study on segmental difference of these undissolved effects in the gastrointestinal tract of the animal with having a hope of finding out a clue to these phenomena. Gastrointestinal tract is consist of many different parts and main of them is stomach, duodenum, jejunum, ileum, colon, and rectum, and each segment has its own physiological function in relation to digestion and absorption of food stuffs. Functions concerning the bidirectional fluid movement through the epithelial border of the intestine and the peculiar effect of glucose may not be identical in all of these segments in the tracts.

In the present report, the studies were undertaken to look for segment in which the transmucosal fluid movement would be excellent and also in which the glucose effect on the drug absorption would be dominant.

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2) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 1856 (1975).
3) T. Komuro, S. Kitazawa, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 909 (1975).
4) S. Kitazawa, H. Ito, and M. Iinuma, *Chem. Pharm. Bull.* (Tokyo), **23**, 2128 (1975).

Experimental

Animal Procedures——Wistar albino strain of male rat, weighing 150 to 170 g, was fasted for an overnight prior to the experimentations, but 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 of the animal. Although the segments of stomach, duodenum, jejunum, ileum, and colon were subjected to be investigated in the present study, the animal employed in the stomach experiment was separated at random and the ligation method[5] was conducted, and the rest of four segments were investigated with four independent *in situ* recirculating perfusions which were conducted in an animal simultaneously. Operating performances which will be described below were conducted with utmost carefulness and any blood vessels were not occluded.

Stomach Experiment——The anesthetized rat was placed on an operating plate and the stomach was exposed by a midline incision. The cardiac opening was ligated exactly by strings on the esophagus to prevent the test solution in the stomach from leakage. Cannulation with silicon tubing was made into the pylorus end, and the cannula was fastened with strings. After washing the interior of the organ by irrigation with 10 ml of an isotonic sodium chloride solution using a syringe equipped to the tubing on the pylorus end, a test solution was stuffed into the stomach through the syringe. The abdominal incision was then closed. The test solution was kept in the organ for following one hour and the final solution was obtained by withdrawing the syringe. The volume of the test solution instilled was 3 ml per head in all of the subject animals.

Recirculating Perfusion Experiment——The anesthetized rat was placed and fastened with strings on an operating plate and the intestinal tract exposed by a midline incision. After cleaning the interior of the tract by a single perfusion from the proximal opening of the duodenum to the colonic-rectal opening with 50 ml of an isotonic sodium chloride solution which had been kept 37°, the tract was divided into four parts according to the following protocols. From the pylorus end of the stomach to the ligament of Treitz was regarded as the duodenum, and from the ligament to as long as 20 cm distal of the upper part of the intestine was regarded as the jejunum, and 20 cm of the intestine from the ileocecal junction proximal was regarded as the ileal segment. The length of these jejunum and ileum was measured along the curvature of the intestine as was in the abdomen with a graduated string. Colon was regarded as the intestine from the cecal-colonic junction to the colonic-rectal flexure. The bile duct in the duodenum was ligated in all experiments to avoid any inflow of fluid into the segment during the perfusion experiment. An intermediate portion of 15—20 cm length of the small intestine between the jejunum and the ileum was left univestigated. Since characteristics relating the drug absorption and the fluid movement in both of the jejunum and the ileum would be mixed in this portion, it might be difficult to elucidate the characteristics in each segment respectively.

Silicon tubing having inside diameter of 2.2 mm and outside diameter of 3.0 mm was cannulated into both ends of these segments and each end of the tubing was jointed to respective perfusion pump (CV-1 type, Tokyo Kagaku Seiki Co.) to close a circuit. Each circuit had a reservoir of 20 ml volume of a conical flask just before the inlet tubing leading to the subjected segment. The perfusion of the test solution was conducted in turn of the proximal to the distal end of each segment. Ten minutes after the beginning of the perfusion, an initial sample was pipetted out from the reservoir. Four simultaneous recirculating perfusions were conducted for additional one hour with a volume of 20 ml in all of the segments and the perfusion rate was always kept at 5 ml per minute for respective segments. The final samples were obtained in the same manner as the initial samples and the assay procedures were followed.

Although four segments were concurrently perfused the same test solution, when an accident such as the effusion of blood into the perfusate occurred in a segment, the data obtained from the segment was eliminated from the experimental results. Hence the number of data obtained from the perfusion studies was not always in agreement with the number of experiments.

Drugs——To compare the results obtained in the present study to those in the previous report[2] without difficulty, the same drugs of sulfanilamide, sulfisoxazole, and metoclopramide were employed in the present study. Metoclopramide was kindly supplied by Fujisawa Pharmaceutical Co., Ltd., and the rest of the drugs and the reagents were of reagent grade and these were used without further purifications.

Although pH value of the perfusate in the segment other than the stomach fluctuated reflecting the virtual pH of respective segments, their values maintained almost constant in range of 6.0 to 7.5 during the perfusion respectively. Based on these evidences, sulfanilamide was considered to be in an unionized form in the perfusate and sulfisoxazole should be in an anionic form and metoclopramide should be in a cationic form in the perfusate of each segment during all the course of the perfusion experiment.

Test Solution——All the test solutions used in the perfusion study contained one millimole of the drug and a certain concentration of phenol red which was used as a nonabsorbable indicator.

The test solution applied in the perfusion of each segment of the intestine contained different concentrations of sodium chloride or glucose, and hypertonic, isotonic, and hypotonic solutions were prepared. Since physiological isotonic concentration of sodium chloride and glucose were 0.9% and 5.0% respectively, perfu-

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

sion solutions having various concentrations more than these isotonic concentrations of the solute were nominated as hypertonic solution, and solutions less than the isotonic concentration were nominated as hypotonic solution in this report.

On the other hand, the test solution used in the stomach experiment was phosphate buffer solution of pH 6.5 to keep the pH value constant at 6.5 during the course of the experiment with a purpose of keeping the drug in respective forms which were similar to those in the intestinal perfusion study. The component of the isotonic buffer solution was $Na_2HPO_4 \cdot 12H_2O$ 7.2 g, KH_2PO_4 6.4 g, and NaCl 5.9 g in distilled water to a volume of 1000 ml. Tonicity of the solution was adjusted by changing the concentration of each component of the buffer solution, and solutions having the concentration of two times and three times of the isotonicity were obtained. Thus three levels in the tonicity of the test solution for the stomach experiment were prepared.

Analyses of Samples

Phenol Red——Five tenth milliliters of the perfusate were pipetted out and immediately after alkalization by adding 5 ml of 1N sodium hydroxide solution, the developed color was determined spectrophotometrically at a wavelength of 550 nm.

Drugs——As stated in the previous study,[2] the method of diazo-coupling using Tsuda's reagent was applicable in all cases. After developing color, their optical densities were determined spectrophotometrically at a wavelength of 550 nm using Hitachi spectrophotometer of model 124.

These analytical procedures were carried out in both of the initial and the final samples of each experiment, and absorption of the drugs and the transmucosal fluid movement were calculated following the equation of Schanker and his co-workers.[6] The equation is as follows:

$$\text{Percent absorbed} = 100 - 100\left(\frac{C_{\text{drug final}}}{C_{\text{drug initial}}} \times \frac{C_{\text{indicator initial}}}{C_{\text{indicator final}}}\right)$$

where C is the concentration of drug or phenol red used as a nonabsorbable indicator in the perfusate and $C_{\text{indicator initial}}/C_{\text{indicator final}}$ in the equation was treated as the ratio of fluid movement in the present study. The illustration of results in figures was followed as the same manner presented in the previous report.[2]

Results and Discussion

Transmucosal Fluid Movement and Drug Absorption in the Stomach

It is well known that ingested foods and drinks first arrive at this organ of the alimentary tract and they are soaked in gastric juice containing digestive enzymes and hydrochloric acid and then eliminated into duodenum through the pylorus opening spasmodically by peristatic motion of this organ.[7]

Considering these basic physiological functions of this organ, it is easily understood that large volume of fluid would be moved transepithelially. Figure 1 shows a relationship between the transmucosal fluid movement and the absorption of respective drugs in the stomach.

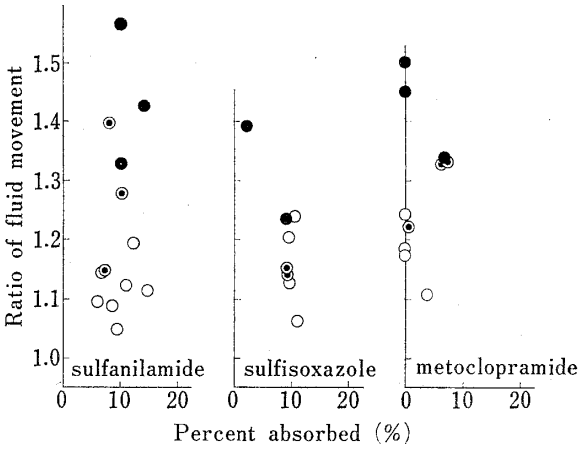

Fig. 1. Relationship between the Absorption of Respective Drugs and the Transmucosal Fluid Movement in the Stomach

phosphate buffer solution of pH 6.5;
○: isotonic concentration
⊙: two times of the isotonic con.
●: three times of the isotonic con.

As illustrated in Fig. 1, absorption of the drugs was essentially scanty in this organ as compared to those in the small intestine which were reported in the previous paper.[2] Once Karel[8] had pointed out that the stomach was definitely not an absorptive organ in the same sense as the intestine. Data obtained in the present report supported his judgement, however, some fluctuations were observed in the absorption of these drugs. Absorption of sulfanilamide

6) C.A.M. Hogben, D.J. Tocco, B.B. Brodie, and L.S. Schanker, *J. Pharmacol. Exper. Therap.*, **125**, 275 (1958).
7) H.W. Davenport, "Physiology of the Digestive Tract," 3rd ed, Year Book Medical Publishers, Chicago, 1971, pp. 44—60.
8) L. Karel, *Physiol. Rev.*, **28**, 433 (1948).

and sulfisoxazole, which were in forms of unionized and anion respectively, came up to about 10%, while the absorption of cationic form of metoclopramide was found not more than 5%.

Aside from these fluctuations in the results of drug absorption, interests should be concentrated in the relation between transmucosal fluid movement and drug absorption. Although the ratio of fluid movement was increased with increasing the tonicity of perfusate, the absorption of the drugs was not changed and maintained almost constant. This evidence suggested that the absorption of these drugs from the stomach was not affected by the transmucosal fluid movement which was artificially occurred by changing tonicities of the test solution and it was different to the absorption of drugs in the small intestine.

Based on these available evidences and Karel's descriptions, a possible conclusion was that the mode of drug absorption in the stomach was quite different in nature to that in the small intestine where dominant absorption was undergoing.

Other findings relating the transmucosal fluid movement in Fig. 1 were an apparent unidirectional movement of fluid. The transmucosal fluid movement in the small intestine was clearly dependent on the tonicity of the perfusion solutions and when a hypertonic solution was applied, an outflow of fluid was observed, and in a case of an isotonic solution, a slight inflow was occurred. As was evident in Fig. 1, the ratio of the fluid movement in all cases was more than 1.0, where the bidirectional fluid movement was balanced and apparent fluid movement was not observed, although the ratio was increased with increasing the tonicity of perfusate. These results indicated that the outflow of fluid was observed even in the cases of instillations of isotonic solutions. Preliminary studies[9] using a hypotonic solution having a half concentration of each component of the test solution used in the present study had shown the ratios came up more than 1.0 also. Although the results were not plotted in the figure, since the alteration in pH value in the instillation solution was observed during the course of the experiment. These evidences demonstrated that fluid secretion was undergoing even in the case of an introduction of hypotonic solution, and it was considerably difficult to look for an appropriate condition relating tonicity of the test solution by which the fluid absorption was brought about. It is well known that there are many secretory glands on the surface of the stomach epithelium.[10] This well known evidence suggests that fluid secretion may always exceed fluid absorption in this organ. As the results of these fundamental physiological functions of the stomach, the ratio of fluid movement was always obtained more than 1.0, *i.e.*, the fluid outflow into the lumen was always found, even when an isotonic or hypotonic solution was introduced in this organ.

Another characteristic evidence revealed in the present study was the strength of cell surface of this organ enduring to the tonicity of the test solution. As reported in the previous report,[2] cell surface of the small intestine was damaged and peeled off when a hypertonic solution having concentration of more than 1.8%, that is, 2 times of an isotonicity of sodium chloride, was perfused. However, the results obtained in the present report revealed that the epithelial cell surface should be strong enough to endure the hypertonicity of the solution having 3 times of an isotonicity and no damages and peeling of the epithelial cells were observed. These results were satisfied in considering the physiological functions of this organ, since osmolarities of foods and drinks were not enough regulated up to arrive first in this organ, and stayed here until they were digested into a status of chyme, so that the epithelial surface should be strong enough to endure hypertonicity of contents in the stomach.

Transmucosal Fluid Movement and Drug Absorption in the Duodenum

Simultaneous perfusion experiment was conducted in the four segments of the tract and the results obtained in the duodenal segment, which is the first segment in the small intestine, were depicted in Fig. 2.

9) Unpublished data.
10) C.S. Tidball, *Am. J. Digest. Disease*, **16**, 745 (1971).

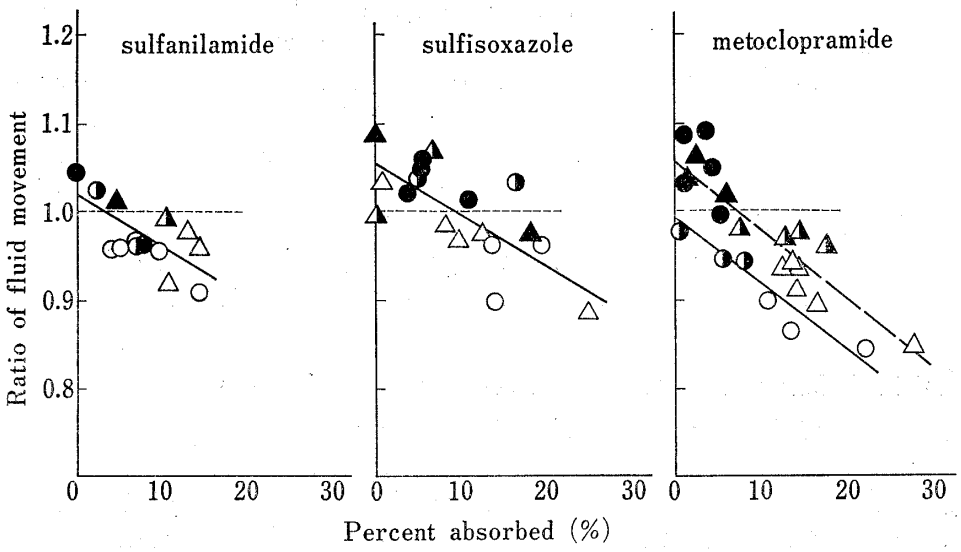

Fig. 2. Relationship between the Absorption of Respective Drugs and the Transmucosal Fluid Movement in the Duodenum

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

The chyme released from the stomach through the pylorus end comes to this segment and is mixed well with bile and other digestive fluid which are predominantly alkaline in nature.[11] Thus preparations for absorption of nutrients which will occur thereafter must be proceeded such as adjustment of pH and modulating tonicity of the contents. However, works of Borgstrom and his collaborators[12] demonstrated an evidence that triglyceride was absorbed in this segment and Dowell and his co-workers[13] revealed that iron was absorved in this part of the intestine. On the other hand, Code and his co-workers[14] presented their findings demonstrating an evidence that fluid was also absorbed from this organ. These lines of evidences apparently demonstrated that the duodenum is functioning as one of the absorption organs.

Reflecting these informations relating the functions of the duodenum, Figure 2 demonstrates that drug absorption also do occur in this organ and, moreover, the absorption is apparently affected by the transmucosal fluid movement. In the case of sulfanilamide, the scattered plots obtained with sodium chloride or glucose perfusate were overlapped to each other and the regression equation calculated with the least squares method was $y=-0.0058\cdot x+1.0169$ (n: 14, r: −0.715). As well as found in sulfanilamide absorption, the regression equation for sulfisoxazole was $y=-0.0057\cdot x+1.0532$ (n: 18, r: −0.723). On the contrary, two separate regression lines were obtained from both sodium chloride and glucose media in the case of metoclopramide. The regression equation calculated from the data obtained with the perfusate whose sodium chloride concentration is less than 1.3% was $y=-0.0074\cdot x+0.9923$ (n: 7, r: −0.910). Another regression equation was $y=-0.0077\cdot x+1.0538$ (n: 13, r: −0.908). From these facts, the straight regression lines are almost parallel in all cases, the extent of the affection of the fluid movement must be identical to any drug subjected in the experiment.

However, in detailed investigations on the absorption at the intercept of the respective regression line and the 1.0 of the fluid movement ratio, it was elucidated that there were some

11) T.R. Hendrix and T.M. Bayless, *Ann. Rev. Physiol.*, **32**, 139 (1970).
12) B. Borgstrom, A. Dahlqvist, G. Lundh, and J. Sjovall, *J. Clin. Invest.*, **36**, 1521 (1957).
13) E.B. Dowell, D. Schachter, and H. Schenker, *Am. J. Physiol.*, **198**, 609 (1960).
14) C.F. Code, P. Bass, and A.L. Orvis, *Am. J. Physiol.*, **199**, 281 (1960).

fluctuations in the absorption of these three drugs from the perfusate containing sodium chloride. Sulfisoxazole was absorbed in an extent of approximate 10%, while sulfanilamide was about 5% and in the case of metoclopramide, the absorption was as low as not more than 5%. It seemed to be impossible to conclude relating the difference in absorbability of these drugs with these fragment results. However, the differences seemed not to be negligible.

The glucose effect which was observed in the absorption of ionized drugs from the entire intestine could be recognized in the absorption of metoclopramide in this segment, but not of sulfisoxazole. Since detailed mechanism of the glucose effect was not elucidated yet, authors would prefer to avoid deep discussion concerning the differences in the glucose effect in this organ.

Transmucosal Fluid Movement and Drug Absorption in the Jejunum

Too much knowledges are accumulated concerning morphology and functions of this main segment of the upper small intestine.[15] Absorption of nutrients as well as drugs are undergoing in this segment. Farqular and Palade[16] demonstrated an evidence that water could penetrate through tight junction of epithelial cell lines of this part of the small intestine. This evidence suggested that the transmucosal fluid movement might occur in larger extent than other segment in the intestine.

Reflecting these evidences, the results obtained had revealed appreciable absorption and the bidirectional fluid movement occurred in all of these drugs during the course of the perfusion experiment. The results were illustrated in Fig. 3.

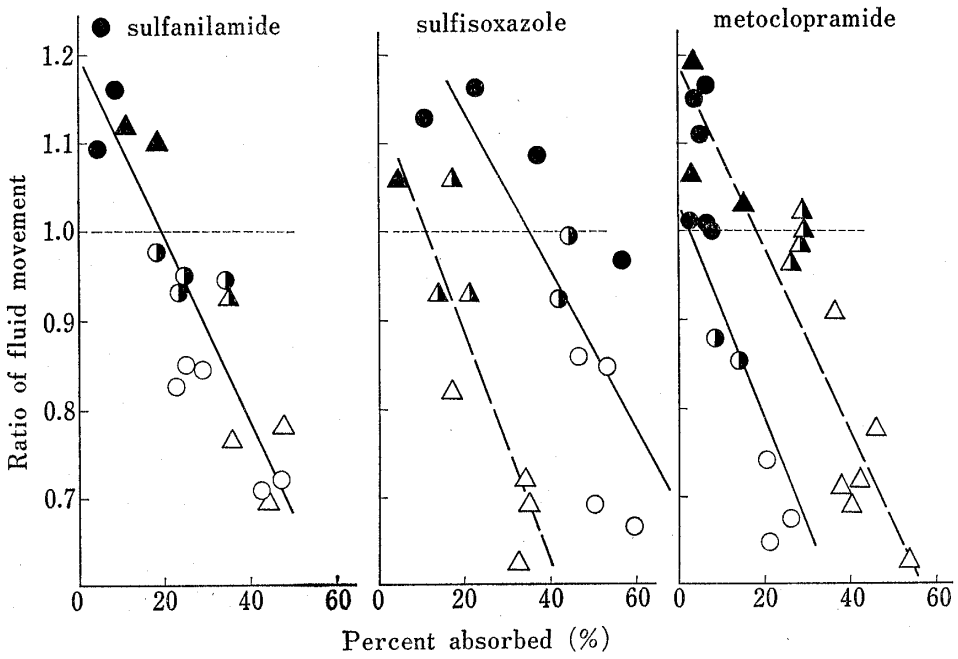

Fig. 3. Relationship between the Absorption of Respective Drugs and the Transmucosal Fluid Movement in the Jejunum

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

Essentially similar illustrations to those in the previous report[2] were obtained. In the absorption of sulfanilamide, the regression line of sodium chloride was overlapped to that of glucose and the glucose effect was not observed. One regression line was obtained with the

15) W.O. Dobbins, III, *Am. J. Med. Sci.*, **258**, 150 (1969); S.P. Balcerzak, W.C. Lane, and J.W. Bullard, *Gastroenterology*, **58**, 49 (1970).
16) M.G. Farqular and G.E. Palade, *J. Cell. Biol.*, **17**, 375 (1963).

perfusate containing sodium chloride or glucose and the regression equation was $y=-0.0104\cdot x+1.1982$ (n: 18, r: −0.898). On the other hand, the glucose effect was apparently demonstrated in the other two drugs. In the case of sulfisoxazole, the regression equations were $y=-0.0088\cdot x+1.3069$ (n: 10, r: −0.792) for sodium chloride medium and $y=-0.0131\cdot x+1.1420$ (n: 8, r: −0.878) for glucose medium. The regression equation for metoclopramide calculated from the scattered plots obtained with the perfusate whose sodium chloride concentration is less than 1.3% was $y=-0.0118\cdot x+1.0275$ (n: 9, r: −0.861), and that obtained from glucose medium was $y=-0.0103\cdot x+1.1864$ (n: 13, r: −0.865). The regression lines of sulfisoxazole and metoclopramide obtained from the perfusate containing glucose were shifted to their respective directions compared to those obtained from sodium chloride medium.

Transmucosal Fluid Movement and Drug Absorption in the Ileum

Ileum, the lower small intestine, is considered as one of important sites of absorption, however, substances absorbable from this site were rather limited such as vitamin B_{12}[17] or bile salt.[18] Fordtran[19] reported his findings relating the differences of the upper and the lower part of the intestine that effective pore size in the upper intestine was at least twice of that in the lower part of the small intestine in man. These findings were supported by Ross and his co-workers[20] in the rabbit small intestine. Of course, these lines of evidences do not suggest directly that the drug absorption would not occur, nor the fluid would move transepithelially.

As a matter of fact, the results obtained using the ileal segment revealed the occurrences of these absorption and fluid movement, as illustrated in Fig. 4. Contrary to our expectations, the regression lines of ionized drugs in the sodium chloride solution were overlapped to those of the glucose solution in a wide range of the fluid movement. The regression equations were

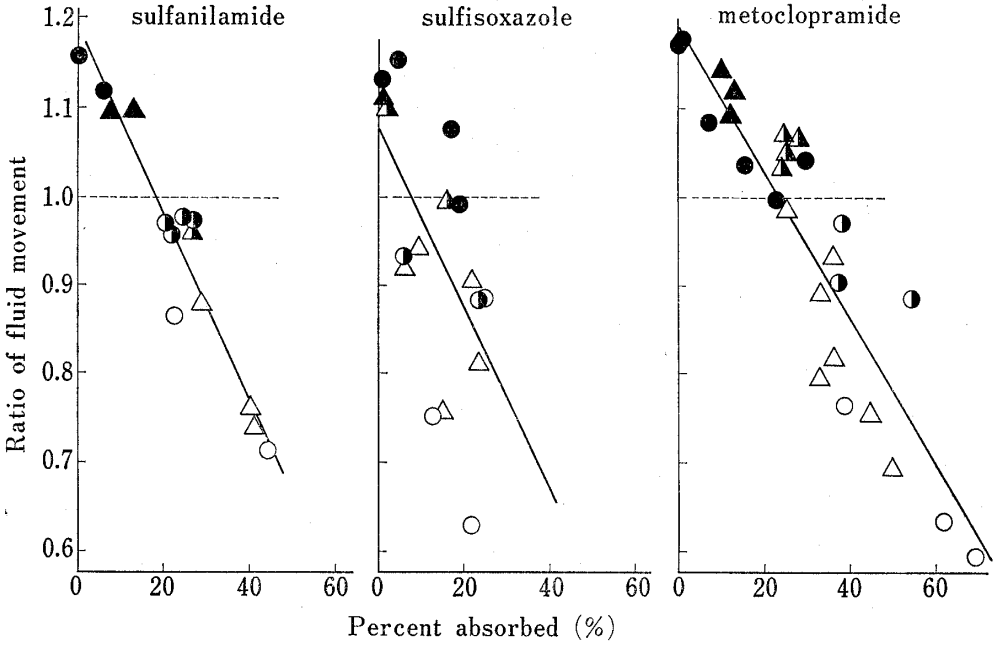

Fig. 4. Relationship between the Absorption of Respective Drugs and the Transmucosal Fluid Movement in the Ileum

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

17) C.C. Booth and D.L. Mollin, *Lancet*, **2**, 1122 (1957).
18) J. Sjovall and I. Akesson, *Acta Physiol. Scand.*, **34**, 273 and 279 (1955).
19) J.S. Fordtran, F.C. Rector, M.F. Ewton, N. Soter, and J. Kinney, *J. Clin. Invest.*, **44**, 1935 (1965).
20) A. Ross, A.W. Rubin, and J.J. Deren, *J. Clin. Invest.*, **51**, 2414 (1972).

$y=-0.0103\cdot x+1.1859$ (n: 14, r: -0.963) for sulfanilamide, $y=-0.0098\cdot x+1.0767$ (n: 18, r: -0.642) for sulfisoxazole, and $y=-0.0082\cdot x+1.1940$ (n: 26, r: -0.904) for metoclopramide, respectively.

These results did indicate that the glucose effect on the absorption of ionized drugs was not functioning in the lower part of the small intestine. It is of interest that the effect of glucose on the absorption of ionized drug was quite different between the upper and the lower part of the small intestine of the animal. It is well known that glucose is absorbed by the process of active transport[21] and the upper portion of the small intestine has a great capacity in absorbing glucose rather than the lower, while the stomach and colon absorb glucose in a scarce extent.[22] These evidences do suggest that the site of glucose absorption coincides where the glucose effect is developed on the drug absorption. If these coincidences were true, the reason why the glucose effect was not observed in the ileal segment would be understood without difficulties and, moreover, this speculation must be one of the keys to open the way of elucidating mechanism of development of the glucose effect on the drug absorption.

Detailed surveys on the absorption of the drugs from this segment demonstrated another differences to that from the jejunal segment. The absorption of respective drugs at the intercept in Fig. 4 was compared to that in Fig. 3. In the case of sulfanilamide, the absorption in both of the jejunum and the ileum were found almost the same percentage of around 20%. This indicated that sulfanilamide was absorbed from both of the segments identically and, moreover, sulfanilamide might be absorbed from all over the surface of the small intestine evenly and no difference was observed between the upper and the lower of the small intestine.

Equality in the drug absorption in both of these segments was not observed in the case of sulfisoxazole. The absorption of this drug at the intercept obtained with sodium chloride medium showed 34.9% in the jejunum, while in the ileum the absorption decreased to as low as 9.0%. The uneven absorption was enough to suggest that the absorption of this drug would proceed more in the upper than in the lower part of the small intestine. A reversed unequality was observed in the case of metoclopramide. The absorption of metoclopramide at the intercept exhibited to be negligible in the jejunum and 24.7% in the ileum. These site depending differences in the absorption of this drug suggested that metoclopramide was absorbed more in the lower than in the upper part of the small intestine.

Many reports had concerned in selective absorbability or site depending difference in the absorption of nutrients such as glucose[23] or amino acids,[24] however, little informations had accumulated in literature relating drug absorption. Although the reason why there was such site dependency in the drug absorption in the small intestine was, of course, not elucidated clearly in this report and many reasons should be considered in unravelling of these phenomena, there was one possibility that the site dependency might be related to the ionized form of drug in the perfusate. Anyhow the results presented in this report might suggest valuable informations in considering the drug absorption to investigators and especially to formulators in designing pharmaceutical dosage form.

Transmucosal Fluid Movement and Drug Absorption in the Colon

Physiological functions of the colon had been revealed by many researchers, in the first place a series of works by Parsons[25] had contributed in revealing the functions of this segment.

21) B.A. Barry, J. Matthews, and D.H. Smyth, *J. Physiol.*, **157**, 279 (1961).
22) R.B. Fisher and D.S. Parsons, *J. Physiol.*, **110**, 36, 281 (1949).
23) R. Lium and H.W. Flory, *Quart. J. exp. Physiol.*, **29**, 303 (1939).
24) H.P. Schedl, D.L. Miller, H.D. Wilson, and P. Flores, *Am. J. Physiol.*, **216**, 1131 (1969); R.A. Parkins, A. Dimitriadou, and C.C. Booth, *Clin. Sci.*, **19**, 595 (1960).
25) D.S. Parsons and C.R. Paterson, *Quart. J. exp. Physiol.*, **41**, 410 (1956); *idem*, *Biochim. Biophys. Acta*, **41**, 173 (1960); D.S. Parsons and G.V.R. van Rossum, *Quart. J. exp. Physiol.*, **46**, 353 (1961); D.S. Parsons and D.L. Wingate, *Biochim. Biophys. Acta*, **46**, 170 (1961); D.S. Parsons and C.R. Paterson, *Quart. J. exp. Physiol.*, **50**, 220 (1965).

These reports indicated that the colon exhibited an important function as a terminal site of absorbing fluid and electrolytes on the way of forming from chyme to feces. Davidson and Garry[26] had presented an evidence that glucose was not absorbed in this part of the tract. These lines of evidences suggested that the colon, a part of the large intestine, is functioning not as a site of the absorption but as a site of preparing in forming feces.

Some conflicting results were obtained in the perfusion experiments, which were illustrated in Fig. 5.

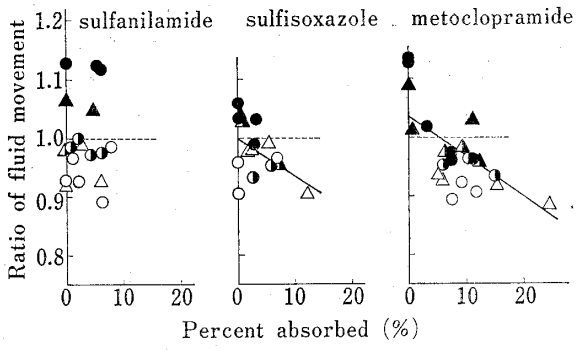

Fig. 5. Relationship between the Absorption of Respective Drugs and the Transmucosal Fluid Movement in the Colon

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

As depicted in Fig. 5, the scattered plots obtained with both of the media were overlapped to each other in all cases, so that the glucose effect could not be also found in this segment. Although the ratio of fluid movement in both of the media was increased with increasing the tonicity of perfusate, sulfanilamide absorption was almost constant. This results were similar to the findings obtained in the stomach mentioned above. On the other hand, sulfisoxazole and metoclopramide absorptions were influenced by the transmucosal fluid movement. The regression equations were $y=-0.0062\cdot x+0.9985$ (n: 17, r: -0.475) for sulfisoxazole and $y=-0.0068\cdot x+1.0358$ (n: 24, r: -0.657) for metoclopramide. This evidence did suggest that the drugs might be absorbed also in this segment. However, there might be some restrictions in the absorption and the fluid depending on the ionized form of the drug. As a matter of fact, sulfanilamide, an unionized form of substance, was hardly absorbed and the absorption was not affected by the transmucosal fluid movement. This mode of absorption was varied in the case of sulfisoxazole and was more exaggerated in the case of metoclopramide. Based on the slope of the regression lines, it is apparent that the absorption of these drugs might be affected by the transmucosal fluid movement.

As illustrated in Fig. 5, the ratio of fluid movement was not high enough corresponding to the hypertonicity of the perfusate, as had been observed in the segment of jejunum and ileum, and the regression line was only appeared in the region of fluid absorption. The secreted fluid in the colon is sticky and viscous in nature,[27] and secretion, in other words, outflow of fluid from tissue would not occur easily in corresponding to the tonicity of the perfusate and this might be one of the reasons why the ratio of fluid movement did not become high enough.

Simultaneous Four *in Situ* Recirculating Perfusions

The characteristics relating the drug absorption and the transmucosal fluid movement in four segments in the small intestine were investigated using simultaneous four *in situ* recirculating perfusions method which was originally developed in our laboratories. This method required minute and delicate technologies in the processes of animal preparations. However, the results obtained by the perfusion method presented the relative characteristic of respective segments in the whole intestine.

Many evidences relating the characteristic comparative studies on the morphology and the physiological function of the segment in the alimentary tract had been accumulated in literature. In most cases of these studies, an isolated segment was investigated in *in vitro* method and even *in vivo* method, only a target segment of the intestine was subjected for

26) J.N. Davidson and R.C. Garry, *J. Physiol.*, **97**, 509 (1939).
27) R.D. Wright, H.W. Florey, and M.A. Jennings, *Quart. J. exp. Physiol.*, **28**, 207 (1938).

investigations. In these cases the physiological function of the target segment would be exaggerated in the results, because the absorption of a certain substance would be conducted through limited surface of the whole intestine, that is, the target segment, to the large volume of distribution in the whole body, and bulk volume of fluid in the whole body would be faced to the perfusion solution through restricted surface area of the investigated segment in the entire intestine.

The physiological function of a certain segment should be considered in the period, since other segments are also in functioning. From this reason, the relative characterictics should be elucidated in revealing the role of the segment in the whole intestine which is in normal functioning.

The method applied in the present study should be one of the methods elucidating the relative characteristic of each segment in relation to the function of the whole intestine. However, authors are left in regret, because the volume of the perfusate was determined without taking the surface area available for absorption into account, and the rate of perfusion was determined equal in all of the segments without taking the respective transit time of contents in the segment into account. These measurements of available surface area for absorption and the transit time of contents in the respective segments were considerably difficult and the appropriate method for these determinations was not established as yet. Adjustments for these variables are possible only in modulating the volume of the perfusate and the rate of the perfusion in the presented method. The simultaneous four *in situ* recirculating perfusions method which was devised originally in our laboratories might open the way in investigating the relative characteristics concerning absorption and bidirectional fluid movement in the small intestine.