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Effects of Glucose on Intestinal Drug Absorption in Alloxan Diabetic Rats

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The effects of increased glucose concentration in blood on the glucose effect proposed in our previous papers were studied with sulfisoxazole, metoclopramide, and sulfanilamide using the *in situ* recirculating perfusion method with perfusion solution having three different tonicities which were adjusted to hypertonic, isotonic, and hypotonic with sodium chloride or glucose. To achieve the purpose, alloxan-induced diabetic rats were introduced in the present study. No effect of the increase of glucose concentration in blood in the diabetics on the glucose effect was found, since the regression lines representing the relation between the transmucosal fluid movement and the drug absorption with the controls were overlapped to each other with those of the diabetics in both of the media.

Simultaneously, the effects of diabetes on the transmucosal fluid movement and the absorption of the drugs were examined. The fluid movement and the absorption of the drugs in the diabetics were always significantly greater than in the controls in all of the experimental conditions. It is reasonable to understand that the increased drug absorptions might be based on only the increment in the transmucosal fluid inflow.

Keywords——alloxan diabetic rat; blood glucose; diabetes; glucose effect on drug absorption; intestinal drug absorption; in situ recirculating perfusion method; metoclopramide; sulfanilamide; sulfisoxazole; transmucosal fluid movement

In our previous studies,[2] it had been clarified that the absorption of drugs was increased with increasing the absorption of fluid, that is, one of the transmucosal fluid movement, and it was also found that *D*-glucose which was employed to adjust the osmolality of perfusion solution played a peculiar role in the absorption of ionized drugs in the physiological pH[3] of the small intestine in the rat. The absorption of sulfisoxazole, an anionic drug in the physiological pH, was decreased when sodium chloride in the perfusate was replaced by glucose, nevertheless the fluid movement was equivalent. Namely the straight regression line representing the relation between the transmucosal fluid movement and the drug absorption obtained with glucose medium was shifted to a region where the absorption of the drug was decreased, and two corresponding regression lines which were nearly parallel to each other were obtained. On the contrary, absorption of metoclopramide, a cationic drug, was increased when the solute in the perfusion medium was changed from sodium chloride to glucose. The regression line obtained from plots of the transmucosal fluid movement *versus* the drug absorption with glucose medium was shifted to a region where the drug absorption was increased. On the other hand, the absorption of unionized drug, such as sulfanilamide, was not affected by the components, *i.e.*, sodium chloride or glucose, of perfusion medium. These effects of glucose on the absorption of the drugs were nominated glucose effect in our previous studies[2b,c] and the glucose effect, moreover, was supported by our previous findings[2b,4] using *in vivo* technique, such as an intraduodenal administration method, in mice with ephedrine hydrochloride, a cationic drug.

1) Location: *Kawara-cho, Shogoin, Sakyo-ku, Kyoto.*
2) *a*) S. Kitazawa and H. Ito, "In Absorption, Metabolism, and Excretion of Drugs," Ed. by K. Kakemi, Hirokawa Publishing Co., Tokyo, 1972, p. 30—47; *b*) S. Kitazawa and H. Ito, "In Proceedings of the Fourth Symposium on Drug Metabolism and Action," Ed. by H. Ozawa, the Pharmaceutical Society of Japan, Tokyo, 1973, pp. 11—24; *c*) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 1856 (1975).
3) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).
4) S. Kitazawa, H. Ito, and M. Iinuma, *Chem. Pharm. Bull.* (Tokyo), **23**, 2128 (1975).

To examine that the glucose effect might be exerted when glucose was much more in the body of animal, alloxan pretreated diabetic rats whose blood glucose level was much higher than normal rats were introduced into our study.

It has been reported previously[5] that the transmucosal fluid inflow in alloxan-induced diabetic rats using perfusion solutions whose tonicities were adjusted with sodium chloride was always significantly greater than in control rats, because of the increase in plasma osmolality due to the hyperglycemia, and the absorption of sulfanilamide in the diabetics was also significantly greater than in the controls. The hyperglycemia which is one of the pathophysiological characteristics in diabetes mellitus provided to us an interesting finding concerning the transmucosal fluid inflow and the absorption of the drug. The effect of diabetes on the absorption of sulfanilamide, however, was not studied with glucose medium and, moreover, the drug absorption was not affected by the components of perfusion solution as mentioned above.

Hence three drugs, sulfisoxazole, metoclopramide, and sulfanilamide having different charges in the physiological pH of the small intestine were also selected and examined with sodium chloride medium and glucose medium in the present study. The effects of diabetes on the transmucosal fluid movement and the absorption of the drugs under all experimental conditions were discussed concurrently.

Materials and Methods

Materials——Sulfisoxazole used was the J.P. VIII grade and metoclopramide was kindly supplied by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan. All other drugs and chemicals used in this study were of reagent grade and obtained from commercial sources, and they were employed in the study without further purifications.

Animals——Male albino rats of the Wistar strain weighing about 180 g were used in all experiments. Rats were divided randomly into two groups for each study. The group to be made diabetic was injected intraperitoneally with a freshly prepared solution of alloxan monohydrate in sterile water (200 mg/kg of 100 mg/ml solution, Nakarai Chemicals, Ltd., Kyoto, Japan). The control group received matched equal volume of sterile water intraperitoneally. Animals were housed individually in metabolic cages[6] and a standard laboratory diet and tap water were given freely until the perfusion experimentation. It was thought necessary that animals having similar physiological conditions should be subjected for such an experimentation, since effect of alloxan administration varied in wide range ob blood glucose and other physiological conditions such as body weight.[5a,7] To evaluate the physiological condition of the animal, body weight, food and water consumption of all rats including the diabetic group and the control group were measured daily. Animals indicating similar results as that presented in the previous report[5a] were selected and were subjected to the experiment. The criteria for diabetes in these rats were 1) decreased growth rate; 2) glycosuria (Tes-Tape, Eli Lilly and Co.); and 3) hyperglycemia (more than 300 mg/100 ml). Control rats had normal blood glucose level (less than 200 mg/100 ml) and showed a steady body weight growth. On the seventh day after the injection, perfusion experiments were conducted.

Perfusion Procedures——According to the recirculating perfusion method which was devised by Schanker, Tocco and others,[8] amount of the drug disappeared in the perfusate was regarded as the amount absorbed from the site of the lumen. The technique was used throughout in this study. After anesthesia with intraperitoneal sodium pentobarbital in a dose of 40 mg/kg, the abdomen was opened through a midline incision. As a mean to clean the entire small intestine, physiological sodium chloride solution was gently passed through it until the effluent solution became clear. Inlet and exit cannulas were tied into both ends of the proximal duodenum and the distal ileum. The pylorus, bile duct, and the distal terminal ileum were ligated. Forty milliliters of drug solution which had been kept at 37° were infused into the duodenum at 5 ml per minute with a pump (CV-1 type, Tokyo Kagaku Seiki Co.). Following the cylinder method which was devised by Kitazawa and his co-workers,[9] volumetric cylinder having a volumn of 50 ml used as a reservoir for perfusate was calibrated before and after each run. The perfusion was followed for one hour and then a final sample was pipetted out and analytical procedures were followed. The measurement of dry weight of the small

5) *a*) S. Kitazawa and I. Johno, *Chem. Pharm. Bull.* (Tokyo), **24**, 2832, (1976); *b*) *Idem, ibid.*, **25**, 115 (1977).
6) S. Kitazawa and T. Komuro, *Igaku No Ayumi*, **100**, 521 (1977).
7) L.E. Schneider and H.P. Schedl, *Am. J. Physiol.*, **223**, 1319 (1972).
8) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. exp. Therap.*, **123**, 81 (1958).
9) S. Kitazawa, M. Ishizu, and K. Kimura, *Chem. Pharm. Bull.* (Tokyo), **25**, 590, (1977).

intestine was presented in detail in our previous report.[5a] Blood for blood glucose and/or drug analyses was collected from the femoral artery which was cannulated polyethylene tubing at a given interval. Heparin solution (200 U/kg of a 1000 U/ml solution, Novo Industri: A/S Copenhagen, Denmark), if necessary, was intravenously injected to obtain the blood sample consecutively.

Perfusion Solution——The concentration of each drug was set to 1 mM in all of the perfusion solutions. The solute used to adjust tonicities of the solution was sodium chloride or D-glucose and the concentrations of these solutes were 1.2, 0.9, and 0.6% for sodium chloride, and 6.7, 5.0, and 3.3% for glucose, respectively, to obtain hypertonic, isotonic and hypotonic perfusion solutions.

Analytical Methods——Blood Glucose: Blood for glucose analysis was collected prior to the perfusion studies in all cases. As mentioned in full in the previous study,[5a] blood glucose was analyzed chemically employing the modified *o*-aminodiphenyl-borate method by Sasaki and others.[10]

Drug: As all of the drugs used in this study had an aromatic amino group, so the drugs in the perfusate and the blood samples were diazotized with the regular manner[11] and coupled with 2-diethylaminoethyl-1-naphthylamine (Tsuda's reagent). After developing color, their optical densities were determined spectrophotometrically at a wave length of 550 nm with Hitachi spectrophotometer model 124.

Calculations——The drug absorption was calculated as follows:

$$\% \text{ of drug not absorbed} = 100\ (C_f/C_i)\ [\{40-(V_i-V_f)\}/40]$$

% of drug absorbed = 100—the percentage of drug not absorbed

where C_i and C_f are the initial and the final concentrations of the drug and V_i and V_f are the initial and the final volumes in the cylinder used as a reservoir for the perfusate, and (V_i-V_f) in the equation is the volume of transmucosal fluid movement across the small intestine.

Differences between groups were compared using the t test. P values less than 0.05 were considered to be statistically significant.

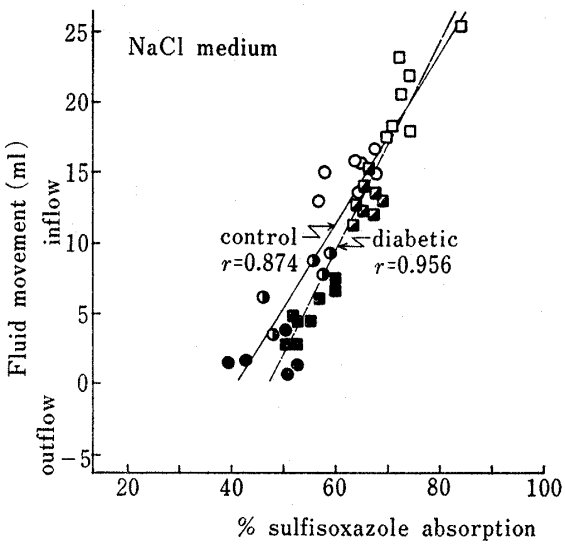

Fig. 1. The Relation between Transmucosal Fuid Movement and Sulfisoxazole Absorption with Sodium Chloride Medium in the Control and the Diabetic Animals

The perfusion was followed for one hour using the entire small intestine. The tonicities of perfusion solution were adjusted to hypertonic (1.2%), isotonic (0.9%), and hypotonic (0.6%) with sodium chloride, respectively. Inflow means apparent fluid flow into the animal during the course of the experiment. The regression lines were obtained by the least squares method. The solid line and the broken line in the figure were obtained from the controls and the diabetics, respectively.

control diabetic
○ hypotonic □
◑ isotonic ◪
● hypertonic ■

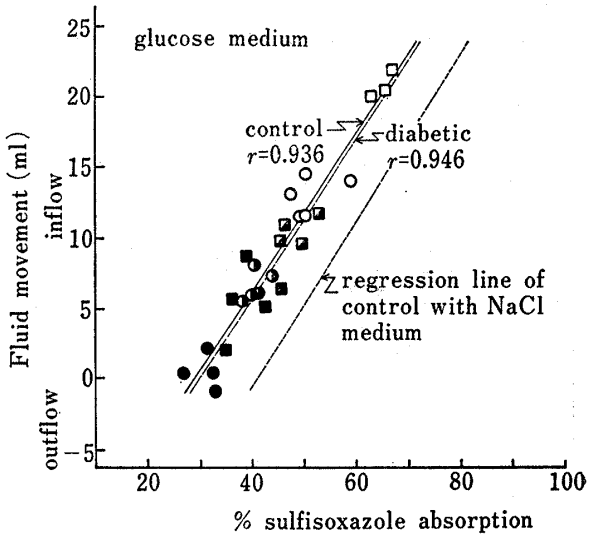

Fig. 2. The Relation between Transmucosal Fluid Movement and Sulfisoxazole Absorption with Glucose Medium in the Control and the Diabetic Animals

The perfusion was followed for one hour using the entire small intestine. The tonicities of perfusion solution were adjusted to hypertonic (6.7%), isotonic (5.0%), and hypotonic (3.3%) with glucose, respectively. The regression lines were obtained by the least squares method. The solid line and the broken line in the figure were obtained from the controls and the diabetics, respectively. The regression line of the controls with sodium chloride medium, which was represented with the solid line in Fig. 1, was illustrated with the dotted line.

control diabetic
○ hypotonic □
◑ isotonic ◪
● hypertonic ■

10) M. Sasaki, Y. Ohba, and N. Ito, *Rinsho Byori*, **Suppl. 15**, 55 (1968).
11) A.C. Bratton and E.K. Marshall, Jr., *J. Biol. Chem.*, **128**, 537 (1939).

Results and Discussion

Effects of Glucose on Drug Absorption

Sulfisoxazole which is considered to exist in the form of an anionic compound in the perfusate during the perfusion experimentations was selected to attain the purpose of this study. The relation between the transmucosal fluid movement and the drug absorption in the control and the diabetic animals using three different levels in concentration of sodium chloride was depicted in Fig. 1. The regression equation obtained with control rats was $y=0.60\ x\ -24.24$ (x: drug absorption, y: volume of fluid moved transmucosally; n: 17, r: 0.874) and that obtained with the diabetic animals was $y=0.75\ x-35.64$ (n: 23, r: 0.956). Strict calculations indicated such differences in slope and intercept of these regression lines of the controls and the diabetics. However, as illustrated in Fig. 1, it might be possible to expect that these scattered plots including the controls and the diabetics might have one regression line. Calculation for the regression line conducted and an equation for the line was obtained as $y=0.63\ x-27.19$ (n: 40, r: 0.916). Considering from the coefficient of correlation of 0.916, there might be enough foundations concluding that all the scattered plots had one regression line. This evidence demonstrated that although individual values in the drug absorption were different between the controls and the diabetics as was found in the previous report,[5a] the regression lines were not influenced whether the animal was the controls or the diabetics.

Similar findings were obtained when sodium chloride was replaced to glucose. The results obtained were shown in Fig. 2. The regression equation of the controls calculated according to the least squares method was $y=0.56\ x-16.06$ (n: 14, r: 0.936) and that of the diabetics

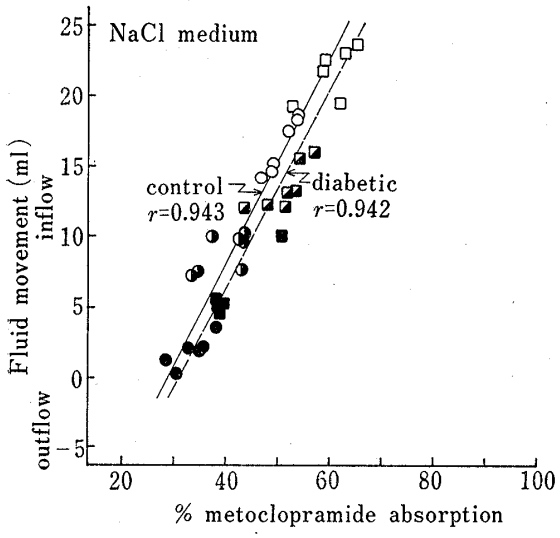

Fig. 3. The Relation between Transmucosal Fluid Movement and Metoclopramide Absorption with Sodium Chloride Medium in the Control and the Diabetic Animals

The perfusion was followed for one hour using the entire small intestine. The tonicities of perfusion solution were adjusted to hypertonic (1.2%) isotonic (0.9%), and hypotonic (0.6%) with sodium chloride, respectively. The regression lines were obtained by the least squares method. The solid line and the broken line in the figure were obtained from the controls and the diabetics, respectively.

control diabetic
○ hypotonic □
◑ isotonic ◪
● hypertonic ■

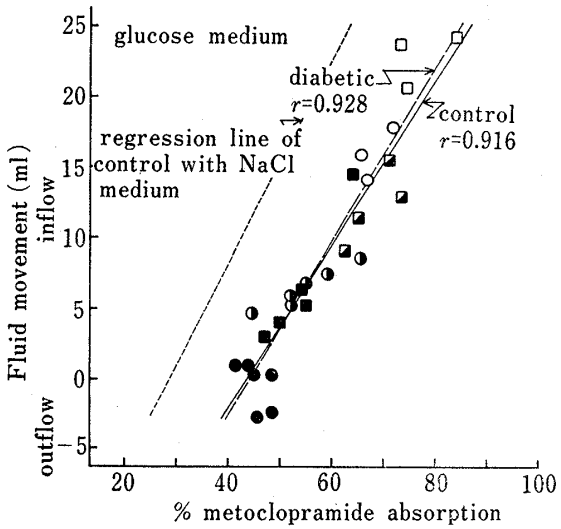

Fig. 4. The Relation between Transmucosal Fluid Movement and Metoclopramide Absorption with Glucose Medium in the Control and the Diabetic Animals

The perfusion was followed for one hour using the entire small intestine. The tonicities of perfusion solution were adjusted to hypertonic (6.7%), isotonic (5.0%), and hypotonic (3.3%) with glucose, respectively. The regression lines were obtained by the least squares method. The solid line and the broken line in the figure were obtained from the controls and the diabetics, respectively. The regression line of the controls with sodium chloride medium, which was represented with the solid line in Fig. 3, was illustrated with the dotted line.

control diabetic
○ hypotonic □
◑ isotonic ◪
● hypertonic ■

was $y=0.55\,x-16.46$ (n: 12, r: 0.946). The straight regression lines in both of the groups were overlapped to each other. This evidence suggested as in the case of the perfusate containing sodium chloride which was mentioned above that the effect of diabetes on the regression line was not observed in this case of the perfusate containing glucose as a solute to adjust the tonicities.

However, the regression line of glucose was apparently shifted to the region where the absorption of the drug was decreased when compared to that of sodium chloride which was illustrated in a dotted line in Fig. 2. This evidence demonstrated that similar glucose effect which had been observed in intact animals in our previous paper[2b,c] was observed in the diabetic animals.

Metoclopramide which exists in the form of a cationic substance in the physiological pH of the rat small intestine was also selected to compare with sulfisoxazole which exists an anionic compound in the perfusate. Plots of the transmucosal fluid movement *versus* the drug absorption in both of the controls and the diabetics using sodium chloride medium were illustrated in Fig. 3. The regression equation in the control rats was $y=0.72\,x-20.72$ (n: 19, r: 0.943) and that of the diabetic animals was $y=0.70\,x-21.70$ (n: 18, r: 0.942). As well observed in the case of sulfisoxazole, the regression lines in both the controls and the diabetics were overlapped to each other over a wide range of the transmucosal fluid movement.

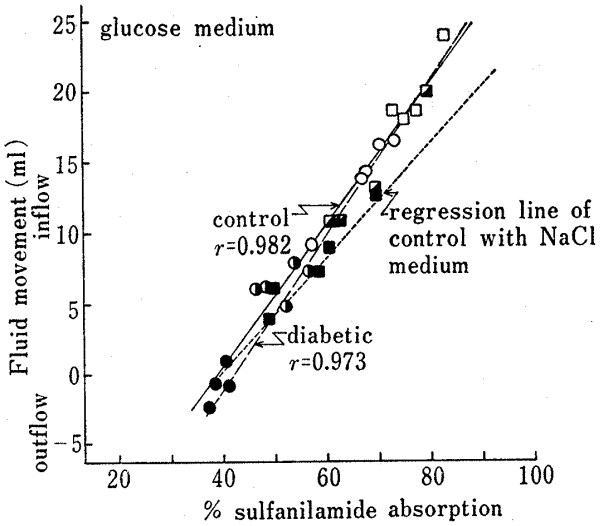

Fig. 5. The Relation between Transmucosal Fluid Movement and Sulfanilamide Absorption with Glucose Medium in the Control and the Diabetic Animals

The perfusion was followed for one hour using the entire small intestine. The tonicities of perfusion solution were adjused to hypertonic (6.7%), isotonic (5.0%), and hypotonic (3.3%) with glucose, respectively. The regression lines were obtained by the least squares method. The solid line and the broken line in the figure were obtained from the controls and the diabetics, respectively. The regression line of the controls with sodium chloride medium, which was quoted from our previous report,[5a] was illustrated with the dotted line.

control diabetic
○ hypotonic □
◑ isotonic ◪
● hypertonic ■

When the solute of perfusion solution was changed from sodium chloride to glucose, the relating the transmucosal fluid movement to the drug absorption in both of the groups was illustrated in Fig. 4. The regression equation obtained with the controls was $y=0.58\,x-25.40$ (n: 15, r: 0.916) and that obtained with the diabetics was $y=0.61\,x-26.81$ (n: 12, r: 0.928). Contrary to the case of sulfisoxazole, the regression lines of glucose medium in both of the groups were shifted to the region where the absorption of the drug was increased, that is, the lines were on the region of the right hand side of those of sodium chloride medium in this case of metoclopramide.

The relation between the transmucosal fluid movement and the absorption of sulfanilamide, an unionized form, in the controls and the diabetics with sodium chloride medium was reported in our previous study.[5a] The regression equation obtained with the control animals was $y=0.38\,x-14.94$ (n: 20, r: 0.739) and that obtained with the diabetic animals was $y=0.51\,x-22.48$ (n: 18, r: 0.816). On the other hand, the regression equations obtained with glucose medium were $y=0.50\,x-19.66$ (n: 14, r: 0.982) for the controls and $y=0.54\,x-23.08$ (n: 14, r: 0.973) for the diabetics, respectively. The results obtained were illustrated in Fig. 5. Moreover, the regression equation obtained from all scattered plots including the controls and the diabetics with both of the media was $y=0.47\,x-19.20$ (n: 66, r: 0.896). From this evidence, it might be reasonable to conclude that these scattered plots

had one regression line. Therefore, differently from the results obtained with ionized drugs in the physiological pH of the small intestine as mentioned above, these four regression lines were overlapped to each other over a wide range of the transmucosal fluid movement.

Many clinical signs and symptoms as well as biochemical and pathophysiological changes which are brought about by diabetes mellitus are well known in literature, and one of the prominent changes is thought increase in blood concentration of glucose in the animal.[12] This pathophysiological change was expected to modify the glucose effect which was disclosed in the previous paper[2b,c] concerning the absorption of ionized drug in the presence of glucose in the perfusate. Essentially similar results, however, were obtained with the controls and the diabetics, *i.e.*, no effect of the increase of glucose concentration in blood on the glucose effect was found.

On the other hand, the glucose effect was found as expected when the solute of perfusion solution was replaced from sodium chloride to glucose. Therefore, it might be able to conclude that the existence of glucose in the perfusate is requisite for the glucose effect. Thereupon, the exsitence of glucose in the perfusate in the case of sodium chloride medium was examined in both the control and the diabetic rats. The hexose, however, was not always detected in the perfusate. This evidence will be reported in detail in future.[13]

Mayersohn and Gibaldi[14] shown that the transfer of riboflavin across the everted rat intestine was significantly decreased in the presence of glucose in both mucosal and serosal sides. Material causing tissue fluid uptake such as glucose inhibited the riboflavin transfer, because the transfer of the drug across the extracellular space of the intestine would be markedly inhibited by cellular swelling. Their considerations, however, might not be applicable to our findings, since the absorption of metoclopramide obtained with glucose medium was always greater than that obtained with sodium chloride medium, and the absorption of sulfanilamide was a similar value in both of the media when the transmucosal fluid movement was equivalent.

Table I. Effects of Diabetes on the Transmucosal Fluid Movement During the Perfusion for One Hour Using Entire Small Intestine

Drug	Group	Hypertonic		Isotonic		Hypotonic	
		NaCl medium	Glucose medium	NaCl medium	Glucose medium	NaCl medium	Glucose medium
Sulfisoxazole	control	1.8± 1.1(5)	0.5± 1.1(4)	7.1± 2.1 (5)	6.6± 0.9(5)	14.9± 1.2(7)	13.0± 1.2(5)
	diabetic	4.9± 1.6(8)	5.6± 2.2(5)	13.0± 1.2 (8)	10.6± 0.9(4)	20.6± 2.8(7)	20.8± 0.9(3)
	p	<0.005	<0.01	<0.001	<0.001	<0.001	<0.001
Metoclopramide	control	1.9± 1.0(6)	0.0± 1.0(6)	8.9± 1.2 (7)	6.5± 1.3(6)	16.4± 1.8(6)	15.9± 1.6(3)
	diabetic	6.1± 2.0(5)	6.6± 4.1(5)	13.4± 1.6 (7)	12.3 2.3(4)	21.7± 1.7(6)	22.8± 1.6(3)
	p	<0.005	<0.01	<0.001	<0.005	<0.001	<0.02
Sulfanilamide	control	1.5± 1.7(4)[a]	−0.4± 0.8(4)	7.4± 2.3(12)[a]	6.5± 1.1(5)	13.7± 2.4(4)[a]	14.0± 2.6(5)
	diabetic	7.7± 1.6(5)[a]	7.8± 2.9(5)	12.0± 2.2(10)[a]	13.1± 3.6(5)	22.3± 1.8(3)[a]	19.8± 2.5(4)
	p	<0.005	<0.005	<0.001	<0.01	<0.01	<0.025

Values, ml. Entries are mean ± S.D. Numbers in parentheses indicate number of experiments. Minus sign suggests outflow which means apparent fluid flow into the lumen during the course of the experiment.
a) The data were quoted from our previous report.[5a]

12) W.F. Ganong, "Review of Medical Physiology," 4th ed., Maruzen Co., Ltd., Tokyo, 1969, p. 275.
13) S. Kitazawa and I. Johno, "in preparation."
14) M. Mayersohn and M. Gibaldi, J. Pharm. Sci., 60, 225 (1971).

Effects of Diabetes on the Transmucosal Fluid Movement

The effect of diabetes on the transmucosal fluid movement in the entire small intestine was shown in Table I. This table was written on the basis of the data presented in Fig. 1—5. The transmucosal fluid movement in the diabetic rats with sodium chloride medium was always significantly greater than in the control rats. Similar tendency was found when glucose was used as a solute of the perfusion solution and the differences between the controls and the diabetics were seemed to be almost constant indifferent to the tonicity of the perfusate.

The transmucosal fluid movement during the perfusion of the entire small intestine in the diabetic animals was compared with that of the control animals on the basis of dry-weight of the intestine. The results obtained were shown in Table II. The transmucosal fluid movement in the diabetics was constantly greater than in the controls under all experimental conditions.

Table II. Effects of Diabetes on the Transmucosal Fluid Movement per Unit Dry-weight of Intestine During the Perfusion for One Hour Using Entire Small Intestine

Drug	Group	Hypertonic		Isotonic		Hypotonic	
		NaCl medium	Glucose medium	NaCl medium	Glucose medium	NaCl medium	Glucose medium
Sulfisoxazole	control	1.31±0.74	0.35±0.80	5.07±1.46	4.93±0.54	11.82±0.71	9.89±1.21
	diabetic	3.45±1.12	4.04±1.50	8.74±0.48	7.76±0.26	15.36±2.05	14.29±0.63
	p	<0.005	<0.01	<0.001	<0.001	<0.005	<0.005
Metoclopramide	control	1.44±0.77	0.00±0.66	6.25±0.96	4.63±0.67	11.68±1.78	10.28±0.46
	diabetic	4.41±1.45	4.45±2.64	8.72±0.98	8.68±1.23	14.99±1.42	15.52±1.85
	p	<0.005	<0.01	<0.001	<0.001	<0.01	<0.02
Sulfanilamide	control	0.97±1.02[a]	−0.11±0.53	5.49±1.59[a]	4.68±0.75	10.15±1.83[a]	10.33±1.84
	diabetic	5.60±1.06[a]	5.00±1.94	9.13±1.47[a]	8.99±2.43	15.04±0.21[a]	14.03±1.51
	p	<0.001	<0.005	<0.001	<0.02	<0.02	<0.025

Values, ml/g dry-weight. Entries are mean±S.D. Number of experiments was represented in Table I. Minus sign suggests outflow which means apparent fluid flow into the lumen during the course of the experiment.
a) The data were quoted from our previous report.[5a]

Gerson and others[15] had reported that the addition of glucose in perfusate containing sodium chloride as a solute resulted in a significant increase of water absorption in the jejunum from human using a triple lumen tube perfusion system. However, in the present study, glucose was not detected in the luminal fluid in both of the controls and the diabetics as mentioned above. These lines of evidences suggest that the increased fluid inflow in the diabetics with sodium chloride medium might not be based on the findings of Gerson and others, since glucose did not exist in the perfusate during the perfusion study.

In our previous study,[5b] it has been demonstrated that the increased transmucosal fluid inflow in the diabetic rats might be caused by the increase of plasma osmolality due to the hyperglycemia. A good positive correlation between the transmucosal fluid movement and the blood glucose was also obtained in all experimental conditions used in the present study. The results obtained are shown in Table III. Therefore, it is proper to understand that the increased fluid inflow in the diabetics might be caused by a similar mechanism as obtained previously.[5b]

Lifson and Parsons[16] had demonstrated that the rate of water absorption using isolated jejunum loops of rat intestine was increased with increasing glucose concentration in serosal fluid. An isosmotic solution in both of sides of the intestine, however, was always used in

15) C.D. Gerson, N. Cohen, G.W. Hepner, N. Brown, V. Herbert, and H.D. Janowitz, *Gastroenterology*, **61**, 224 (1971).
16) N. Lifson and D.S. Parsons, *Proc. Soc. Exp. Biol. Med.*, **95**, 532 (1957).

Table III. Correlation between Blood Glucose and the Transmucosal Fluid Movement During the Perfusion for One Hour Using Entire Small Intestine

Drug	Medium	Hypertonic				Isotonic				Hypotonic			
		$n^{a)}$	$A^{b)}$	$B^{b)}$	$r^{c)}$	$n^{a)}$	$A^{b)}$	$B^{b)}$	$r^{c)}$	$n^{a)}$	$A^{b)}$	$B^{b)}$	$r^{c)}$
Sulfisoxazole	NaCl	13	54.27	114.86	0.857	14	42.45	−91.42	0.868	14	44.53	−485.90	0.868
	Glucose	10	35.67	188.26	0.892	10	45.64	−107.51	0.923	11	37.84	−319.84	0.977
Metoclopramide	NaCl	15	40.37	116.64	0.707	20	42.36	−190.64	0.886	13	32.38	−327.23	0.790
	Glucose	13	24.96	195.22	0.850	12	31.40	−25.59	0.936	7	27.62	−250.16	0.925
Sulfanilamide	NaCl[d)]	11	52.27	126.76	0.916	25	41.64	−126.68	0.872	9	37.97	−325.96	0.944
	Glucose	11	30.98	191.11	0.883	12	32.80	−23.24	0.972	11	31.29	−239.87	0.782

p values of the t test for coefficient of correlation was less than 0.01 in all cases.
Data which were obtained from the rats whose blood glucose was between 200 mg/100 ml and 300 mg/100 ml at the time of experiment were used as well as the controls and the diabetics to calculate the regression equation and the coefficient of correlation.
a) number of experiments
b) The regression equations between blood glucose on the vertical axis and transmucosal fluid movement on the horizontal axis under each experimental condition were obtained by the least squares method and represented as following equation: $y=A(x)+B$.
c) coefficient of correlation
d) The data were quoted from our previous report.[5b)]

Table IV. Effects of Diabetes on the Absorption of Drugs During the Perfusion for One Hour Using Entire Small Intestine

Drug	Group	Hypertonic		Isotonic		Hypotonic	
		NaCl medium	Glucose medium	MaCl medium	Glucose medium	NaCl medium	Glucose medium
Sulfisoxazole	control	47.1±5.2	31.1±2.5	53.2±5.2	40.6±1.9	63.3±4.0	51.1±4.1
	diabetic	54.9±3.4	39.9±3.9	65.7±2.0	51.4±4.1	74.0±4.4	65.3±1.7
	p	<0.02	<0.02	<0.001	<0.005	<0.001	<0.005
Metoclopramide	control	33.4±3.2	45.5±2.5	39.8±4.0	54.8±6.5	50.7±2.7	68.0±2.7
	diabetic	41.1±4.9	54.1±5.8	51.4±4.1	68.3±4.4	60.2±3.9	77.2±4.8
	p	<0.02	<0.02	<0.001	<0.02	<0.005	0.05<P<0.1
Sulfanilamide	control	54.1±7.6[a)]	39.5±1.6	57.7±8.4[a)]	51.6±3.7	65.3±5.8[a)]	67.1±5.5
	diabetic	65.4±4.9[a)]	57.6±7.6	66.6±7.2[a)]	67.1±7.1	80.7±4.4[a)]	77.2±3.6
	p	<0.05	<0.005	<0.025	<0.005	<0.025	<0.05

Values, %. Entries are mean±S.D. Number of experiments was represented in Table I.
a) The data were quoted from our previous report.[5a)]

Table V. Effects of Diabetes on the Absorption of Drugs per Unit Dry-weight of Intestine During the Perfusion for One Hour Using Entire Small Intestine

Drug	Group	Hypertonic		Isotonic		Hypotonic	
		NaCl medium	Glucose medium	NaCl medium	Glucose medium	NaCl medium	Glucose medium
Sulfisoxazole	control	346.4±30.7	218.9±23.8	380.7±32.4	304.8±18.7	502.5±23.7	388.7±30.6
	diabetic	386.1±25.2	287.0±29.7	442.3±29.9	378.5±27.2	552.0±25.9	449.3±23.8
	p	<0.05	<0.02	<0.01	<0.005	<0.005	<0.05
Metoclopramide	control	251.8±24.5	314.0±29.4	276.7±21.1	394.5±28.2	360.1±39.9	441.6±23.0
	diabetic	298.8±35.1	369.1±30.3	334.1±34.1	487.1±28.8	416.7±32.4	523.1±27.9
	p	<0.05	<0.025	<0.005	<0.005	<0.05	<0.05
Sulfanilamide	control	397.1±36.7[a)]	275.4±33.3	432.4±39.5[a)]	373.0±36.8	484.9±41.3[a)]	494.5±35.6
	diabetic	474.4±20.4[a)]	370.2±53.2	508.2±36.7[a)]	462.6±62.3	562.7±12.9[a)]	548.1±16.9
	p	<0.01	<0.05	<0.001	<0.05	<0.05	<0.05

Values, μM/g dry-weight. Entries are mean±S.D. Number of experiments was represented in Table I.
a) The data were quoted from our previous report.[5a)]

their experiments. This experimental condition could not be applicable to the *in situ* method used in the present study, since no one can change only the glucose level in blood without any alterations of the osmolality. However, the increased glucose level in blood with the dia-diabetics might cause the increase in the transmucosal fluid inflow in addition to the increase of plasma osmolality as described previously.[5b]

Effects of Diabetes on the Drug Absorption

The effects of diabetes on the absorption of sulfisoxazole, metoclopramide, and sulfanilamide in the entire small intestine with both of the media were shown in Table IV. This table was also written on the basis of the data presented in Fig. 1—5. These three drug absorptions in the diabetics with sodium chloride medium were always significantly greater than in the controls. Similar results were obtained with glucose medium.

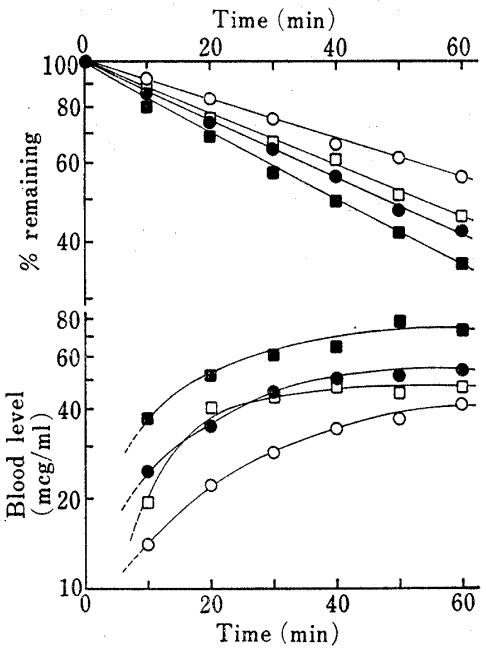

Fig. 6. Time Course Observations of Sulfisoxazole Level in the Perfusate and Blood in the Control and the Diabetic Animals with both Isotonic Sodium Chloride Medium and Isotonic Glucose Medium using Entire Small Intestine

A typical data was illustrated in this figure. The transmucosal fluid movement was 6.5 ml for sodium chloride medium and 6.2 ml for glucose medium in the controls, and 12.1 ml for sodium chloride medium and 12.5 ml for glucose medium in the diabetics. The blood glucose was 164 mg/100 ml for sodium chloride medium and 171 mg/100 ml for glucose medium in the controls, and 500 mg/100 ml for sodium chloride medium and 485 mg/100 ml for glucose medium in the diabetics, respectively.

control diabetic
○ isotonic glucose □
● isotonic NaCl ■

The absorptions of the drugs during the perfusion are shown per unit weight in Table V. The absorptions in the diabetics with both of the media were always significantly greater than in the controls on a dry-weight basis.

In our previous studies,[2] it has been clarified that the drug absorption was increased with increasing the transmucosal fluid inflow. Gerson and others[15] found a good positive correlation between folate absorption and water absorption. On the other hand, several reports in the literature[17] suggested that the increased intestinal absorption of actively transported sugars in diabetic animals might be caused by the increase of a transport activity in the intestine. If the increased drug absorption in the diabetics would be caused by these two factors, such as the increases in the fluid inflow and the transport activity, the regression lines representing the relation between drug absorption and transmucosal fluid movement with the diabetics ought to shift to a region where the absorption of drug was increased. The regression lines obtained with the diabetics, however, were always nearly equal to those obtained with the controls as shown in Fig. 1—5. Therefore, it seems to be reasonable that the increased drug absorption in the diabetics might be based on only the increased fluid inflow. These observations obtained strongly support the findings of our previous studies.[5]

To support these findings further in detail, time course observations on sulfisoxazole concentrations in the perfusate and the blood with both of the media were conducted with the diabetic and the control animals. The results obtained were illustrated in Fig. 6. To

17) *a*) R.K. Crane, *Biochem. Biophys. Res. Commun.*, **4**, 436 (1961); *b*) I.E. Vinnik, F. Kern, and K.E. Sussman, *J. Lab. Clin. Med.*, **66**, 131 (1965); *c*) H.P. Schedl and H.D. Wilson, *Am. J. Physiol.*, **220**, 1739 (1971).

minimize the effect of the transmucosal fluid movement on the drug absorption, the rats in which the transmucosal fluid movement was a similar value in the controls, and the blood glucose was a similar degree in the diabetics were chosen in this experimentation. The disappearance of the drug from isotonic sodium chloride perfusate with the diabetic animal was the greatest and the blood level was also the highest value. On the other hand, when isotonic glucose medium was perfused with the control animal, the disappearance of the drug from the perfusate was the least and the blood level was the lowest. Namely, the increase in the disappearance of the drug resulted in the increase in the blood level.

Although alloxan diabetic rats showed depressed body growth, the intestinal growth was enhanced progressively with time.[17c,18] Similar findings concerning the intestinal growth were reported by several workers.[5a,19] The absorption of a substance across the intestine in diabetic animals was expected to be modified due to the intestinal growth stimulation, one of peculiar characteristics in alloxan diabetic animals.

As one of the examples on the absorption characteristics in diabetes mellitus, it had been reported[17c,20] that intestinal tissue accumulation of actively transported sugars and amino acid was increased in diabetic animals. Data on which the measurements of blood level of sulfisoxazole in both of the groups, however, suggest the increase of the drug transport into the serosal side, *i.e.*, the blood, across the intestinal membrane in the diabetic animals. These lines of evidences also strongly support the findings of our previous report.[5a]

18) H.P. Schedl and H.D. Wilson, *J. Exptl. Zool.*, **176**, 487 (1971).
19) E.L. Jervis and R.J. Levin, *Nature*, **210**, 391 (1966).
20) P. Flores and H.P. Schedl, *Am. J. Physiol.*, **214**, 725 (1968); W.A. Olsen and I.H. Rosenberg, *J. Clin. Invest.*, **49**, 96 (1970).