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Transmucosal Fluid Movement and Intestinal Drug Absorption in Alloxan Diabetic Rats

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The effects of alloxan-induced diabetes on the relation between transmucosal fluid movement and its effect on sulfanilamide absorption were studied with the *in situ* recircurating perfusion method using the small intestine of rats with perfusion solution having three different tonicities which were adjusted to hypertonic (1.2%), isotonic (0.9%), and hypotonic (0.6 %) solution with sodium chloride, respectively. Sulfanilamide absorption in both of control and diabetic rats was increased with increasing the apparent transmucosal fluid movement. The regression lines obtained from both of them were almost overlapped in a wide range of drug absorption on the horizontal axis and fluid movement on the vertical axis. The transmucosal fluid movement using the entire small intestine in diabetic rats were always significantly greater than in control animals. On both the wet- and the dry-weight basis, the fluid movement was also significantly greater with the diabetics. The total drug absorption was significantly increased in the diabetics than in the controls. The drug absorption in the diabetics was also significantly incresaed than in the controls when compared on the dry-weight basis. The increased drug absorption in the diabetics might be based on the increase of fluid inflow rather than increased transport activity per unit weight of the intestine, since the drug absorption was increased only the increased portion of fluid inflow in the diabetics along the regression line of the controls. These evidences and findings so far obtained might allow us to conclude that the effect of transmucosal fluid movement on the drug absorption was the same characteristic in nature indifferent to the induced mechanisms which were due to artificial and natural devices.

Transmucosal fluid movement and its effect on drug absorption in the rat small intestine had been a purpose of the study in our laboratory, and based on the evidences found during the course of the study it had been emphasized that the transmucosal fluid movement played sometimes an important role in the process of drug absorption. The results so far obtained have indicated that the increase in drug absorption was observed with increasing the fluid absorption.[2] However, our findings were based on the condition in which the fluid movement was occurred artificially by adjusting the tonicities of the perfusion solution with changing concentrations of solutes. On the other hand, polydipsia is observed as one of symptoms of certain diseases. Diabetes mellitus is one of the examples, and physiological and pathological characteristics concerning the polydipsia and polyuria of diabetes in man and animals had been accumulated. Levinson and Englert[3] showed that water absorption decisively increased in alloxan-induced diabetic rats in contrast to that of non-treated controls in the *in situ* loop of jejunal and ileal segments of the intestinal tract of the experimental animals. Schneider and Schedl[4] also reported an evidence that a large volume of water was absorbed from the duodenal part in the diabetic rats, while water secretion was observed from the same segments in the control animals. A similar observation was also represented by Aulsebrook in his *in vitro* study.[5] In addition to these comparative studies concerning water absorption in

1) Location: *Kawara-cho, Shogo-in, 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; *b*) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 1856 (1975).
3) R.A. Levinson and E. Englert, Jr., *Diabetes*, **19**, 683 (1970).
4) L.E. Schneider and H.P. Schedl, *Am. J. Physiol.*, **223**, 1319 (1972).
5) K.A. Aulsebrook, *Experientia*, **21**, 346 (1965).

diabetic and control animals, excretion characteristics of diabetic animals were investigated by several workers[4,6] and they revealed that the urine volume was increased in alloxan diabetic rats. These lines of evidences suggest that diabetes enhanced water intake and urine volume, in other words, a large volume of water might be passed through the body of the diabetic animals.

The present study was undertaken to reveal the effect of these increases in fluid movement on intestinal drug absorption in alloxan-induced diabetic rats.

Materials and Methods

Materials——Sulfanilamide and other chemicals used in the present study were reagent grade and obtained from commercial sources. They were used without further purifications. Ten percent sterile aqueous solution of alloxan monohydrate (Nakarai Chemicals, Ltd., Kyoto, Japan) was prepared at each time of dosing.

Animals——Wistar strain male albino rats weighing about 180 g were used in all experiments. Animals were randomly divided into two groups. One group was intraperitoneally injected the alloxan solution in a dose of 200 mg/kg of body weight of animals and was styled as diabetic group, while the other group was also injected an equal volume of sterile water and was called as control group. Animals were housed in metabolic cages individually and fed a standard laboratory diet (Oriental Yeast Co., Ltd., Tokyo, Japan) ad libitum and given tap water freely prior to the perfusion study. During these period daily measurement in body weight, food and water intake, and hematocrit were conducted in all the rats, and those rats in the diabetic group whose blood glucose was over 300 mg/100 ml of blood and urine reacted positive to glycosuria (Tes-Tape, Eli Lilly and Co., U.S.A.) on the seventh day after the injection were used as the diabetic rats and those rats in the control group whose blood glucose was less than 200 mg/100 ml and urine reacted negative to glycosuria were used as the control rats in the experiments.

Measurements of Hematocrit——Blood samples for hematocrit determination were collected into a heparinized small beaker by cutting off the end of the tail of the animals.[7] Then approximate 50 μl of the blood were placed in a heparinized glass capillary and the capillary was centrifuged for 5 minutes at 12000 rpm using a centrifuge[8] (HEMATO KH-120, Kubota Manufacturing Industry Co., Ltd., Osaka, Japan).

Animal Procedures and Perfusion Studies——Following the recirculating perfusion method which had been initially devised by Schanker, Tocco, and others,[9] amount of 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 throughout this study. The rats were anesthetized by an intraperitoneal injection of sodium pentobarbital in a dose of 40 mg/kg. The small intestine was exposed by a midline abdominal incision and cannulated at the both ends of the proximal duodenum and distal ileum openings with silicon tubings. The bile duct was also ligated in all experiments to avoid any inflow of fluid into the lemen during the course of the perfusion study. The small intestine was washed with about 100 ml of physiological sodium chloride solution which had been maintained at 37°. The intestine was stored again in the abdomen and the incision was closed with metal clips. These animal procedures were performed with utmost cautions especially to avoid any interferences of mesenteric blood flow. The recirculating system adopted in the perfusion experiment was consisted of the entire small intestine, silicon tubings, glass tubings, a perfusion pump (CV-1 type, Tokyo Kagaku Seiki Co.), and a 50 ml of volumetric cylinder which was used as a reservoir of the perfusate. The glass tubings and the reservoir were immersed in a water bath having a temperature of 37° to keep the temperature of the perfusate constant. Forty milliliters of the perfusion solution were recirculated in order of duodenum to ileum of the intestine at a rate of 5 ml per minute. The recirculation was followed for one hour and then the perfusate was withdrawn and recovered attentively into the volumetric cylinder as complete as possible and the difference between the initial and the final volume of the perfusate in the cylinder was accounted as the amount of fluid moved transepithelially.[2a,10] Then the entire small intestine was removed gently from the mesentery and immediately blotted with a filter paper and weighed. The dry weight was obtained after drying the intestine for 24 hours at 100°.[11] With the purpose of determining drug and/or glucose concentration in blood at a given interval during the perfusion experiments, 0.1 ml of heparin solution (1000

6) *a*) M.K. Younoszai and H.P. Schedl, *J. Lab. Clin. Med.*, **79**, 579 (1972); *b*) W.F. Ganong, "Review of Medical Physiology," 4th ed., Maruzen Co., Ltd., Tokyo, 1969, p. 275.
7) H.C. Grice, *Lab. Anim. Care*, **14**, 483 (1964).
8) T. Komuro, S. Kitazawa, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 909 (1975).
9) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. exp. Therap.*, **123**, 81 (1958).
10) *a*) K. Inui, M. Horiguchi, T. Kimura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1781 (1974); *b*) T. Komuro, S. Kitazawa, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 400 (1975).
11) H.P. Schedl and H.D. Wilson, *Am. J. Physiol.*, **220**, 1739 (1971).

Unit/ml solution, Novo Industri, A/S Copenhagen, Denmark) was injected intravenously and blood samples were collected through a polyethylene tubing which was cannulated in the femoral artery prior to the perfusion.

Perfusion Solution——Perfusion solution contained sulfanilamide in a concentration of 1 mM and sodium chloride with an aim of adjusting tonicities of the solution. Three levels of concentrations of the salt (1.2, 0.9, and 0.6%) were used in the present study to obtain hypertonic, isotonic, and hypotonic solutions, respectively.

Analytical Procedures——Drug: Sulfanilamide in the perfusate and in the blood samples was diazotized with the regular manner[12] and coupled with 2-diethylaminoethyl-1-naphthylamine (Tsuda's reagent). The color developed was determined spectrophotometrically at a wave length of 550 nm using Hitachi spectrophotometer model 124.

Blood Glucose: Blood glucose was analyzed chemically using the modified *o*-aminodiphenyl-borate method developed by Sasaki and others.[13] The blood sample of 0.05—0.1 ml was hemolyzed by addition of a sufficient distilled water to make 0.6 ml. The solution was deproteinized with 0.5 ml of 10 w/v% trichloroacetic acid solution and then centrifuged. A half milliliter of the supernatant was mixed with 5 ml of *o*-aminodiphenyl reagent (2 w/v% *o*-aminodiphenyl and 8 v/v% saturated aqueous boric acid solution are contained in acetic acid). After being stored in a bath of boiling water for exactly 15 minutes, the mixture was immediately chilled in an ice-cold bath and the developed color was determined at a wave length of 660 nm by the spectrophotometer.

Calculations——The transmucosal fluid movement was calculated from the difference between the initial (V_i) and the final (V_f) volume of the perfusate in the volumetric cylinder used as a reservoir in the perfusion experiments. Drug absorption was calculated by the following equation: % abscrorbed $=100-100[C_f/C_i \times \{40-(V_i-V_f)\}/40]$ where C_i and C_f stand for the intial and the final concentrations of the drug in the perfusate, respectively, and (V_i-V_f) was the transmucosal fluid movement during the course of the perfusion study. Data were analyzed by standard statistical methods and p values of less than 0.05 were considered to be significant.

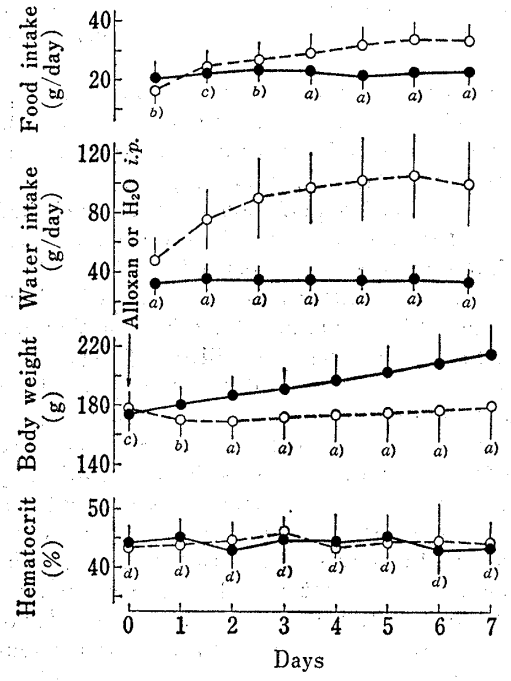

Fig. 1. Changes of Food and Water Intake, Body Weight, and Hematocrit in the Control and the Alloxan-induced Diabetic Rats

The rats were intraperitoneally injected 200 mg/kg of alloxan monohydrate or sterile water. All figures are mean ± S.D. Number of experiments (control 20; diabetic 18).
a) $p<0.005$, *b*) $p<0.05$, *c*) $p>0.1$, *d*) $p>0.2$
—●—: control ---○---: diabetic

Results

Differences in Food and Water Consumption, Body Weight, and Hematocrit in Diabetic and Control Rats

Daily changes in food and water intake, body weight, and hematocrit in both of the groups are shown in Fig. 1. In the control rats, food intake and water intake per head of the animals were maintained almost constant during the period of observations of 20 g/day and 30 g/day, respectively. These results demonstrated eventually to coincide with the data in normal rats obtained in a separate study in our laboratory.[14] The body weight was also increased as the same manner as that of the rate of normal growth in rats reported by Widdowson and McCance.[15] These findings suggest that the intraperitoneal injection of sterile water had little effect on the food and water consumption, and increasing in body weight of the rats. However, the effects of alloxan administration were observed in all of these results. Increase in water intake was observed as quickly as on the first day of the experiment and since that time significant differences were always

12) A.C. Bratton and E.K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).
13) M. Sasaki, Y. Ohba, and N. Ito, *Rinsho Byori Suppl.* **15**, 55 (1968).
14) S. Kitazawa and T. Komuro, *Chem. Pharm. Bull.* (Tokyo), submitted.
15) E.M. Widdowson and R.A. McCance, *Proc. Roy Soc.* (*London*), *Ser. B.*, **152**, 188 (1960).

recorded between those of the results of the controls and the diabetics ($p<0.005$). Despite these pronounced increase of water consumption in the diabetic animals, hematocrit in both of the groups kept constant throughout the period. Increase was also noted in food intake of the diabetics. These increases in both food and water consumption were recognized to reach a plateau after the fourth day of the administration. In spite of these increases in both of them, the body weight did not increase, but kept rather constant during the observations, in other words, the increases in food and water consumption did not reflect in parallel with the increase of weight of animal bodies, and significant differences in the results were noted between the controls and the diabetics after the second day of injection ($p<0.05$).

Wet weight of the entire small intestine in the diabetic animals was significantly greater than that of the controls as shown in Table I, although the diabetics showed depressed body growth. However, the difference in the dry weight could not be regarded as significant.

Considering these results on food and water consumption, body weight, and hematocrit measurements, physiological characteristics developed by the administration of alloxan seemed to be a steady state on the seventh day of the injection. The perfusion experiments were performed on the day in both the control and the diabetic animals.

TABLE I. Wet Weight and Dry Weight of the Entire Small Intestine in Control and Diabetic Rats

Group	n	Wet weight (g)	Dry weight (g)
Control	20	9.1±1.5	1.34±0.13
Diabetic	18	10.7±1.0	1.36±0.10
		$p<0.005$	$p>0.9$

Entries represented mean ± S.D.
Entire small intestine was perfused intraluminally prior to weighing.

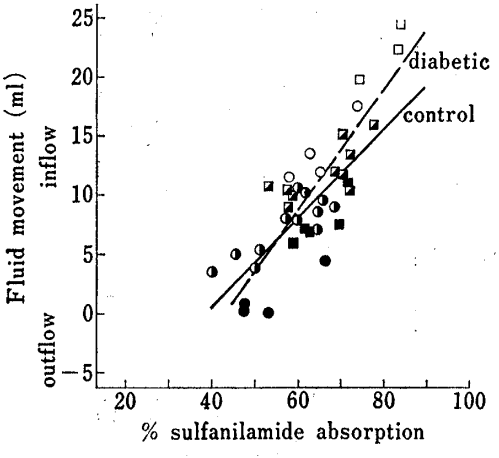

Fig. 2. Relation between Transmucosal Fluid Movement and Sulfanilamide Absorption in both of the Control and the Diabetic Rats

The regression lines were drawn by the least squares method. The tonicities of the perfusate were adjusted to hypertonic (1.2%), isotonic (0.9%), and hypotonic (0.6%) with sodium chloride, respectively.

control
○: hypotonic
◑: isotonic
●: hypertonic

diabetic
□: hypotonic
◪: isotonic
■: hypertonic

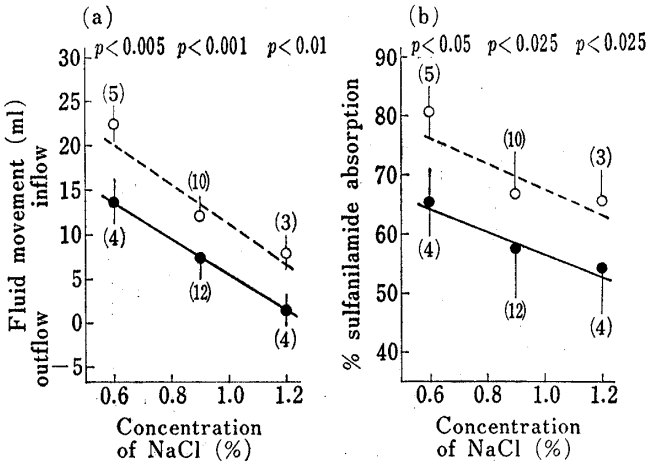

Fig. 3. Apparent Fluid Movement and Sulfanilamide Absorption by Entire Small Intestine in the Control and the Diabetic Rats at Different Tonicities of the Perfusate

The lines presented were drawn following the least squares method. Verticals bars indicate S.D. Numbers in parenthesis indicate number of experiments.

●: control ○: diabeti

Transmucosal Fluid Movement and Drug Absorption in both of the Groups

The control and the diabetic rats were subjected in the perfusion studies and the results depicted in Fig. 2 were obtained. Sulfanilamide absorption in both of the groups was increased with increasing the apparent transmucosal fluid movement. The regression line obtained in the control animals was $y=0.38x-14.94$, while in the diabetics was $y=0.51x-22.48$, and both of these lines were seemed to be almost overlapped in a wide range of drug absorption on the horizontal axis and fluid movement on the vertical axis in Fig. 2. However, as the results of detailed surveys, it was revealed that plots in the diabetic rats were shifted in the direction of increase in the drug absorption along the regression line as compared with those of the corresponding control animals.

To promote better understanding the relations between the tonicities of the perfusate and transmucosal fluid movement, and drug absorption, Fig. 3 was illustrated on the basis of the data presented in Fig. 2. Two figures in Fig. 3 strongly demonstrated characteristic change in the fluid movement and the drug absorption brought about by diabetes mellitus. The inflow of the fluid was always significantly greater with the diabetics ($p<0.01$) and the differences were seemed to be almost constant in wide range of the tonicity of the perfusate.

Reflecting these increases in the transmucosal fluid movement, the drug absorption was also significantly increased ($p<0.05$) in all cases of the tonicity employing in the present study.

The data from perfusion studies are shown per unit weight in Fig. 4. The transmucosal fluid inflow and the drug absorption were also always significantly greater in the diabetic animals than in the controls on a dry-weight basis.

To support the evidences more clearly, the drug concentrations in the perfusate and the blood in both of the groups were determined successively during the perfusion, and the results are presented in Fig. 5. The increase of the drug absorption in the diabetics was also demon-

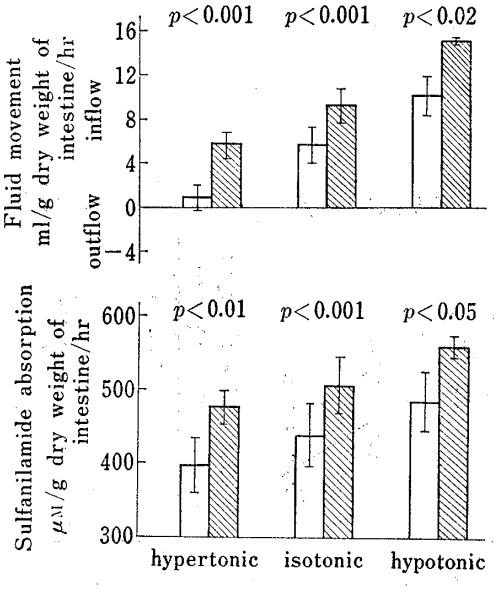

Fig. 4. Transmucosal Fluid Movement and Sulfanilamide Absorption per Unit Weight of Intestine during the Perfusion of Entire Small Intestine in both the Control and the Diabetic Rats

The initial drug concentration in the perfusion solution was 1 mM. The tonicities of the perfusate were adjusted to hypertonic (1.2%), isotonic (0.9%), and hyporonic (0.6%) with sodium chloride, respectively.

□: control ▨: diabetic ⊥: mean±S.D.

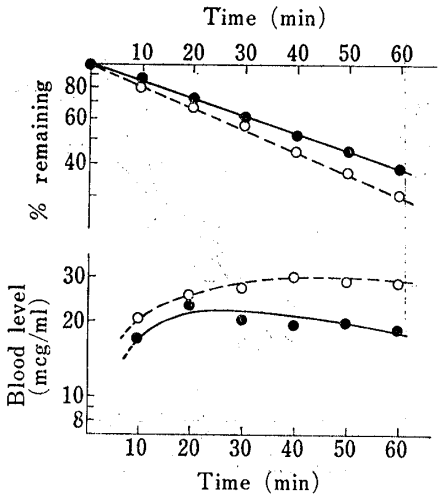

Fig. 5. Time Course Observations of Sulfanilamide Level in the Perfusate and Blood in both the Control and the Diabetic Rats in the Case of an Isotonic Perfusion Solxtion

Essentially similar patterns were obtained for all tonicities of the perfusate.

—●—: control —○—: diabetic

strated in the disappearance of the drug in the perfusate during the perfusion experiment. The disappearance obeyed the first order kinetics which was essentially the same as that of the controls. The increase in the disappearance of the drug in the perfusate was recognized in the increase in the blood level in the diabetic animal. These evidences clearly demonstrated that the drug was not stored in mucosal tissue of the small intestine on the way of transport through the epithelial tissue.

Taking these findings and the results represented in the previous reports[2] into considerations, it might be able to conclude that the drug absorption in the diabetic animals was enhanced and the reason of this enhancement was due to the increase in the transmucosal fluid movement which was significantly greater in the diabetics.

Discussion

Several reports[3-5,11,16] in literature had mainly concerned with effects of diabetes on the intestinal absorption of actively transported sugars and electrolytes and they suggested that the absorption of these substances was significantly greater in diabetic animals than in controls. Hypoglycemic agents are often prescribed with other drugs which are passively transported from the gastrointestinal tract to a patient which was diagnosed as diabetes mellitus. A comparative absorption study between diabetics and controls on these drugs seems to be important from the standpoint of the efficacy and/or the safety on a drug treatment, however, limited findings have accumulated concerning to effects of diabetes on the intestinal absorption of drugs.

One of a series of study undertaken in our laboratory had elucidated the effect of transmucosal fluid movement on the intestinal drug absorption. However, our findings were based on an artificially initiated transepithelial fluid movement, since the fluid movement was caused by changing tonicities of perfusion solution in the *in situ* perfusion experiments using the rat small intestine. This should be an example in which transmucosal fluid movement would occur physiologically and/or pathologically in the cases of some diseases, and surveys for such an example were undertaken in our laboratory, and as the results, diabetes mellitus was picked up as a suitable example for our studies.

The mechanism of increase of water intake in diabetic animals had been explained that excretion of the osmotically active glucose molecules entails the loss of a large amount of water and the resultant dehydration activates the mechanisms regulating water intake, leading to polydipsia.[17] Younoszai and Schedl[6a] reported that water intake and urine volume remarkably increased with diabetic rats. These lines of evidences were also supported in the results obtained in the present study, water intake in the diabetics was observed significantly greater than that of the controls in the first 24 hours after the alloxan injection (Fig. 1), although hematocrit was the same in both of the groups, and the fluid movement was always significantly greater in the diabetics (Fig. 3a). These findings would support to understand that the increase of water intake in the diabetics resulted an increase in water absorption across the gastrointestinal tract and the increased water absorption might not be stored as the body fluid such as plasma and as a resultant urine volume might be increased.

Wet weight of the entire small intestine in the diabetics was significantly increased than in the controls, but dry weight was the same in both of the groups (Table I). The results obtained suggest that the increased wet weight in the diabetics might be due to the increase of water content in the intestinal membrane. Schedl and Wilson[11] had shown that a difference

16) *a*) P. Flores and H.P. Schedl, *Am. J. Physiol.*, **214**, 725 (1968); *b*) W.A. Olsen and I.H. Rosenberg, *J. Clin. Invest.*, **49**, 96 (1970).

17) W.F. Ganong, "Review of Medical Physiology," 4th ed., Maruzen Co., Ltd., Tokyo, 1969, pp. 276—277.

between control rats and diabetics on dry weight at 5—8 days after diabetes induction could not be found because of increase in tissue water content of the small intestine in diabetics. The rats used in the present study were with 7 days of diabetic age, so that it seemed to be reasonable that the evidences found by them could be applicable.

The transmucosal fluid movement was always significantly greater in the diabetics than in the controls (Fig. 3a). The small intestinal growth stimulation in the diabetics as mentioned above might cause the increase of fluid absorption across the tract. The average increase of the wet weight, however, was only 17.6%; on the other hand, the average increase of the fluid absorption was 62.8% (ml: controls, 13.7; diabetics, 22.3) at hypotonicity, 62.2% (ml: controls, 7.4; diabetics, 12.0) at isotonicity, and 413.3% (ml: controls, 1.5; diabetics, 7.7) at hypertonicity, respectively. Moreover, the fluid movement was significantly greater in the diabetics than in the controls when compared on the dry-weight basis (Fig. 4). Also, the fluid movement was significantly increased in the diabetics at all three test solutions ($p<0.05$) when compared on the wet-weight basis, although the data were not shown in this paper. The findings are in agreement with those of Levinson and Englert[3] and Aulsebrook[5] who observed an increase of water absorption *in situ* and *in vitro* in alloxan-induced diabetic rats. They, however, could not demonstrate clearly the mechanism of enhanced intestinal water absorption in diabetes. Schneider and Schedl[4] presented a factor related to these phenomena, they illustrated that serum osmolality was greater in diabetics than in controls because of the hyperglycemia, in other words, the osmotic gradient for fluid absorption was much greater in the diabetics than in the controls. These lines of evidences suggest that the increased fluid absorption in the diabetics might be caused by the increase of osmotic gradient for the movement, but not be depended on a factor such as the intestinal growth.

Reflecting these evidences concerning the transmucosal fluid movement, a difference between the controls and the diabetics on the drug, sulfanilamide, absorption was found. Regardless of sodium chloride concentrations the drug absorption was always significantly greater in the diabetics than in the controls (Fig. 3b). In addition, the drug absorption was always significantly increased with the diabetic animals on dry-weight basis (Fig. 4), but a difference in the two groups could not be regarded as significant on wet-weight basis, although the data were not depicted. Schedl and Wilson[11] had reported that a difference of 3-O-methyl-D-glucose transport across the intestine between control and alloxan or streptozotocin diabetic animals was found the greatest on the 5- to 8-day diabetic groups and the absorption per unit weight of intestine was significantly greater with the diabetics on both wet- and dry-weight basis. The increase of the sugar absorption in diabetics was depended on increased transport activity per unit weight of tissue. They also pointed out that the data decribing absorption by the entire small intestine reflected not only effects of the intestinal growth but also the sugar transport stimulation, so that transport data were also best compared on the dry-weight basis, since transport activity of the intestine from diabetics was underestimated when based on wet weight. Crane[18] had reported that enhanced intestinal glucose absorption in alloxan diabetic rats using *in vivo* technique was a functional alteration characteristic of the diabetes. Similar mechanism on increased glucose absorption in diabetic patient was proposed by Vinnik and others.[19] If the increased drug absorption in the diabetic group would be caused by the factors, such as some functional change based on their findings and the fluid inflow proposed in our previous paper,[2] the regression line representing the relation between drug absorption and fluid movement in the diabetics ought to shift on the right hand side. The regression line obtained from the diabetic group, however, was nearly equal to that of the controls (Fig. 2), and the drug absorption was increased only the increased portion of fluid inflow in the diabetics along the regression line of the controls. Hence the

18) R.K. Crane, *Biochem. Biophys. Res. Commun.*, **4**, 436 (1961).
19) I.E. Vinnik, F. Kern, and K.E. Sussman, *J. Lab. Clin. Med.*, **66**, 131 (1965).

increased drug absorption in the diabetics might be based on the increase of fluid inflow rather than increased transport activity per unit weight of the intestine or some other factors. From these considerations, it might be proper to understand that the mechanism of increased absorption of sulfanilamide, a passively transported substance, and actively transported sugars discussed previously[11,18,19] in diabetic animals might differ essentially.

The enhancement of the transmucosal fluid movement in the diabetics might influence the drug absorption by solvent drag,[20] an important absorption mechanism. Previous report in our laboratory[2b] had suggested that solvent drag hypothesis might be applicable not only to substances of small molecule such as electrolytes, but also to those of comparatively large and organic molecule, since the absorption of the latters was increased with increasing fluid movement indifferent to size and charge of the molecules. They had also pointed out that solvent was not able to drag all kinds of organic substances, because phenol red was not quite absorbed in spite of the conspicuous increase of fluid absorption. Similar phenomenon had been demonstrated by Ochsenfahrt and Winne.[21] On the other hand, Flores and Schedl[16a] had reported that solvent drag would not appear to be an important factor in increased 3-O-methyl-D-glucose transport in diabetic rats, since the increase in net water movement in diabetes is proportional to the increase in the sugar transport. From these lines of evidences, it might be premature to conclude whether the increased sulfanilamide absorption in diabetes is due to solvent drag or some other factors in this paper, because more detailed experimentations might be necessary in this point.

We had encountered so far two kinds of transmucosal fluid movement, one was tonicity induced movement which had been reported in the previous paper,[2] and the other was physiologically or pathologically induced movement in the present study, and found that these fluid movements had the same effect on the drug absorption from the rat small intestine, although their induced mechanisms were quite different each other. These evidences strongly suggest that the effect of transmucosal fluid movement on the drug absorption was the same characteristic in nature indifferent to their induced mechanisms which were due to artificial or natural devices.

An amount of sulfanilamide disappeared from the intestinal perfusate was in essence the absorption of the drug from the site of the lumen which was exposed to the perfusate in the present study, so that it was not found whether the increase of the drug disappearance in diabetics was the increase of accumulation into the small intestinal tissue or that of transport into serosal side. Flores and Schedl[16a] demonstrated employing the *in vitro* everted sac that intestinal accumulation of 3-O-methyl-D-glucose in diabetic rats was increased, and at the same time, absorption of the sugar was also increased. Increased intracellular accumulation of amino acid, as well as galactose and 3-O-methyl-D-glucose, was reported by Olsen and Rosenberg.[16b] Data on which the measurement of blood levels of sulfanilamide in both a control and a diabetic rats (Fig. 5) suggests the increase of the drug transport from mucosal to serosal side across the small intestinal membrane.

Sulfanilamide which is not hypoglycemic agent was selected as a drug in the present study. The reason of this selection was not only that the drug had been used in the series of our studies,[2] but that the drug does not effect on blood glucose level which is subtlely affected by the hypoglycemic agents such as biguanide and sulfonylurea groups,[22] so that an accurate difference between control and diabetic groups on the intestinal absorption might be discussed in such an experimentation. Based on these reasons, hypoglycemic agents

20) H.H. Ussing, *Physiol. Rev.*, **29**, 127 (1949); V. Koefoed-Johnsen and H.H. Ussing, *Acta. Physiol. Scand.*, **28**, 60 (1953).
21) H. Ochsenfahrt and D. Winne, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **281**, 175 (1974); *idem, ibid.*, **281**, 197 (1974).
22) E. Nelson, E.L. Knoechel, W.E. Hamlin, and J.G. Wagner, *J. Pharm. Sci.*, **51**, 509 (1962); L.J.P. Ducan and B.F. Clark, *Ann. Rev. Pharmacol.*, **5**, 151 (1965).

were not employed in the present study.

It is well known that diabetes mellitus often leads to several complications, so that a hypoglycemic agent is often coadministered with other drugs. The present study also indicated absorption of these drugs other than hypoglycemic agents might be increased in diabetic animals. This report suggests that medications in diabetes mellitus should be promoted with utmost carefulnesses.