

Effects of Blood Glucose and Plasma Osmolality on Transmucosal Fluid Movement and Intestinal Drug Absorption

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It had been clarified in our previous study that the transmucosal fluid inflow and the absorption of sulfanilamide in alloxan diabetic rats were increased significantly than in control rats using the *in situ* recirculating perfusion method. The present study was designed to elucidate further in full the effects of diabetes on these intestinal absorptions. Plasma osmolality was increased with increasing blood glucose and a good correlation was obtained over a wide range of the blood glucose. The increase of blood glucose resulted in the increment of the transmucosal fluid inflow and the absorption of the drug. To examine these effects of blood glucose, rats having hyperglycemia, one of physiological characteristics in diabetic animals, were prepared by the administration of D-glucose. The fluid movement and the drug absorption in the glucose administered rats were significantly increased than in the controls and were not found significant difference compared to those of the diabetics on the basis of dry-weight of the small intestine of the experimental animal. From the results obtained in the present study and in our previous findings, it might be able to conclude that a possible mechanism on the increase of the drug absorption in the diabetics would be that the enhancement of plasma osmolality due to the hyperglycemia caused the increment of the transmucosal fluid inflow to compensate the abnormally high osmolality in blood and this inflow might lead the drug absorption increase consequently.

Keywords—alloxan diabetic rat; blood glucose; D-glucose injection; *in situ* recirculating perfusion method; intestinal drug absorption; plasma glucose; plasma osmolality; sulfanilamide; transmucosal fluid movement

Studies concerning the effects of the transmucosal fluid movement on drug absorption from the small intestine of rat employing the *in situ* recirculating perfusion method²⁾ with perfusion solution of which the tonicities were adjusted by the different concentrations of sodium chloride have been reported from our laboratories.³⁾ The results obtained showed that the intestinal absorption of drugs was increased with increasing the transmucosal fluid inflow. In addition to these observations, it was found that as the transmucosal fluid movement in alloxan-induced diabetic rats was compared with that of normal rats, the former was always significantly greater than the latter and consequently the absorption of sulfanilamide used as a marker drug was also significantly increased in the diabetics. Moreover, the transmucosal fluid movement and the drug absorption in the diabetics were always significantly increased than in the controls when compared on the basis of dry-weight of the small intestine of the experimental animal.⁴⁾ Several reports in literature revealed that water absorption across the intestinal membrane was increased in diabetic animals in both the *in situ*⁵⁾ and the *in vivo*^{5b,6)} techniques. The mechanisms of the increased water absorption, however, were not demonstrated clearly.

1) Location: *Kawara-cho, Shogo-in, Sakyo-ku, Kyoto.*

2) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. exp. Therap.*, **123**, 81 (1958).

3) S. Kitazawa and H. Ito, "In Absorption, Metabolism, and Excretion of Drugs," Ed. by K. Kakemi, Hirokawa Publishing Co., Tokyo, 1972, p. 30; S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 1856 (1975).

4) S. Kitazawa and I. Johnno, *Chem. Pharm. Bull.* (Tokyo), **24**, 2832 (1976).

5) a) R.A. Levinson and E. Englert, *Diabetes*, **19**, 683 (1970); b) P. Flores and H.P. Schedl, *Am. J. Physiol.*, **214**, 725 (1968).

6) K.A. Aulsebrook, *Experientia*, **21**, 346 (1965).

The present study was designed to clarify a further possible mechanism on the increase of fluid inflow and drug absorption in diabetic rats.

Materials and Methods

Materials—Sulfanilamide and all other chemicals used in this study were reagent grade and obtained from commercial sources. These were used without further purifications.

Animals—Male albino rats of Wistar strain weighing about 180 g were purchased. They were divided randomly into two groups. The group to be made diabetic was injected intraperitoneally a freshly prepared solution of alloxan monohydrate in sterile water (200 mg/kg of a 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 and they were fed a standard laboratory diet and given tap water freely prior to each experiment. Body weights, food and water intake in both the groups were measured daily. The results obtained were essentially similar to that found in our previous study.⁴⁾ Each experiment was performed on the seventh day after the pretreatment.

Perfusion Procedures—According to the *in situ* recirculating perfusion method which was devised by Schanker, Tocco and others²⁾ on the rat small intestine, an amount of drug disappeared in the perfusate was regarded as the amount absorbed from the site of the lumen exposed to the perfusate. This method was employed in the present study with some modifications. The descriptions of the method and the test solutions were presented fully in the previous report from our laboratory.⁴⁾

Enhancement of Blood Glucose in the Control Rats—To reveal the effects of blood glucose more in detail, it was necessary to increase the blood glucose level during the course of the perfusion experiment. However, there was rigorous condition not to increase the volume of blood. The method developed and applied in the present study was as follows. One gram of D-glucose was placed in a syringe having a volume of 5 ml, and 2 ml of the blood collected from the rat under the perfusion experiment through an intraartery cannulation in the femur of the left side. Blood and glucose were mixed well in the syringe and the mixture was again administered slowly taking about 5 minutes through the femoral vein of the right side. This technique was found to be applied not only the rat under the perfusion experiments but that of an intact condition. After observing plasma osmolality and blood glucose of the rat at a given time, this method was found satisfactory to enhance the blood glucose of the rat without increase the blood volume.

Analytical Methods—Blood Glucose: As mentioned in full in the previous study,⁴⁾ blood glucose was analyzed chemically employing the modified *o*-aminodiphenyl-borate method developed by Sasaki and co-workers.⁷⁾

Plasma Glucose: An aliquot of supernatant of the blood centrifuged as will be mentioned below was pipetted and analyzed in a similar manner as the blood glucose analysis.

Drug: Sulfanilamide in the perfusate withdrawn at a given time was diazotized with the regular manner⁸⁾ 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.

Plasma Osmolality: Blood was collected from the femoral artery in a heparinized test tube and after a centrifugation for 20 minutes at 3000 rpm, 0.2 ml of the plasma were suffered to the measurement with Advanced Osmometer model 3D applying freezing point depression.

Calculations—The drug absorption was calculated as follows:

$$\% \text{ of drug not absorbed} = 100(C_f/C_i)[\{40 - (V_1 - V_f)\}/40]$$

$$\% \text{ of drug absorbed} = 100 - \text{the percentage of drug not absorbed}$$

where C_i and C_f are the initial and the final concentration of the drug in the perfusate and V_1 and V_f are the initial and the final volume in volumetric cylinder having a volume of 50 ml using as a reservoir for the perfusate, and $(V_1 - V_f)$ in the equation is the transmucosal fluid movement across the entire small intestine.

Data were compared using the *t* test. Differences were considered to be significant for $p < 0.05$.

Results

To elucidate the enhancement of fluid inflow across the small intestine in diabetic rats, which was found in our previous study,⁴⁾ the relations between plasma osmolality and blood glucose, and plasma glucose including both of the groups of the animals were examined in the first place and the results obtained were depicted in Fig. 1. A good correlation whose correlation coefficient was 0.893 ($n: 23$, $p < 0.001$) was found between the plasma osmolality

7) M. Sasaki, Y. Ohba, and N. Ito, *Rinsho Byori*, Suppl. 15, 55 (1968).

8) A.C. Bratton and E.K. Marshall, Jr., *J. Biol. Chem.*, 128, 537 (1939).

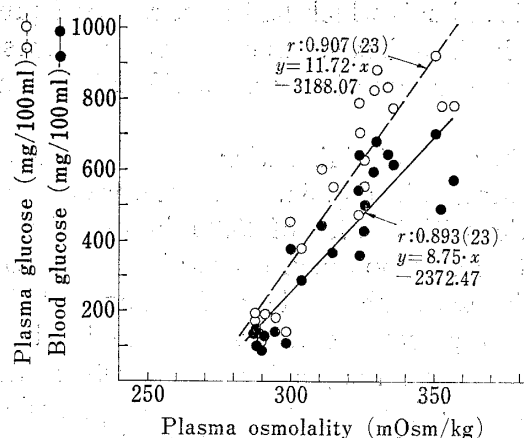


Fig. 1. The Relation between Plasma Osmolality and Blood Glucose, and Plasma Glucose

The regression lines were calculated by the least squares method and the values of the Student t test for each correlation coefficient were $p < 0.001$. Number in parentheses suggests number of experiments.

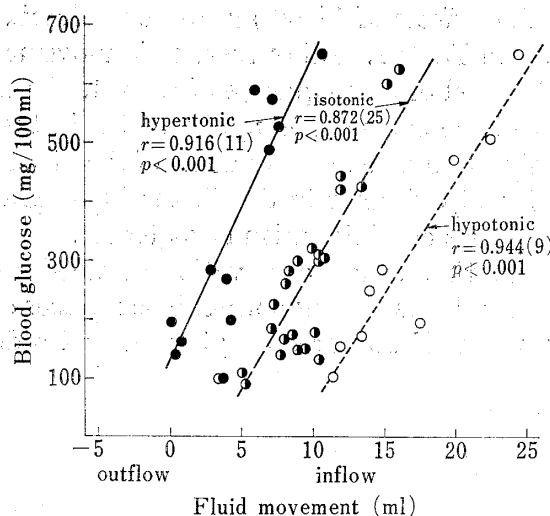


Fig. 2. The Effects of Blood Glucose on Transmucosal Fluid Movement

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 plotted in this figure as well as the controls and the diabetics. The tonicities of the 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 drawn following the least squares method. Number in parentheses indicates number of experiments.

and the blood glucose and the increase of blood glucose which was brought about by the diabetics resulted in the increment of the plasma osmolality. A good correlation between the plasma osmolality and the plasma glucose was also found ($n: 23, r: 0.907, p < 0.001$).

The effects of initial blood glucose on the apparent transmucosal fluid movement which was observed during the course of the *in situ* perfusion experiment were shown in Fig. 2. Data which were obtained from the rats whose the blood glucose was between 200 mg/100 ml and 300 mg/100 ml at the time of experiment were plotted in this figure as well as the controls and the diabetics. The fluid movement across the small intestinal membrane was apparently affected by the initial blood glucose, that is, the fluid inflow was increased according to the increase of the blood glucose and the three regression lines which were obtained using the perfusion solution having three different tonicities with sodium chloride were nearly parallel to each other. These effects on the transmucosal fluid movement were directly affected on the drug absorption.

TABLE I. Effects of Blood Glucose on the Intestinal Absorption of Sulfanilamide in the Rats using Perfusion Solution whose Tonicities were Adjusted with Sodium Chloride

Tonicity	$n^a)$	$A^b)$	$B^b)$	$r^c)$	$p^d)$
Hypertonic ^{e)}	11	16.86	-629.15	0.724	$p < 0.02$
Isotonic ^{e)}	25	11.75	-460.39	0.690	$p < 0.001$
Hypotonic ^{e)}	9	19.49	-1085.20	0.896	$p < 0.005$

a) number of experiments

b) The regression lines between blood glucose on the vertical axis and drug absorption 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) p values are the Student t test for the coefficient of correlation.

e) hypertonic (1.2%); isotonic (0.9%); hypotonic (0.6%)

The effects of blood glucose on sulfanilamide absorption under different conditions are shown in Table I. All of these fluid movements and also the drug absorption were depended on the level of blood glucose of the rat. These lines of evidences strongly suggested that the cause of the enhanced drug absorption in the diabetic animals which had been reported previously⁴⁾ would be in the increase of the blood glucose in the animals. In order to clarify these relationships further in detail, the blood glucose of the rat was increased artificially by an intravenous administration of D-glucose.

The time course observation of blood glucose after the administration of 1 g glucose to the control rats having normal blood glucose was illustrated in Fig. 3a. The increased blood glucose was disappeared obeying the first-order kinetics with half life of 35.3 minutes. From

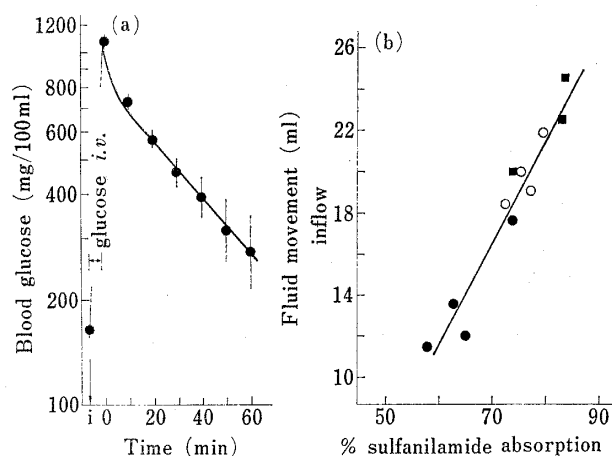


Fig. 3. The Time Course Observation of Blood Glucose after Intravenous Administration of One Gram of D-Glucose with Control Rats (a); and the Effects of the Glucose Administration on the Relation between Transmucosal Fluid Movement and Sulfanilamide Absorption using Hypotonic Perfusate (b)

Initial mean blood glucose was 164.4 mg/100 ml and the values shown for blood glucose represent the mean \pm S.D. on four animals. The regression line in (b) was obtained from the control and the diabetic animals using the least squares method. The data of the controls and the diabetics in (b) were quoted from our previous paper.⁴⁾

●: control ■: diabetic ○: glucose *i.v.*

the result obtained concerning the blood glucose, it should be understood that the high glucose level was not maintained constant throughout one hour which was the minimum period of the perfusion study, and the results of the fluid movement and the drug absorption obtained using this technique, which was illustrated in Fig. 3b, did not present solely the effect of the blood glucose. However, the plots in Fig. 3b placed fairly on the regression line which had been obtained from the control and the diabetic animals. The transmucosal fluid inflow in the glucose administered rats was significantly increased ($p < 0.01$) than in the control rats and was not found significant difference ($p > 0.1$) compared to that of the diabetic rats. As well the effect of the glucose administration on the transmucosal fluid movement, the absorption of the drug was significantly increased ($p < 0.025$) by the administration of glucose, compared with that of the control animals, significant difference between the glucose injected

and the diabetic animals on the drug absorption, however, could not be found ($p > 0.2$).

Moreover, the data from perfusion studies with hypotonic perfusate are shown per unit weight in Fig. 4. The fluid and the drug absorptions were significantly greater ($p < 0.02$ and $p < 0.05$, respectively) in diabetics and glucose administered group than in controls on a dry-weight basis. Their absorptions, however, did not differ between the diabetics and the group administered glucose. These results demonstrated that the rats administered glucose presented the data more similar to that of the diabetics than the control animals.

To ensure the effect of high blood glucose more precisely, glucose was administered intravenously during the course of the *in situ* perfusion experiments, and time course observations on both of the volume of the perfusate and the drug absorption were conducted with control animal. Essentially similar results were obtained in all of these experiments and a typical result obtained when hypertonic perfusion solution was used was presented in Fig. 5. As illustrated in Fig. 5, the slope of the regression line became more steeper than that of the nontreatment and the rate of fluid disappearance from the perfusate in the first 30 minutes was 0.0666 ml/min and after the glucose administration the rate was 0.1086 ml/min. The same tendency was observed in the drug absorption. The drug in the perfusate was disappear-

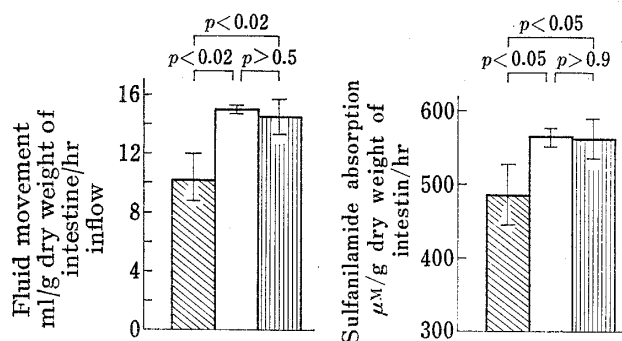


Fig. 4. Transmucosal Fluid Movement and Sulfanilamide Absorption per Unit Weight of Intestine during the Perfusion of Entire Small Intestine in Control and Diabetic Groups, and Group Administered Glucose Intravenously

Transmucosal fluid movement and sulfanilamide absorption are significantly greater in the diabetics and the group administered glucose than the controls. These absorptions did not differ between the diabetics and the group administered glucose. The data of the controls and the diabetics were quoted from our previous paper.⁴⁾

▨: control, □: diabetic, ▤: control+glucose *i.v.*
 ⊥: mean ± S.D.

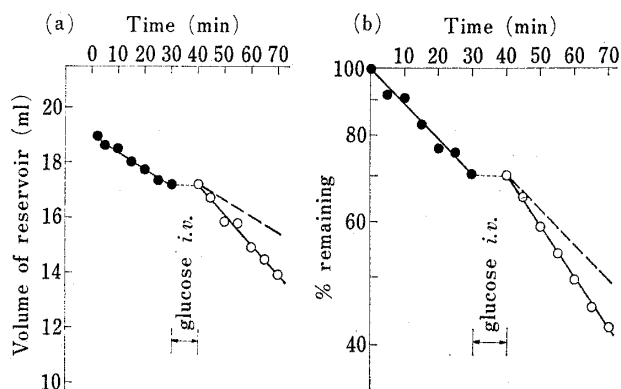


Fig. 5. The Time Course Observations of Fluid and Sulfanilamide Disappearances from Hypertonic Perfusate before and after Intravenous Administration of One Gram of D-Glucose with Control Rat

The broken lines were illustrated parallel to the regression lines of closed circles. The broken lines in the figures show data which might be obtained when D-glucose was not administered intravenously. The differences between the broken lines and the regression lines of open circles suggest the effect of glucose injection. Each solid line was obtained following the least squares method.

ed obeying the first-order kinetics in both before and after the glucose injection. The disappearance rate of the drug from the perfusate was 0.00499 min^{-1} in the first 30 minutes and the rate was 0.00745 min^{-1} in the second 30 minutes. This result suggests that the disappearance of the drug after the administration was increased 49.3% to that of the nonadministration.

Discussion

A good correlation between blood glucose and plasma osmolality was observed over a wide range of the blood glucose including both of the diabetic and the control animals. Moreover, a good correlation was also found between plasma glucose and plasma osmolality, and the plasma osmolality was increased with increasing the plasma glucose or the blood glucose (Fig. 1). These evidences would be rather reasonable, since glucose had been revealed as an active solute which could affect tonicities of solutions and, as a matter of fact, glucose was used as a solute in perfusion solutions in the series of this study.^{3,9)} However, the evidence obtained suggests that glucose acts osmotically active solute even in blood which is not so simple as the perfusion solutions. The increase in plasma osmolality due to the increment of plasma glucose from 100 mg/100 ml to 500 mg/100 ml might be corresponded to increasing theoretically in 22.2 mOsm/kg assuming that the plasma is an ideal glucose solution. On the other hand, the increase in plasma osmolality obtained experimentally following the regression equation ($y=11.72 \cdot x-3188.07$) between plasma glucose and plasma osmolality as illustrated in Fig. 1 was 34.1 mOsm/kg. Thus a difference between the increase in plasma osmolality calculated theoretically and that obtained experimentally was found without knowing.

McMillan¹⁰⁾ had reported that the increase of serum total protein level was observed in diabetic patients as well the increase of serum glucose level. Takahashi¹¹⁾ had reviewed

9) S. Kitazawa, H. Ito, and M. Iinuma, *Chem. Pharm. Bull.* (Tokyo), **23**, 2128 (1975).

10) D.E. McMillan, *J. Clin. Invest.*, **53**, 1071 (1974).

11) M. Takahashi, *Saishin Igaku*, **15**, 1988 (1960).

that extracellular osmolality in diabetes mellitus is increased due to the hyperglycemia and the ketosis. From these observations, although there are various factors affecting the plasma osmolality, evidences obtained in the present study disclosed that hyperglycemia might have a determinant role in increasing the plasma osmolality.

A good correlation was also observed between the blood glucose and the transmucosal fluid movement (Fig. 2), and this correlation was always obtained in all cases of the perfusion solutions having three different tonicities and respective three regression lines corresponded to the tonicity were to be parallel to each other. Moreover, there could not find out any difference in the correlation between the diabetic and the control groups was not disclosed, and all of the results including both of the groups formed one straight regression line. This suggests that the effect of blood glucose on the fluid movement should be continuous in a wide range of the blood glucose indifferent to the diabetics or the controls. These results present important evidences suggesting that glucose in plasma might be able to move the fluid transepithelially in the small intestine. In the series of the study of transmucosal fluid movement, authors' attention had been directed to tonicity of perfusion solution which was perfused in the small intestine and this tonicity had been considered to be sole cause of the transmucosal fluid movement. This mode of thinking was supported by many investigators. Curran and Solomon¹²⁾ had demonstrated that water was found to move freely in response to gradients of osmotic pressure in the ileum of rats and Vaughan¹³⁾ pointed out after skillful experiments using Thiry-Vella loops in the intestine of dog that water net transfer rate was inversely proportional to luminal osmolality and net absorption of water takes place at all luminal osmolalities up to 400 mOsm/l of the perfusion solution. Smyth and Taylor¹⁴⁾ also presented a similar phenomenon. Also, it had been reported that the fluid movement followed the osmotic gradient was occurred not only across the intestinal membrane, but also across the red cell membrane. Sidel and Solomon¹⁵⁾ measured the rate of water entrance into human red cells under an osmotic pressure gradient and confirmed that cell swelling was recognized when the cells were mixed with a hypotonic solution. Contrary to the cell swelling, cell shrinking was found as the cells were mixed with a hypertonic solution. Hence it is reasonable to understand that if an osmotic gradient between inside and outside across a membrane was caused by various factors, the fluid movement might always be provoked to supplement the gradient. However, the evidence obtained in the present study indicated that the cause of the transmucosal fluid movement would exist in the body, that is, blood glucose. Once Schneider and Schedl¹⁶⁾ had pointed out that serum osmolality was greater in diabetic rats than in control rats because of the hyperglycemia, and this osmotic gradient might cause fluid absorption. These evidences suggest that blood glucose played an important and decisive role in the transmucosal fluid movement. Hence the significant increase of the fluid inflow in the diabetic rats might not be caused directly by diabetes mellitus, but the increment of plasma osmolality due to the increase of glucose concentration in blood itself.

The absorption of sulfanilamide was increased with increasing blood glucose and a fair correlation between these was also observed (Table I). The increase of absorption of the drug in the diabetics was markedly depended on the increment of the transmucosal fluid inflow, since the least-squares lines representing the relation between the fluid movement and the drug absorption in both the control and the diabetic animals were overlapped each other as shown in our previous report.⁴⁾ This result demonstrates that the regulation of the blood glucose in the treatment of diabetes mellitus is an important factor from standpoint on the drug absorption.

- 12) P.F. Curran and A.K. Solomon, *J. Gen. Physiol.*, **41**, 143 (1957).
- 13) B.E. Vaughan, *Am. J. Physiol.*, **198**, 1235 (1960).
- 14) D.H. Smyth and C.B. Taylor, *J. Physiol.*, **136**, 632 (1957).
- 15) V.W. Sidel and A.K. Solomon, *J. Gen. Physiol.*, **41**, 243 (1957).
- 16) L.E. Schneider and H.P. Schedl, *Am. J. Physiol.*, **223**, 1319 (1972).

To examine further particularly these considerations, rats having the hyperglycemia, one of physiological characteristics in diabetic animals, were prepared by intravenous administration of D-glucose to the control rats. The transmucosal fluid inflow and the absorption of sulfanilamide in the glucose administered rats were significantly greater than in the control rats, but the significant differences were not found, compared with the diabetics (Fig. 3b). Each data obtained in the glucose injected rats was plotted on the least-squares line relating the transmucosal fluid movement to the absorption of the drug in both the control and the diabetic animals. Although duration of the hyperglycemia in the glucose treated rats was rather short, because glucose administered intravenously was disappeared immediately obeying to the first-order kinetics¹⁷⁾ (Fig. 3a), the blood glucose over 200 mg/100 ml lasted during a period of the perfusion experiment of one hour.

Moreover, the fluid and the drug absorptions were significantly greater in the diabetics and the glucose administered rats than in the controls on a dry-weight basis. However, these absorptions did not differ between the diabetics and the glucose administered animals (Fig. 4). The results obtained strongly suggest that the increase in the transmucosal fluid inflow and the drug absorption caused by the glucose injection was not depended on a factor such as intestinal growth^{4,18)} which was found in alloxan-induced diabetic animals.

These results were supported by the time course studies of the disappearances of fluid and sulfanilamide from the hypertonic perfusate before and after the intravenous administration of D-glucose (Fig. 5). The fluid and the drug disappearances were increased 63.1% and 49.3%, respectively after the glucose injection. If the glucose was not administered in the midst of the perfusion study, these data might be plotted along the broken lines which were drawn parallel to the regression lines of closed circles illustrated in Fig. 5a and b. Hence the differences between the broken lines and the regression lines of open circles suggest the effect of the treatment.

Effects of volume of intravenous injection on the absorption of sulfanilamide had been examined using normal rats in our laboratories¹⁹⁾ and the results obtained suggested that the drug absorption across the intestine was decreased with increasing the volume of injection. So glucose was injected after well-mixed with the blood which had been collected from the test animals. Since the difference between the diabetic model rats and the diabetic rats in both the fluid inflow and the drug absorption was not found, the characteristics on these absorptions across the intestinal membrane in the diabetic animals might be based on the increase of plasma osmolality because of the hyperglycemia.

From the results obtained in the present study and in our previous report,⁴⁾ it might be able to conclude that a possible mechanism on the increase of the drug absorption in the diabetics would be that the enhancement of plasma osmolality due to the hyperglycemia caused the increment of the transmucosal fluid inflow to compensate the abnormal high osmolality in blood and this inflow might lead the drug absorption increase consequently following to our proposed considerations.^{3,9)}

17) S.L. Hollobaugh, M. Bhaskar Rao, and F.A. Kruger, *Diabetes*, **19**, 45 (1970).

18) E.L. Jervis and R.J. Levin, *Nature*, **210**, 391 (1966); H.P. Schedl and H.D. Wilson, *Am. J. Physiol.*, **220**, 1939 (1971).

19) S. Kitazawa, H. Ito, and T. Takahashi, "in preparation."