

Effects of Insulin on Transmucosal Fluid Movement and Intestinal Drug Absorption in Alloxan Diabetic Rats

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The effects of insulin on the transmucosal fluid movement and the intestinal absorption of sulfisoxazole and metoclopramide in alloxan diabetic rats were studied with perfusion solution adjusted to isotonicity with sodium chloride using the *in situ* recirculating perfusion method. Insulin was intravenously administered to diabetic rats in a dose of 3 unit/kg of body weight and the perfusions were started at one and half an hour after the hormone administration.

When insulin was injected to the diabetics to decrease the high blood glucose, the transmucosal fluid inflow and the drug absorption were decreased along the corresponding regression line representing the relations between the blood glucose and the transmucosal fluid movement, and the drug absorption. The fluid inflow and the drug absorption from the entire small intestine and per unit dry weight of the intestine were always significantly less in the insulin received group than in the diabetic group.

Moreover, the effect of insulin on an actively transported sodium absorption was also studied to compare with that of the drugs. A similar observation was obtained in this case.

A possible mechanism of these decreased absorptions by insulin was discussed.

Keywords—alloxan diabetic rat; blood glucose; diabetes; metoclopramide; insulin; intestinal drug absorption; sulfisoxazole; transmucosal fluid movement; transmucosal sodium movement

It has been elucidated previously in our laboratory²⁾ that the transmucosal fluid inflow was always in greater extent in alloxan-induced diabetic rats than the matched non-treated control animals, and that volume of the fluid inflowed was increased with increasing in blood glucose, and that the increment in fluid inflow might be based on the increase in plasma osmolality due to hyperglycemia in diabetics. To support the evidence further in detail, D-glucose was administered intravenously to the control animals and the effects of such an artificial increment in blood glucose on the transmucosal fluid movement and drug absorption were studied.^{2b)} No significant difference was observed in both of the fluid movement and drug absorption between the diabetic group and the glucose administered group, however, a significant difference was found in those between the hexose received group and the non-treated control animals. These fluctuations in both of the transmucosal fluid movement and the drug absorption obtained from all of the animals formed a straight regression line in an illustration depicting the relationship of the transmucosal fluid movement and the drug absorption. From these evidences, it is proper to understand that the blood glucose played an important role affecting on the transmucosal fluid movement and on the drug absorption.

Insulin is well known as one of hypoglycemic agents and, in fact, is used widely in therapy of diabetes mellitus. Along with our expectations, the effects of insulin may not limit only to decrease the blood glucose, but may alter the transmucosal fluid movement and the drug absorption from the small intestine in diabetic animals.

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

2) a) S. Kitazawa and I. Johno, *Chem. Pharm. Bull.* (Tokyo), **24**, 2832 (1976); b) *Idem, ibid.*, **25**, 115 (1977); c) *Idem, ibid.*, **25**, 601 (1977).

In the present study the effects of insulin on the transmucosal fluid movement and the drug absorption were studied in alloxan-induced diabetic rats and, based on the evidences obtained in the course of the experiments, the drug therapy of diabetes mellitus under insulin therapy was discussed.

Experimental

Materials—Sulfisoxazole having pK_a value of 1.55 and 5.10³⁾ was expected to exist as an anionic compound in the physiological pH⁴⁾ of the rat small intestine. On the other hand, metoclopramide was a cationic substance considering from the pK_a value of 8.97.⁵⁾ These two compounds having reverse charge in the physiological pH were selected in the present study to compare with the results obtained previously^{2a)} with control and alloxan-induced diabetic animals.

Sulfisoxazole and insulin for injection (40 unit/ml, ISZILIN "Shimizu," Lot No. 225SB, manufactured by Shimizu Seiyaku Co., Ltd., Shimizu, Japan, and distributed by Takeda Chemicals Ind., Ltd., Osaka, Japan) were the J.P. VIII grade and obtained from commercial sources. All other chemicals used in the present study were reagent grade. All chemicals were used without further purification.

Animals and Perfusion Experiment—Wistar strain male albino rats weighing about 180 g were used. The animals were given food and water *ad libitum* until perfusion studies. Rats were made diabetic by injecting freshly prepared solution of alloxan monohydrate (Nakarai Chemicals, Ltd., Kyoto, Japan) intraperitoneally with a dose of 200 mg/kg of body weight. Control rats received intraperitoneally a matched volume of sterile water. The diabetic rats showed hyperglycemia (more than 300 mg/100 ml), decreased of body weight, glycosuria (Tes-Tape, Eli Lilly and Co., U.S.A.), and increase of food and water consumption as well as has been reported previously.^{2a)} In the control rats, blood glucose was less than 200 mg/100 ml and urine was negative to glycosuria. Alloxan received rats whose blood glucose was between 200 mg/100 ml and 300 mg/100 ml at the time of perfusion experiment were also employed similarly to the control and the diabetic animals. The animals were employed to the perfusion studies at seventh day after the injection.^{2a)}

Perfusion studies were conducted with the method of Schanker and his co-workers.⁶⁾ The rats were anesthetized with intraperitoneal injection of pentobarbital sodium (Nembutal, 40 mg/kg of body weight) and the abdomen was opened by a midline incision. Incisions were made in the proximal duodenum and the terminal ileum. The entire small intestine was rinsed clearly with physiological saline solution, which had been maintained at 37°, through the proximal incision. Both of the ends were cannulated with silicon tubings. The pylorus, bile duct, and ileo-cecal junction were tied with carefulness to avoid any inflow of fluid into the lumen during the perfusion experiments. Forty milliliters of perfusion solution were infused into the duodenum and recirculated at a rate of 5 ml/min for one hour. At the end of the perfusion, the intestine was emptied and the animal was sacrificed by bleeding. The entire small intestine was stripped gently from the mesentery and immediately blotted with filter paper. The wetting intestine was dried at 100° over a period of 24 hours⁷⁾ and the dry weight of the intestine was obtained.

The intestinal absorption data in control and diabetic animals were usually compared by means of per unit dry weight of the intestine, since the intestinal growth rate and tissue water content were greater in diabetic animals than in control animals.^{7,8)} Hence the data obtained in the present study were represented on the basis of dry weight of the intestine.

Perfusion Solution—The tonicity of perfusion solution was adjusted to isosmotic with sodium chloride. The drug concentration in the perfusion solution was set to 1 mM. Isotonic sodium chloride solution containing sodium of 154 mEq/liter, which did not contain any medicament, was used as a perfusion solution to examine transmucosal sodium movement.

Determination of Dose of Insulin—Before insulin administration, it was verified that the blood glucose in alloxan received animals was more than 300 mg/100 ml. Insulin solution was intravenously administered to the diabetic rats with a microsyringe to decrease the blood glucose down to the same level as that in control rats. Three different doses, such as 1, 2, and 3 unit/kg of body weight, were examined and the results obtained were illustrated in Fig. 1. As was evident from Fig. 1, the effects of insulin on the blood glucose were dose dependent, *i.e.*, the blood glucose in the diabetics was decreased with increasing the dose of insulin. The maximum effect of insulin on the blood glucose was found at 1.5—2.0 hours after the administration of the hormone in all cases. The value of blood glucose, however, varied widely in the cases of 1 and 2 unit/kg. When insulin was administered in a dose of 3 unit/kg, the variance of decreased blood glucose was the least

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

5) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 1856 (1975).

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

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and the initial high blood glucose in the insulin received group was decreased down to the same level as that of control rats. In addition, the minimum value of blood glucose continued over more than one hour. Based on these evidences, the dose of insulin was determined to 3 unit/kg and perfusion experiment was initiated at one and half an hour after the administration.

Analyses—Blood Glucose: Blood for glucose analysis was collected from the femoral artery cannulating polyethylene tubing. Initial blood glucose was obtained prior to insulin injection. After intravenous administration of insulin, blood was sampled at just before, middle (30 min), and final of the perfusion. The average of these three determinations was treated as blood glucose during the perfusion.

Blood collected for glucose determination was analyzed chemically with the modified *o*-aminodiphenylborate method devised by Sasaki and his co-workers.⁹⁾ The processes of the method were mentioned previously^{2a)} in detail.

Drugs: The drugs used in the present study had an aromatic amino group, so the drug in the perfusate was diazotized with the regular manner¹⁰⁾ and coupled with 2-diethylaminoethyl-1-naphthylamine (Tsuda's reagent). Developed color was determined spectrophotometrically at a wave length of 550 nm with Hitachi spectrophotometer model 124.

Sodium: Sodium concentration in the perfusate was determined using a Hitachi flame photometer model 205.

Plasma Osmolality: At the end of the perfusion study, blood was collected in a heparinized test tube from the femoral artery. After a centrifugation, plasma osmolality was measured with Advanced osmometer model 3D.

Calculations—The drug and sodium absorptions were calculated as follows:

$$\% \text{ of drug absorbed} = 100 - 100[C_f/C_i \times \{40 - (V_i - V_f)\} / 40]$$

$$\text{mEq of sodium absorbed} = 40 \times (Na_i, \text{ mEq/ml}) - \{40 - (V_i - V_f)\} \times (Na_f, \text{ mEq/ml})$$

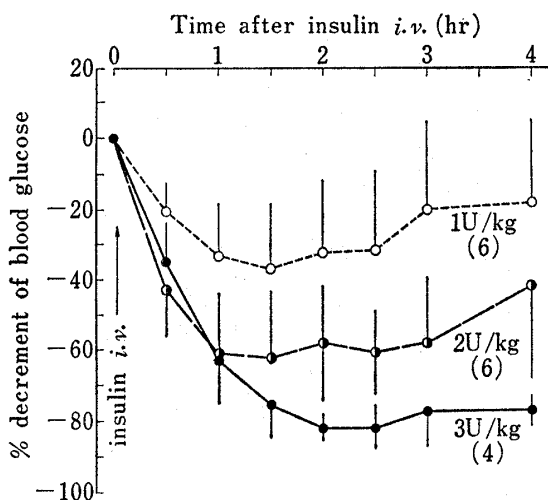


Fig. 1. Time Course Observations of Percent Decrement of Blood Glucose after Intravenous Administration of Insulin

Three different doses such as 1, 2, and 3 unit/kg of body weight of animals were examined. Numbers in parentheses indicate number of experiments. Vertical lines indicate S.D.

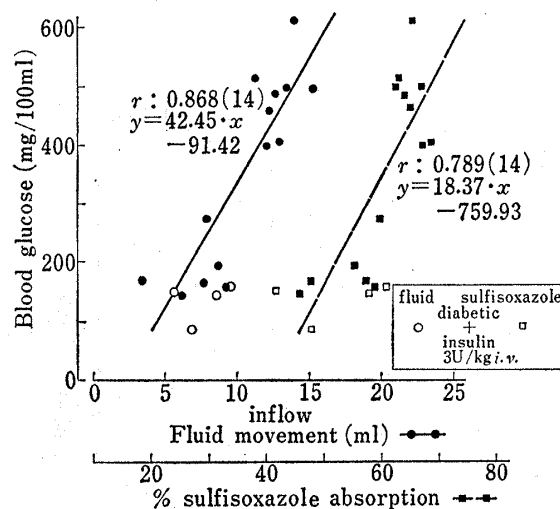


Fig. 2. Effects of Insulin on the Relations Between Blood Glucose and Transmucosal Fluid Movement, and Sulfisoxazole Absorption

The perfusion experiments were started at 1.5 hours after the administration of insulin. The entire small intestine was perfused intraluminally the perfusion solution, whose tonicity was adjusted to the isotonicity with sodium chloride, for one hour. Inflow means apparent fluid flow into the animal during the perfusion. The data, which were illustrated with closed circle, representing the relation between blood glucose and transmucosal fluid movement were quoted from our previous report.^{2a)} The regression equations were obtained from the corresponding closed scattered plots, respectively. Numbers in parentheses indicate number of experiments.

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The subscripts i and f mean the initial and the final of each value. C and Na are the drug and sodium concentrations in the perfusate, respectively. V is the volume of perfusate in the volumetric cylinder used as a reservoir of the perfusate.

Differences between groups were compared using the t test and p values of less than 0.05 were considered to be significant.

Results

The relations between the blood glucose and the transmucosal fluid movement, and the intestinal absorption of sulfisoxazole from both of the alloxan received and the control rats using an isotonic perfusion solution were shown in Fig. 2. As has been reported previously,^{2c)} a good positive correlation between the blood glucose and the transmucosal fluid movement was found over a wide range of blood glucose and the regression equation obtained from plots illustrated with closed circle in Fig. 2 was $y=42.45x-91.42$ ($n: 14, r: 0.868$). As the results of intravenous administration of given amount of insulin to the diabetic group of rats, the blood glucose was eventually decreased as was expected. The decrease in blood glucose apparently accompanied the decrease in the transmucosal fluid inflow and, as the results of these decreasing in both of the indices, the plots appeared just around those of the control rats which were plotted at the lower parts of the regression line.

Similarly, a good correlation between the blood glucose and the drug absorption was obtained and the regression equation obtained from plots shown with closed square was $y=18.37x-759.93$ ($n: 14, r: 0.789$). When the blood glucose in the diabetic animals was

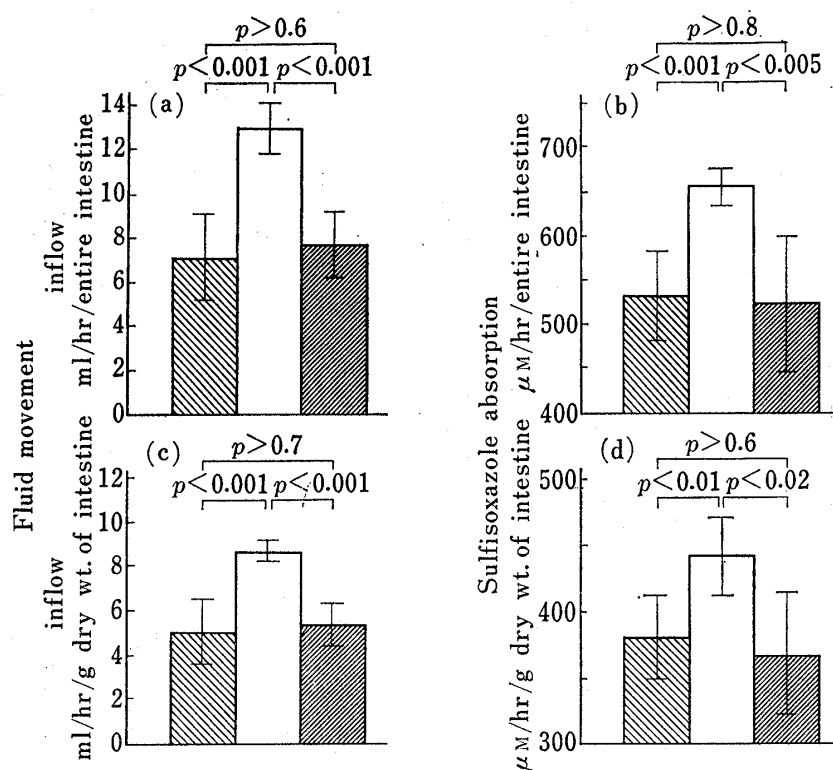


Fig. 3. Transmucosal Fluid Movement and Sulfisoxazole Absorption from the Entire Small Intestine (a and b) and per Unit Weight of the Intestine During the Perfusion (c and d): Comparison of Animals Made Diabetic with Alloxan with Controls, or Insulin Received Animals

The initial drug concentration in the perfusion solution was 1 mM. The tonicity of the perfusion solution was adjusted to the isotonicity with sodium chloride. The data obtained with control and diabetic animals were quoted from our previous report.^{2c)}

▨: control □: diabetic ▩: diabetic+insulin ┆: mean ± S.D.

decreased by insulin down to the same level as that of the controls, the drug absorption was also decreased, compared with that which would be expected from the initial high blood glucose, and the results obtained, which were illustrated with open square, were plotted on the regression line.

The effects of insulin on the transmucosal fluid inflow and the drug absorption by the entire small intestine and per unit weight of the intestine during the perfusion were illustrated in Fig. 3. The transmucosal fluid inflow from the entire small intestine was significantly greater ($p < 0.001$) in the diabetic rats than in the control rats and the insulin received diabetic rats as shown in Fig. 3-a. Sulfisoxazole absorption through the entire small intestine was also significantly greater ($p < 0.005$) in the diabetics than in the controls and the insulin received diabetic animals (Fig. 3-b).

The data from the perfusion of the entire small intestine were expressed per unit dry weight of the intestine (Fig. 3-c and d). The transmucosal fluid inflow and the absorption of the drug were significantly greater ($p < 0.001$ and $p < 0.02$) in the diabetic group than in the other two groups, respectively.

The untoward effects of insulin are principally the hypoglycemia and several symptoms such as increased sympathetic activity and muscular twitching may induce,¹¹⁾ so it is proper to understand that the administration of the hormone to the control group might lead to undesirable behavior. In practice, the severe case at intravenous administration of 3 unit/kg of the hormone resulted in the death of the animal in the present study. From these considerations, the absorption data obtained with insulin administered control rats were not shown in this report.

Figure 4 shows the effects of insulin on the relations between the blood glucose and the transmucosal fluid movement, and metoclopramide absorption in the alloxan received and the control animals with isotonic sodium chloride medium. A good correlation between the blood glucose and the transmucosal fluid movement was obtained as has been mentioned previously^{2c)} and the regression equation was $y = 42.36x - 190.64$ ($n: 20, r: 0.886$). Moreover, a good positive correlation between the blood glucose and the drug absorption was found and the regression equation was $y = 15.78x - 436.49$ ($n: 20, r: 0.839$). The transmucosal fluid inflow and the absorption of the drug in the insulin administered group were decreased compared to those which would be expected from the initial high blood glucose. The results obtained, which were illustrated with open circle and square in Fig. 4, were plotted on the corresponding regression line.

The effects of insulin on the transmucosal fluid movement and the drug absorption from the entire small intestine and per unit weight of the intestine during the perfusion were shown

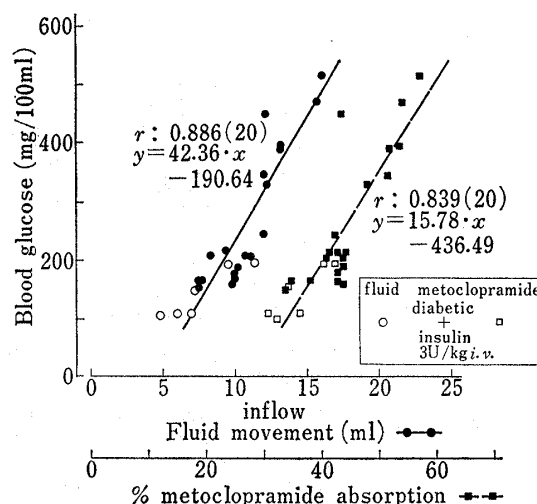


Fig. 4. Effects of Insulin on the Relations Between Blood Glucose and Transmucosal Fluid Movement, and Metoclopramide Absorption

The perfusion experiments were started at 1.5 hours after the administration of insulin. The entire small intestine was perfused intraluminally the perfusion solution, whose tonicity was adjusted to the isotonicity with sodium chloride, for one hour. The data, which were illustrated with closed circle, representing the relation between blood glucose and transmucosal fluid movement were quoted from our previous report.^{2c)} The regression equations were obtained from the corresponding closed scattered plots, respectively. Numbers of parentheses indicate number of experiments.

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in Fig. 5. The transmucosal fluid inflow and the drug absorption from the entire small intestine were significantly greater ($p < 0.001$) in the diabetic animals than in the control and

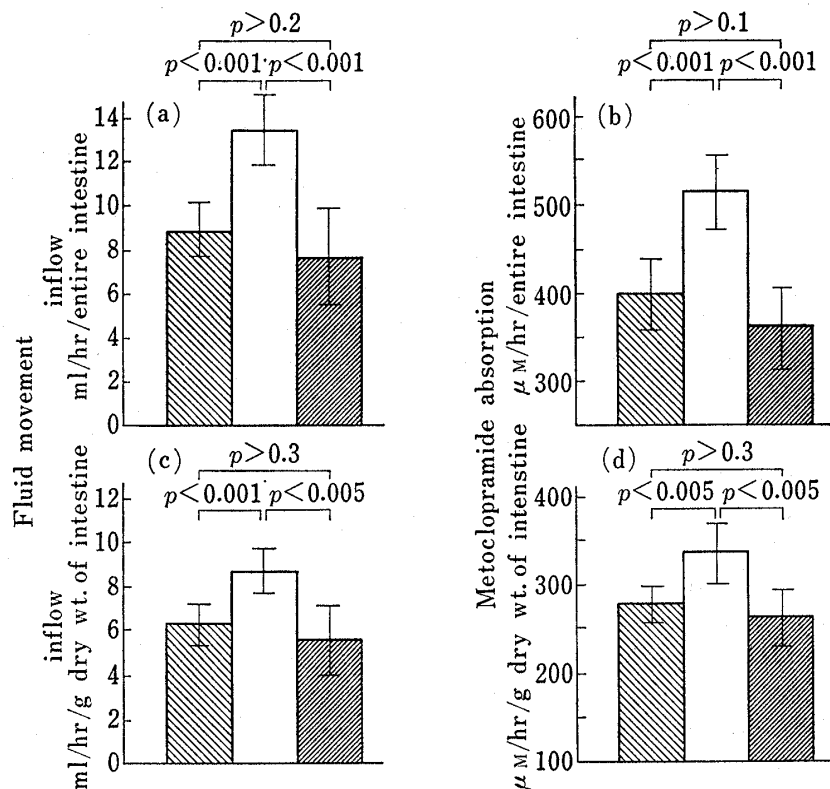


Fig. 5. Transmucosal Fluid Movement and Metoclopramide Absorption from the Entire Small Intestine (a and b) and per Unit Weight of the Intestine During the Perfusion (c and d): Comparison of Animals Made Diabetic with Alloxan with Controls, or Insulin Received Animals

The initial drug concentration in the perfusion solution was 1 mM. The tonicity of the perfusion solution was adjusted to the isotonicity with sodium chloride. The data obtained with control and diabetic animals were quoted from our previous report.^{2c)}
 ▨: control □: diabetic ▩: diabetic+insulin ⊥: mean S.D.

TABLE I. Effects of Insulin on Transmucosal Fluid Movement and Drug Absorption from the Entire Small Intestine

Drug	n ^{a)}	Initial blood glucose	Blood glucose during the perfusion	Fluid movement (ml)			Drug absorption (%)		
				A	B	(B-A)	C	D	(D-C)
Sulfisoxazole	4	446.3 ± 85.8	136.2 ± 29.1	7.8 ± 1.5	12.7 ± 2.0	4.9	52.21 ± 7.79	65.67 ± 4.67	13.46
		383.6 ± 94.2	143.9 ± 40.5	7.7 ± 2.2	13.6 ± 2.2	5.9	36.10 ± 4.36	51.97 ± 5.97	15.87

Entries represented mean ± S.D.

The perfusion was followed for one hour using the entire small intestine.

A, C = experimental data

B = Data which were calculated from the regression equations ($y = 42.45x - 91.42$ for sulfisoxazole; $y = 42.36x - 190.64$ for metoclopramide) between blood glucose on the vertical axis and transmucosal fluid movement on the horizontal axis.

D = Data which were calculated from the regression equations ($y = 13.37x - 759.93$ for sulfisoxazole; $y = 15.78x - 436.49$ for metoclopramide) between blood glucose on the vertical axis and drug absorption on the horizontal axis.

Columns of (B-A) and (D-C) mean the effects of insulin on the transmucosal fluid movement and the drug absorption, respectively.

a) number of experiments

the insulin received diabetic animals (Fig. 5-a and b). The fluid inflow and the drug absorption on the basis of a dry-weight of the intestine were also significantly greater ($p < 0.005$) in the diabetic group than in the other two groups (Fig. 5-c and d).

To explain more in detail the effects of insulin on the transmucosal fluid movement and the drug absorption, Table I was presented. Mean initial blood glucose prior to the administration of insulin was 446.3 mg/100 ml for sulfisoxazole and 383.6 mg/100 ml for metoclopramide. After the intravenous administration of insulin, the blood glucose was decreased and mean blood glucose during the perfusion was 136.2 mg/100 ml for sulfisoxazole and 143.9 mg/100 ml for metoclopramide. The transmucosal fluid inflow which would be expected from the initial blood glucose was theoretically calculated with the regression equation between blood glucose and transmucosal fluid movement as mentioned above. The mean of results calculated were 12.7 ml for sulfisoxazole and 13.6 ml for metoclopramide, respectively. In the case of sulfisoxazole, for example, the value obtained theoretically, 12.7 ml, minus the value obtained experimentally, 7.8 ml, is equal to insulin effect, 4.9 ml. Namely, it means that the suppression of fluid inflow caused by the administration of insulin was 4.9 ml.

The absorption of the drugs which would be expected from the initial blood glucose was theoretically calculated with the regression equation obtained from scattered plots of blood glucose *versus* drug absorption. The results were represented in column D in Table I. The decrement in the drug absorption due to insulin injection was 13.46 % for sulfisoxazole and 15.87 % for metoclopramide as indicated in column (D—C) in Table I.

Moreover, the effect of insulin on the transport of sodium, one of inorganic and actively transported substances, was examined in the present study to compare with that on the absorption of the drugs as mentioned above. As was evident from Fig. 6, a good positive correlation between the blood glucose and the transmucosal fluid movement in the control and the diabetic animals was also found and the regression equation was $y = 42.05x - 118.32$ ($n: 10, r: 0.992$). Sodium absorption was increased with increasing blood glucose and a good correlation between these indices was obtained. The regression equation was $y = 363.11x - 278.28$ ($n: 10, r: 0.981$).

The effects of insulin on the relations between the blood glucose and the transmucosal fluid movement, and the transmucosal sodium movement were illustrated in Fig. 6. As the results of insulin administration to the diabetics, the transmucosal fluid inflow and sodium absorption in the insulin received group were similar to those in the control group. The results obtained were plotted on the corresponding regression line, *i.e.*, the fluid inflow and sodium absorption in the insulin received diabetic animals were decreased along the line.

The effects of insulin on the transmucosal fluid inflow and sodium absorption from the entire small intestine and per unit weight of the intestine during the perfusion were depicted in Fig. 7. The fluid inflow and sodium absorption were always significantly greater ($p < 0.001$) in the diabetic group than in the other two groups.

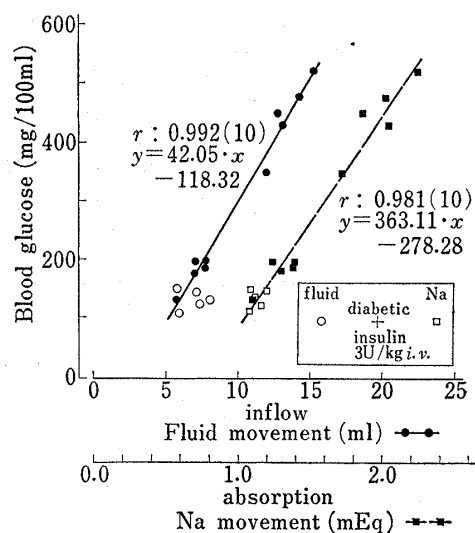


Fig. 6. Effects of Insulin on the Relations Between Blood Glucose and Transmucosal Fluid Movement, and Transmucosal Sodium Movement

The perfusion experiments were started at 1.5 hours after the administration of insulin. The entire small intestine was perfused intraluminally the isotonic sodium chloride solution which did not contain any drug. The regression equations were obtained from the corresponding closed scattered plots, respectively. Numbers in parentheses indicate number of experiments.

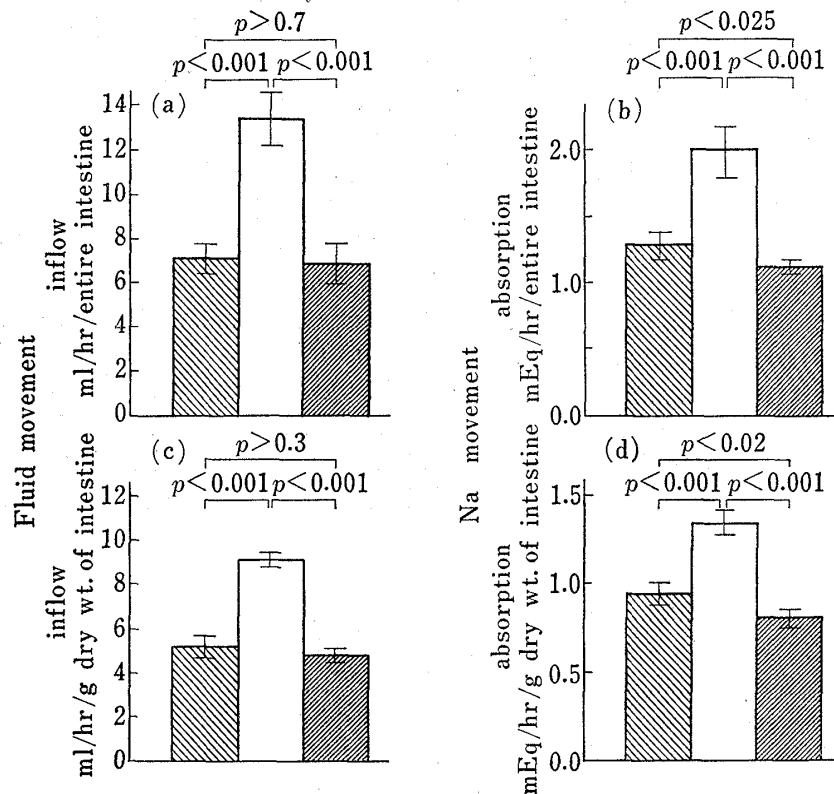


Fig. 7. Transmucosal Fluid Movement and Transmucosal Sodium Movement from the Entire Small Intestine (a and b) and per Unit Weight of the Intestine During the Perfusion (c and d): Comparison of Animals Made Diabetic with Alloxan with Controls, or Insulin Received Animals

The initial sodium concentration in the perfusion solution was about 154 mEq/l. The tonicity of the perfusion solutions was adjusted to isotonic with sodium chloride.
 ▨: control □: diabetic ▩: diabetic+insulin I: mean ± S.D.

Discussion

In our previous reports,^{2b,c)} a good positive correlation between the blood glucose and the transmucosal fluid movement in alloxan received and control rats was found over a wide range of the blood glucose. When insulin, one of hypoglycemic agents, was administered to diabetic rats to decrease the abnormal high blood glucose, the transmucosal fluid movement might be expected to be changed from that before the hormone treatment. Just as our expectation, the transmucosal fluid inflow in the insulin received animals was decreased, compared with that which would be expected from the initial high blood glucose. The data obtained were always plotted on the corresponding regression line representing the relation between the blood glucose on the vertical axis and the transmucosal fluid movement on the horizontal axis (Fig. 2, 4, and 6). The transmucosal fluid inflow from the entire small intestine and per unit dry weight of the intestine during the perfusion was always significantly less in the insulin received group than in the diabetic group, although a significant difference between the insulin received group and the control group was not found (Fig. 3, 5, and 7). Moreover, the blood glucose and plasma osmolality in the insulin received group were measured at the end of the perfusion studies. Insulin decreased simultaneously both the blood glucose and the plasma osmolality in the diabetic animals down to the same level as those in the control animals. The results obtained were plotted on the regression line, which has been obtained previously,^{2b)} representing the relation between blood glucose

on the vertical axis and plasma osmolality on the horizontal axis, although the data were not depicted in the present study. These evidences strongly suggest that the decrease in the transmucosal fluid inflow in the insulin received group might be caused by the decrease in plasma osmolality due to the decrement in blood glucose.

Aulsebrook¹²⁾ had demonstrated that fluid absorption in everted gut sacs was significantly less in diabetic rats given insulin prior to sacrifice than in diabetic animals. This evidence suggests that the decreased fluid absorption in diabetics given insulin is an effect of the hormone. On the other hand, as the results of insulin injection to diabetic rats using an *in situ* technique as shown in the present study, the blood glucose was decreased necessarily and the transmucosal fluid inflow was decreased concurrently. So it was not clear whether the decreased fluid inflow in insulin received group was caused directly by insulin or not. Hence it is proper to understand that his *in vitro* findings might not be applicable immediately into our considerations.

Levinson and Englert¹³⁾ pointed out using an *in situ* method that water absorption was significantly greater in diabetic rats than in control animals. However, they did not demonstrate clearly the mechanism of increased water absorption in the diabetics.

In our previous report,^{2b)} it has been postulated that the increased fluid inflow in diabetic rats might be caused by the increase in plasma osmolality due to the hyperglycemia. A similar observation has been reported by Schneider and Schedl.¹⁴⁾ On the other hand, Aulsebrook¹²⁾ found with *in vitro* method that water absorption was significantly greater with diabetic animals, nevertheless the same buffer solution was used for both serosal and mucosal fluids. If the transmucosal fluid inflow in diabetic animals is fairly affected by factors other than the hyperglycemia, the increased fluid inflow should be hardly decreased by insulin. However, a significant difference between diabetic group and insulin received group on the transmucosal fluid inflow was found in all cases as shown in Fig. 3, 5, and 7. These findings led to the conclusion that blood glucose plays an important and decisive role on the transmucosal fluid movement.

Insulin has various pharmacological effects. Several investigators¹⁵⁾ have pointed out that insulin facilitates the entry of glucose inside the cells. The hormone, moreover, has accelerated the transport of amino acid from the extracellular fluid to the intracellular fluid.¹⁶⁾ On the basis of these findings, studies concerning the effect of insulin on the intestinal absorption of hexose and amino acid have been accumulated.

Beyreiss and his co-workers¹⁷⁾ suggested with an *in vivo* method that the administration of insulin decreased galactose absorption from the intestine of normal rabbit. On the contrary, Manome and Kuriaki¹⁸⁾ reported that insulin increases glucose absorption *in vivo* in normal rats. In addition to these conflicting results, a decrease in glucose absorption *in vivo* after the administration of insulin in diabetic rats was found by Lastz and Vogel.¹⁹⁾ On the other hand, Vinnik and others²⁰⁾ suggested that glucose absorption from the upper small intestine in juvenile diabetic patients was not altered by the intravenous administration of insulin. The findings of Vinnik and others²⁰⁾ were supported by *in vitro* studies of Crane²¹⁾ for galactose and Aulsebrook¹²⁾ for glucose. However, the effect of insulin on the

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intestinal absorption of substances other than actively transported compounds has not been accumulated.

The effect of insulin on the intestinal absorption of sulfisoxazole and metoclopramide was examined in the present study. When insulin of 3 unit/kg of body weight was intravenously administered to diabetic rats to decrease the blood glucose down to the same level as that of control rats, the absorption of both of the drugs in the insulin received group was decreased to the same level as that of the controls (Fig. 2 and 4). The drug absorption by the entire small intestine and per unit dry weight of the intestine during the perfusion was always significantly greater in the diabetic group than in the other two groups (Fig. 3 and 5). Furthermore, the transmucosal fluid inflow and the drug absorption in the insulin received group were similar to those in the control group. In fact, the results obtained from the insulin received group were plotted on the corresponding regression line, which was obtained with perfusion solution having three different tonicities adjusted with sodium chloride in control animals as mentioned previously,²⁰⁾ representing the relation between transmucosal fluid movement on the vertical axis and drug absorption on the horizontal axis, although the data were not depicted in the present study. Namely, the drug absorption in the insulin received group was decreased only the decreased portion of transmucosal fluid inflow along the regression line obtained with the controls. From these lines of evidences, it is able to conclude that the decreased drug absorption in the insulin received group might be caused by the decrease in transmucosal fluid inflow due to the decrement in plasma osmolality as mentioned above.

To support these findings further in detail, the dose of insulin was decreased to 1 or 2 unit/kg of body weight and the effects of decreased blood glucose on the transmucosal fluid movement and the drug absorption were examined. The transmucosal fluid inflow and the drug absorption in insulin injected group were decreased only the decreased portion of the blood glucose along the corresponding regression line mentioned above.

Beyreiss and his co-workers¹⁷⁾ studied the effect of insulin on the absorption of galactose with normal rabbits. They found that decreased absorption of the hexose by the intravenous administration of insulin is not caused by hypoglycemia, because additional intravenous infusion of glucose does not influence the effect of insulin. However, the decrease in blood glucose by insulin was a little compared to that obtained in the present study, since normal animals were used in their experimentations, and moreover, they did not suggest the effect of insulin on transmucosal fluid movement. Vinnik and others²⁰⁾ reported that insulin did not alter glucose absorption in normal and diabetic patients, although it always depressed the blood glucose. They, however, did not discuss the effect of insulin on transmucosal fluid movement. Their findings obtained with glucose were in disagreement with our findings obtained with drugs. These evidences suggest that there might be essentially a difference between actively transported sugar and the drugs used in the present study on the intestinal absorption.

Thereupon, sodium, one of actively transported substances, was introduced in the present study and the effect of insulin on the transmucosal sodium movement was examined. A good positive correlation between the transmucosal fluid movement on the vertical axis and the transmucosal sodium movement on the horizontal axis in both the control and the diabetic rats was found and the regression equation was $y=8.63x-3.80$ ($n: 10, r: 0.988$). Fordtran and others²²⁾ demonstrated that sodium absorption was increased with increasing fluid absorption in man. A similar phenomenon was found by Heaton and Code in dogs.²³⁾ Levinson and Englert¹³⁾ suggested using the *in situ* loop method that water and sodium absorptions were significantly greater in diabetic rats than in control rats and this increased

22) J.S. Fordtran, F.C. Rector, Jr., and N.W. Carter, *J. Clin. Invest.*, **47**, 884 (1968).

23) J.W. Heaton, Jr. and C.F. Code, *Am. J. Physiol.*, **216**, 749 (1969).

sodium absorption may be due in part to physical factors such as solvent drag. Hence it is proper to understand that the increased sodium absorption in the diabetics might be caused by the increase in the transmucosal fluid inflow and it follows perhaps solvent drag.²⁴⁾

Good correlations between the blood glucose and the transmucosal fluid movement, and the transmucosal sodium movement in the control and the diabetic animals were found as shown in Fig. 6. The transmucosal fluid inflow and sodium absorption in the insulin received group were decreased compared to those which would be expected from the initial high blood glucose and the results obtained were plotted on the corresponding regression line. As well as the drug absorption mentioned above, sodium absorption from the entire small intestine and per unit dry weight of the intestine during the perfusion was always significantly greater in the diabetic group than in the other two groups (Fig. 7). These evidences suggested that the effect of insulin on sodium absorption was similar to that on drug absorption.

The sodium absorption was also significantly greater in the control rats than in the insulin received group as illustrated in Fig. 7. This result might be due to the overdecrease of the blood glucose by insulin.

These evidences obtained in the present study and in our previous reports²⁾ led to the conclusion that the increased transmucosal fluid inflow in diabetic rats caused by the increase in plasma osmolality due to the hyperglycemia lead to the increment in the absorption of drugs, which are believed to be passively transported, and sodium, one of actively transported substances. These enhanced absorptions in diabetic animals might be decreased by insulin of 3 unit/kg of body weight down to the same level as those in control animals.

It is well known that the regulation of blood glucose by insulin in diabetes mellitus, especially in a serious case, is difficult,²⁵⁾ and that diabetes mellitus often lead to several complications, so insulin is frequently administered with oral administered drugs. The variation of blood glucose during insulin therapy might change the absorption of drugs according to the change of transmucosal fluid movement. Hence it is proper to understand that special attentions in dose of such drugs as oral administered for the therapy of complications in diabetes mellitus might be necessary and the regulation of blood glucose during insulin therapy in diabetes mellitus might be an important factor from the standpoint of the intestinal absorption of drugs.

24) H.H. Ussing, *Physiol. Rev.*, **29**, 127 (1949); V. Koefoed-Johnsen and H.H. Ussing, *Acta Physiol. Scand.*, **28**, 60 (1953).

25) S. Nakagawa, *Naiha*, **32**, 643 (1973).