Short-term Treatment of Alloxan-Diabetic Rats with Intrajejunal Administration of Water-in-Oil-in-Water Insulin Emulsions

Motoaki Shichiri, M.D., Ryuzo Kawamori, M.D., Michio Yoshida, M.D., Nobuaki Etani, M.D., Mitsuru Hoshi, M.D., Kanji Izumi, M.D., Yukio Shigeta, M.D., and Hiroshi Abe, M.D., Osaka, Japan*

SUMMARY

Alloxan-diabetic rats with fasting blood glucose levels above 300 mg./100 ml. were treated with intrajejunal administration of water-in-oil-in-water (W/O/W) insulin emulsions via an indwelling catheter at a dose of either 25 or 50 U./100 gm. body weight, three times daily for five to fourteen days. The course of diabetes was followed by determinations of glucose levels in blood and urine.

During treatment a significant reduction in urinary glucose levels was observed in all rats studied. In two rats treated with 25 U./100 gm., fasting blood glucose levels did not change significantly. In four of five rats treated with 50 U./100 gm., W/O/W insulin emulsions significantly lessened hyperglycemia during treatment, when compared with the glucose levels before and after treatment. Quantitative estimates suggested that the effectiveness of 50 U./100 gm. of intrajejunal W/O/W insulin emulsions was comparable to that after intramuscular regular insulin at doses between 1 and 2 U./100 gm.

These results would indicate that diabetes can be controlled by enteral administration of insulin preparations. DIABETES *24*:971-76, November, 1975.

Lasch et al.[1,2] were the first to demonstrate improvement of diabetes after oral administration of insulin to diabetic patients. In their experiments, insulin was combined with dyes as a means of protecting insulin molecules from digestive destruction. The results obtained from Murlin et al.[3] paralleled those of Lasch et al.,[1,2] except for the magnitude of the effects. To the best of our knowledge, there have been no other reports in the literature concerning diabetes control by oral administration of insulin, though several investigations have demonstrated that insulin can be absorbed from the intestine of mammals in a physiologically active form.[4-9]

During recent years, studies from our laboratory[10-11] have focused attention on the possibility that diabetes can be controlled by orally administered insulin. Water-in-oil-in-water (W/O/W) insulin emulsions might be considered to exert insulin action by facilitating gastrointestinal absorption and by protecting the insulin molecule from digestive destruction.[12,13]

To determine the potential effectiveness of W/O/W insulin emulsions as an oral insulin preparation, this preparation was given daily via an indwelling catheter to alloxan-diabetic rats in order to control the diabetes. A clear reduction in urinary glucose levels has been demonstrated in alloxan-diabetic rats that received W/O/W insulin emulsions intrajejunally at a dose of either 25 or 50 U./100 gm. body weight three times daily for as long as fourteen days.

MATERIALS AND METHODS

Preparation of Water-In-Oil-In-Water (W/O/W) Insulin Emulsions

The method for preparing W/O/W insulin emulsions reported by Engel et al.[13] was modified as follows in order to make emulsions with a higher insulin concentration: Insulin solution (26.4 U. per milligram, bovine crystalline insulin, Sigma Chemical), 1,000 U. per milliliter, was made in 0.003 M $ZnCl_2$ at pH 2.2. The oil phase was 0.03 M palmitic acid in octyl-decyl triglyceride (Nisshin Seiyu Co., Japan). The oil phase (12 ml.) was placed in a beaker, sonication was begun, and the insulin solution (8 ml.) was allowed to drain from a pipette into the beaker. Soni-

From the First Department of Medicine, Osaka University Medical School, Osaka, Japan.

*Present address: Chief, Endocrinological Section, Department of Medicine, National Osaka Hospital. Osaka, Japan.

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cation was continued for about twenty seconds with a sonifier (model USV-3000 V, 22 KHz). This resulted in a water-in-oil (W/O) insulin emulsion. Twenty milliliters of the resulting W/O emulsion was added to a second aqueous phase (60 ml.) containing 1 per cent sodium lauryl sulfate (Nikko Chemical, Japan). Resonication was carried out for twenty seconds. The resulting emulsions (W/O/W insulin emulsions) were adjusted to pH 6.5 with dilute NaOH and stored at 4° C. for up to one month. The W/O/W insulin emulsions thus prepared contained insulin at a concentration of 100 U. per milliliter emulsion. In a preliminary experiment, no demonstrable loss of insulin activity was observed after sonication of insulin solution. Insulin-free emulsions were prepared as a control.

The diameters of the W/O droplets in water were in the range of 0.6 to 2.0 μm. The incubation study of W/O/W insulin emulsions with pepsin, trypsin, and α-chymotrypsin in vitro showed that W/O/W insulin emulsions were quite resistant to the action of these enzymes. In the presence of pancreatic lipase, however, W/O/W insulin emulsions gradually lost their activity by the action of proteolytic enzymes, so that about 33 per cent of insulin activity was retained after a three-hour incubation in vitro.[12]

Preparation of the Indwelling Catheter

Male albino rats, Wistar strain, weighing from 200 to 250 gm., were used. The rats were deprived of food for twenty-four hours before operation. A midline abdominal incision was made under sodium pentobarbital anesthesia, and an area of the prepyloric portion of the stomach was pierced with a 12-gauge needle. A polyethylene catheter with an internal diameter of 1.0 mm. was inserted through this puncture into the intestine 8 to 10 cm. below the pyloric ring, so that the tip of the catheter was lying in the lumen of the upper jejunum. A purse-string suture was placed through the muscular layers around and adjacent to the entrance site of the tubing. The catheter was anchored to the abdominal wall, and the abdominal and skin incisions were closed with a mattress suture. Finally, the flange of the catheter was fixed on the abdominal wall.

After seven to ten days' recovery, alloxan was injected intravenously at a dose of 4.5 mg./100 gm. One month after alloxan injection, fasting blood glucose values were obtained for four to five consecutive days. The rats were randomly selected if the mean pretreatment blood glucose was greater than 300 mg./100 ml. Alloxan-diabetic rats were then treated with either W/O/W insulin emulsions or W/O/W emulsions as a control by intrajejunal instillation. During the experimental periods, each rat was maintained on constant food intake varying from 15 to 30 gm. of laboratory chow per day (Oriental Co., Japan, 1 gm. = 3.7 cals.) only during the light period (between 9 a.m. and 5 p.m.). The rats had free access to water.

The course of diabetes was followed by determinations of the fasting blood glucose level and the amount of urinary glucose excreted during twenty-four hours, and the change in body weight. In a few rats, diurnal changes in blood glucose levels were also studied by obtaining blood samples every two hours. In an attempt to quantitate the effectiveness of intrajejunal W/O/W insulin emulsions, changes in blood and urinary glucose levels were compared with those after intramuscular injection of regular insulin. Blood and urinary glucose were determined by the method of Somogyi-Nelson.[14]

RESULTS

Diurnal Change in Blood Glucose

The effects of intrajejunal administration of W/O/W insulin emulsions on diurnal changes in blood glucose levels are shown in figure 1. In alloxan-diabetic rats that received no insulin, blood glucose levels increased from fasting levels of about 300 to 400 mg./100 ml. to peaks not in excess of 600 mg./100 ml. during the light period, when the rats had free access to constant food, and then gradually

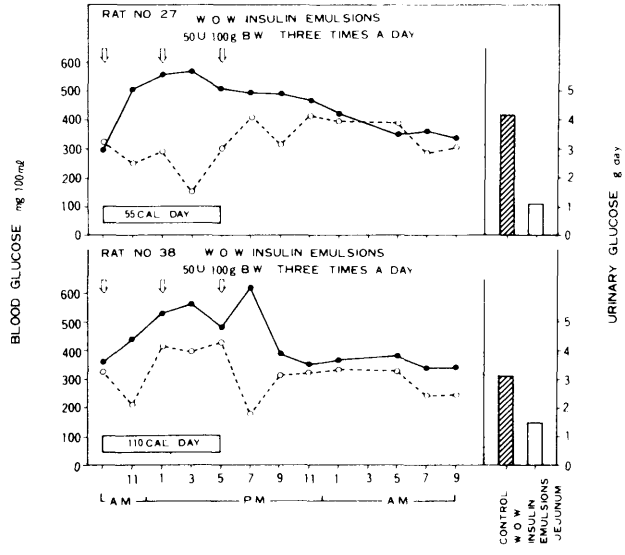

FIG. 1. Diurnal changes in blood glucose in alloxan-diabetic rats treated with intrajejunal administration of W/O/W insulin emulsions. Fifty U. per 100 gm. of W/O/W insulin emulsions was administered three times a day via an indwelling catheter into the upper jejunum of the rat (o– –o). W/O/W emulsions without insulin were infused into controls (●——●). During the experimental period, the caloric intake was kept constant.

returned to the fasting ranges during the dark period. On the following day, with the food kept constant at the same calories, 50 U./100 gm. of W/O/W insulin emulsions was given via an indwelling catheter into the upper jejunum three times a day. After each administration, blood glucose levels were markedly reduced, with concomitant decrease in the amount of urinary glucose.

To estimate the effectiveness of intrajejunal W/O/W insulin emulsions, the results were compared with those with intramuscular injection of regular insulin (figure 2). The effectiveness of 50 U./100 gm. of intrajejunal W/O/W insulin emulsions was comparable to that after intramuscular injection of regular insulin at doses between 1 and 2 U./100 gm.

Short-term Treatment

W/O/W insulin emulsions were given daily to seven diabetic rats for as long as fourteen days. The results in two rats are shown in detail in figures 3 and 4. During treatment, both rats showed a significant reduction in urinary glucose levels. When the emulsions were discontinued, urinary glucose subsequently returned to the pretreatment levels. In rat no. 26 (figure 3), the blood glucose tended to decrease during the treatment for five days. During the subsequent treatment for eight days, however, W/O/W insulin emulsions failed to produce significant changes in blood glucose levels despite the clear reduction in urinary glucose levels. Similar results were obtained by giving regular insulin to the same rat. In rat no. 29

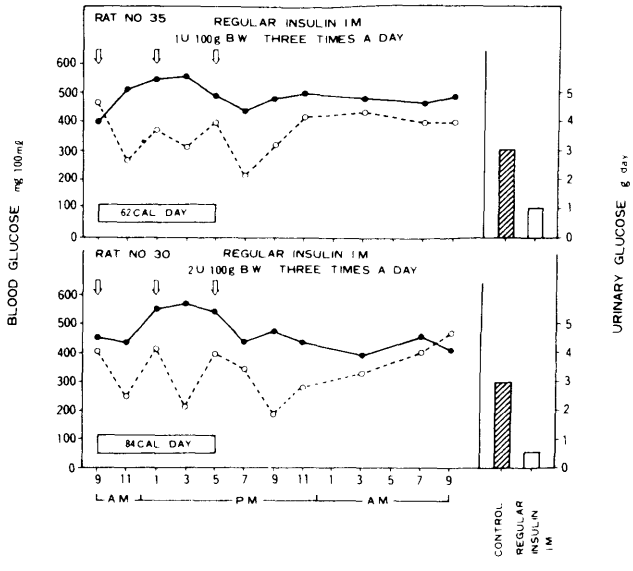

FIG. 2. Diurnal changes in blood glucose in alloxan-diabetic rats treated with intramuscular injection of regular insulin. One to 2 U./100 gm. of regular insulin was injected intramuscularly three times a day (o– –o). Saline solution was injected into controls (●——●).

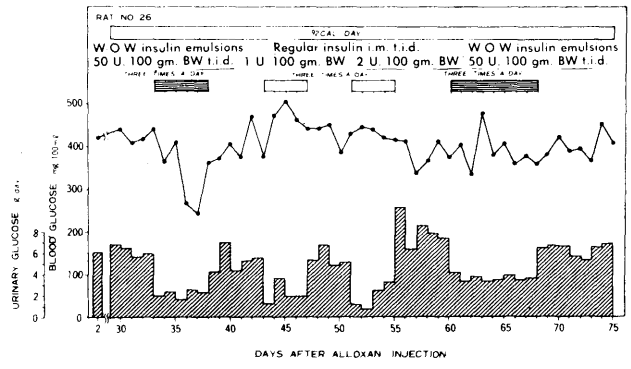

FIG. 3. Changes in blood glucose and urinary glucose levels during the short-term treatment of an alloxan-diabetic rat (no. 26) with intrajejunal administration of W/O/W insulin emulsions or intramuscular injection of regular insulin. Fifty units per 100 gm. of W/O/W insulin emulsions was administered intrajejunally three times daily. Regular insulin was injected intramuscularly at a dose of 1 or 2 U./100 gm. three times daily for four days.

(figure 4), blood glucose levels gradually decreased to levels below 200 mg./100 ml. during treatment for fourteen days and returned to pretreatment levels after the treatment was discontinued.

The mean fasting blood glucose and urinary glucose levels of all seven rats are summarized in table 1. In all rats treated with either 25 or 50 U./100 gm. of W/O/W insulin emulsions, urinary glucose levels were significantly decreased. In rats treated with 25 U./100 gm., fasting blood glucose levels did not change significantly. In four of five rats treated with 50 U./100 gm. however, a significant reduction of fasting blood glucose levels was observed during treatment. As judged by the changes in blood and urinary glucose levels, treatment with intrajejunal W/O/W insulin emulsions at a dose of 50 U./100 gm. was again comparable to that after intramuscular insulin at doses between 1 and 2 U./100 gm.

During the treatment, the body weight did not change significantly in all rats except one; rat no. 29 showed a gain in body weight of 20 gm.

DISCUSSION

The only studies with which present experiments can be compared are those of Lasch et al.[1,2] and Murlin et al.[3] For the oral insulin therapy, these authors developed a lacquer-covered tablet in which insulin was combined with saponin and protective agents such as acidic or basic dyes. When the insulin tablet was given to human diabetics, most of them had a therapeutically recognizable effect of lower blood and urinary glucose and of body weight as well as an improved general clinical condition. Form the standpoint of treatment, their observations should be ap-

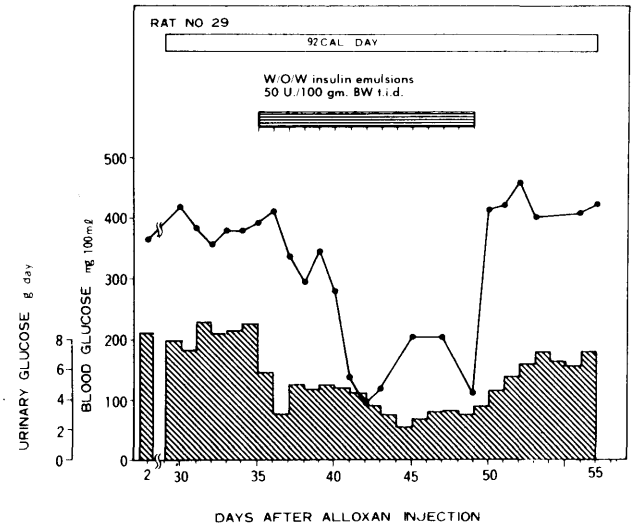

FIG. 4. Changes in blood glucose and urinary glucose levels during short-term treatment of an alloxan-diabetic rat (no. 29). Fifty units per 100 gm. of W/O/W insulin emulsions was administered intrajejunally three times daily for fourteen days.

praised, but the limit of safe application to diabetic patients has not been determined. In addition, enteric-coated tablets containing insulin and the adjuvant materials have not as yet been proved to have any comparable effect.

We have been attempting to prepare insulin derivatives or substitutes that are not affected by digestive enzymes.[10,11] It is interesting to note the possible use of water-in-oil-in-water emulsions as a means of facilitating gastrointestinal absorption of normally nonabsorbed water-soluble biopolymers.[13] Since W/O/W insulin emulsion is considered to exert insulin action by facilitating gastrointestinal absorption and by protecting insulin molecules from digestive destruction, when given intrajejunally or orally to rats and rabbits,[12,13] and is relatively nontoxic, its potential use as an oral insulin preparation has been examined in the present experiments.

In the present experiments, to obtain adequate control of diabetes with enteral administration of insulin, therefore, insulin solution or W/O/W insulin emulsions were administered via an indwelling catheter into the jejunum of rats three times daily, 9 a.m., 12 noon, and 5 p.m. Daily administration of insulin solution into the jejunum at doses between 50 and 150 U./100 gm. did not change either the blood glucose or urinary glucose levels in alloxan-diabetic rats. In contrast, the amount of urinary glucose excreted in twenty-four hours decreased clearly in all rats treated with intrajejunal administration of either 25 or 50 U./100 gm. of W/O/W insulin emulsions. In four of five rats treated with 50 U./100 gm., fasting blood glucose levels decreased significantly. In two rats treated with 25 U./100 gm. and one rat treated with 50 U./100 gm., however, intrajejunal W/O/W insulin emulsions failed to produce significant changes in fasting blood glucose levels, despite the clear reduction in urinary glucose levels.

Increase in food intake might not be responsible for the different responses of fasting blood glucose, since the rats were maintained on constant food intake during the experimental periods. According to our observations on diurnal variations, blood glucose levels re-

TABLE 1

Effects of short-term treatment of alloxan-diabetic rats with either intrajejunal administration of W/O/W insulin emulsions or intramuscular injection of regular insulin on fasting blood glucose levels

Rat No.	Treatment Dose	Days	Blood glucose (mg./100 ml.) Pretreatment	Treatment	Posttreatment
			Insulin, intramuscular administration		
27-2	1 U./100 gm. × 2	4	346 ± 23	379 ± 20 (113.8%)†	319 ± 17
26-2	1 U./100 gm. × 3	4	401 ± 20	473 ± 13 (113.9%)	428 ± 16
31	1 U./100 gm. × 3	8	437 ± 19	481 ± 19 (115.3%)	396 ± 15
27-3	1.5 U./100 gm. × 3	4	319 ± 17	280 ± 76 (76.3%)	414 ± 29
26-3	2 U./100 gm. × 3	4	428 ± 16	430 ± 7 (105.9%)	383 ± 24
32	2 U./100 gm. × 3	8	457 ± 31	285 ± 73* (64.8%)	422 ± 18
			W/O/W Insulin emulsions, jejunal administration		
26-5	25 U./100 gm. × 3	8	425 ± 15	441 ± 18 (108.6%)	387 ± 19
28	25 U./100 gm. × 3	5	325 ± 23	318 ± 19 (97.0%)	330 ± 32
26-1	50 U./100 gm. × 3	5	426 ± 10	329 ± 32* (79.5%)	401 ± 20
26-4	50 U./100 gm. × 3	8	383 ± 24	393 ± 19 (99.7%)	404 ± 13
27-1	50 U./100 gm. × 3	5	354 ± 4	265 ± 32* (75.7%)	346 ± 23
27-4	50 U./100 gm. × 3	8	414 ± 29	275 ± 50* (69.4%)	378 ± 15
29	50 U./100 gm. × 3	14	385 ± 9	232 ± 33* (57.4%)	422 ± 9

Values are expressed as mean ± S.E.M.

*$p<0.05$, as compared with pre- and posttreatment values.

†Change (%) = $[(\text{Treatment})/((\text{Pretreatment} + \text{Posttreatment})/2)] \times 100$.

sponded to each administration of W/O/W insulin emulsions but gradually increased during the dark period. The results of short-term treatment of regular insulin intramuscularly showed the same tendency. The different responses of fasting blood glucose levels might, therefore, account for the duration of insulin effects as well as the pharmacologic amounts of insulin required in the treatment of diabetes. The amounts of insulin might be related to a strong dependency on intestinal absorption of insulin, when given enterally. It is also possible that a much longer period might be necessary to improve the metabolic derangements observed in severely diabetic rats used here.

Quantitative estimates suggest that the effectiveness of 50 U./100 gm. of intrajejunal W/O/W insulin emulsions was comparable to that after intramuscular regular insulin at doses between 1 and 2 U./100 gm. These findings were in keeping with previous results.[12] Less than 5 per cent of W/O/W insulin emulsions was absorbed from the intestine of rabbits during the period of three hours, based on comparisons of plasma insulin responses to a single administration of W/O/W insulin emulsion intrajejunally and insulin intramuscularly.

Since the hyperglycemic state produced by alloxan is often spontaneously reversible, we have used severely diabetic rats, established over a sufficiently long foreperiod with fasting blood glucose levels over 300 mg./100 ml. In the posttreatment period, blood and urinary glucose levels returned to pretreatment levels, indicating that improvement was not due to a spontaneous remission. Histologic evidence at the end of experiments demonstrated that islets in the pancreas of alloxan-treated rats were essentially devoid of beta cells.

No adverse effects, severe hypoglycemia, or gastrointestinal side effects, such as diarrhea, were observed during the short-term experiments reported here. Further confirmation of reliability and of safety with long-term use would be required.

W/O/W insulin emulsion as an oral preparation has been less than satisfactory as a substitute for parenteral administration, since this preparation is still of low efficacy. Additional studies to improve the efficacy of the preparation are therefore necessary. Our present results, however, would indicate that diabetes can be controlled by enteral administration of insulin preparations.

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TABLE 1 (*continued*)

Effects of short-term treatment of alloxan-diabetic rats with either intrajejunal administration of W/O/W insulin emulsions or intramuscular injection of regular insulin on fasting urinary glucose levels

Rat No.	Treatment Dose	Days	Urinary glucose (gm./day) Pretreatment	Treatment	Posttreatment
			Insulin, intramuscular administration		
27-2	1 U./100 gm. × 2	4	2.87 ± 0.22	0.94 ± 0.14* (33.4%)†	2.75 ± 0.36
26-2	1 U./100 gm. × 3	4	5.29 ± 0.51	2.26 ± 0.56* (41.6%)	5.56 ± 0.43
31	1 U./100 gm. × 3	8	5.94 ± 0.19	2.85 ± 0.29* (43.7%)	7.10 ± 0.37
27-3	1.5 U./100 gm. × 3	4	2.75 ± 0.36	0.76 ± 0.42* (17.4%)	5.98 ± 0.98
26-3	2 U./100 gm. × 3	4	5.56 ± 0.43	2.00 ± 0.59* (29.1%)	8.17 ± 0.66
32	2 U./100 gm. × 3	8	7.04 ± 0.48	1.38 ± 0.67* (19.6%)	6.85 ± 0.60
			W/O/W Insulin emulsions, jejunal administration		
26-5	25 U./100 gm. × 3	8	6.16 ± 0.26	3.96 ± 0.33* (63.9%)	6.23 ± 0.28
28	25 U./100 gm. × 3	5	5.25 ± 0.54	3.40 ± 0.12* (60.2%)	6.05 ± 0.55
26-1	50 U./100 gm. × 3	5	6.45 ± 0.30	2.22 ± 0.16* (37.8%)	5.29 ± 0.51
26-4	50 U./100 gm. × 3	8	8.17 ± 0.66	3.75 ± 0.10* (50.9%)	6.55 ± 0.10
27-1	50 U./100 gm. × 3	5	3.64 ± 0.31	0.66 ± 0.07* (20.2%)	2.87 ± 0.22
27-4	50 U./100 gm. × 3	8	5.98 ± 0.98	1.22 ± 0.34* (21.4%)	5.40 ± 0.40
29	50 U./100 gm. × 3	14	8.36 ± 0.28	3.14 ± 0.94* (43.7%)	6.00 ± 0.52

Values are expressed as mean ± S.E.M.

*$p<0.05$, as compared with pre- and posttreatment values.

†Change (%) = $[(\text{Treatment})/((\text{Pretreatment} + \text{Posttreatment})/2)] \times 100$.

REFERENCES

[1]Lasch, F., and Schönbrunner, E.: Experimentelle Untersuchungen über perorale Insulin Therapie unter Beigabe organischer Farbstoffe. Klin. Wochenschr. *34*:117-80, 1938.

[2]Lasch, F.: Bisherige Ergebnisse der peroralen Insulin Therapie. Z. Vitam. Horm. Fermenforsch. *4*:83-93, 1951.

[3]Murlin, J.R., Gibbs, C.B.F., Romanosky, M.J., Steinhausen, T.B., and Truax, F.L.: Effectiveness of per-oral insulin in human diabetes. J. Clin. Invest. *19*:709-22, 1940.

[4]Danforth, E., Jr., and Moore, R.O.: Intestinal absorption of insulin in the rat. Endocrinology *68*:118-23, 1959.

[5]Crane, C.W., and Luntz, George R.W.: Absorption of insulin from the human small intestine. Diabetes *17*:625-27, 1968.

[6]Galloway, J.A., and Root, M.A.: New forms of insulin. Diabetes *21* (suppl. 2): 637-48, 1972.

[7]Shichiri, M., Okada, A., Karasaki, K., Kawamori, R., Shigeta, Y., and Abe, H.: Increase in plasma immunoreactive insulin following administration of insulin to the gastrointestinal tract of rabbits. Diabetes *21*:203-08, 1972.

[8]Shichiri, M., Etani, N., Kawamori, R., Karasaki, K., Okada, A., Shigeta, Y., and Abe, H.: Absorption of insulin from perfused rabbit small intestine in vitro. Diabetes *22*:459-65, 1973.

[9]Shichiri, M., Okada, A., Kawamori, R., Etani, N., Shimizu, Y., Hoshi, M., Shigeta, Y., and Abe, H.: Portal vein insulin responses to the intestinal administration of insulin in rabbits. Endocrinology *93*:131-37, 1973.

[10]Shichiri, M., Okada, A., Kikkawa, R., Kawamori, R., Shigeta, Y., and Abe, H.: β-naphthyl-azo-polystyrene insulin as a means of protecting insulin molecule from digestive enzymes. Biochem. Biophys. Res. Commun. *44*:51-56, 1971.

[11]Shigeta, Y., Shichiri, M., Okada, A., and Karasaki, K.: Plasma immunoreactive insulin after intestinal administration of β-naphthyl-azo-polystyrene insulin to the rabbit. Endocrinology *91*:320-22, 1972.

[12]Shichiri, M., Shimizu, Y., Yoshida, Y., Kawamori, R., Fukuchi, M., Shigeta, Y., and Abe, H.: Enteral absorption of water-in-oil-in-water insulin emulsions in rabbits. Diabetologia *10*:317-21, 1974.

[13]Engel, R.H., Riggi, S.J., and Fahrenbach, M.J.: Insulin: Intestinal absorption as water-in-oil-in-water emulsions. Nature *219*:856-57, 1968.

[14]Nelson, N.: A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. *153*:375, 1944.