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Rate of Insulin Infusion with a Minipump Required to Maintain a Normoglycemia in Diabetic Rats[1] (41529)

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Abstract. It is notoriously difficult to normalize plasma glucose profiles for a prolonged time by conventional methods of insulin administration in both human and animal diabetics. The present study was conducted to determine the dosage of insulin needed to maintain prolonged and around-the-clock normoglycemia as well as normoglucagonemia in streptozotocin diabetic rats with the Alzet osmotic minipump which releases insulin constantly for 14 days. A minipump was inserted into the peritoneal cavity of diabetic rats under chloral hydrate anesthesia. Diabetic rats were treated with several consecutive minipumps and body weights, plasma glucose, and plasma glucagon levels were monitored. Plasma glucose concentrations were determined every 8 hr for several days and were found essentially identical during the active life of the minipump. An average insulin dose of 8.0 to 9.0 U/kg/day was required to normalize body weights, plasma glucose, and plasma glucagon of streptozotocin diabetic rats treated with the Alzet osmotic minipump Model 2002.

The treatment of juvenile-onset diabetics with portable "open loop" insulin infusion pumps makes possible the maintenance of normal or near-normal around-the-clock glucose profiles (1–3) and has corrected certain of the metabolic and endocrine abnormalities (4–6). Streptozotocin diabetic rats have been widely used, as animal models, in studies related to clinical problems in human diabetes mellitus. It has been quite difficult to normalize the plasma glucose profile in diabetic rats by insulin injections as it has been for humans receiving insulin by conventional insulin therapy. The recent development of a small implantable minipump (Alzet osmotic minipump) which releases its contents at a constant rate has made possible an attempt to normalize plasma glucose levels in experimental diabetic animals. Yum *et al.* (7) reported that 2 U/day of insulin subcutaneously delivered from an Alzet osmotic minipump (Model 2001, 7 days of active life) almost normalized plasma glucose levels in streptozotocin diabetic rats but no studies were reported on plasma glucagon levels. However, plasma glucose concentrations reached normal values several days after insertion of the minipump and 3 days later rose above normal values. This suggests that normoglycemia was maintained for only 3 days with the Alzet osmotic minipump (Model 2001) which delivers insulin constantly for 7 days. Therefore, the present study was performed to investigate the dose and rate of administration of insulin required to maintain the plasma glucose levels around the clock and the plasma glucagon concentrations near normal in the streptozotocin diabetic rats by means of the minipump throughout its active life of 14 days (Model 2002).

Materials and Methods. Male Sprague–Dawley rats weighing between 250 and 300 g were used in this study. Animals were housed individually in plastic cages in temperature- and humidity-controlled animal quarters and, unless noted, fed Purina Rat Chow *ad libitum* and given free access to water throughout the experiment. The rats were divided into two weight-matched groups. Diabetes was induced in 18-hr fasted Group I rats by intravenous injection of streptozotocin 65 mg/kg (freshly dissolved in ice-cold 0.05 *M* citrate buffer, pH 4.5). The rats in Group II were fasted for 18 hr and injected with 0.05 *M* citrate buffer alone iv (freshly prepared pH 4.5) and designated as control. All rats were then offered food immediately.

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TABLE I. PLASMA GLUCOSE LEVELS MEASURED AT DIFFERENT TIMES OF DAY IN DIABETIC RATS TREATED WITH INSULIN USING OSMOTIC MINIPUMP

Time of day	Plasma glucose (mg%)
8:00 AM	51.3 ± 16.2
4:00 PM	58.9 ± 22.8
Midnight	64.8 ± 31.5

Note. Results are expressed as mean ± SD. Computed from the data of six consecutive days from three diabetic rats immediately after intraperitoneal implantation of the osmotic minipump filled with insulin representing a mean dose of 16.84 ± 1.18 U/kg/day. The rats were not fasted.

All rats injected with streptozotocin developed glycosuria within 24 hr.

Immediately after the induction of diabetes the diabetic rats were divided into two groups: untreated diabetics and insulin-treated diabetics. The rats in the insulin-treated group were divided into several groups, each of which was treated with insulin (at a different dose) with an Alzet osmotic minipump according to the method described by Bringer *et al.* (8). Insulin solutions were prepared by appropriate dilution of regular purified pork zinc insulin (500 U regular Iletin II, Eli Lilly & Co.) with diluent containing 1.6% glycerine (w/v) and 0.2% phenol (w/v) in distilled water. L-Glutamic acid (Sigma) was added to the diluted insulin solution in a final concentration of 7 mg/ml and pH adjusted to 3.4 with NaOH and HCl. Implantable pumps (Alzet osmotic minipump Model 2002, Alza Corp.) were filled with the diluted insulin. Pumps were weighed before and after filling to determine the initial fluid volume for each pump.

Diabetic rats were anesthetized with chloral hydrate (350 mg/kg ip). A small incision (1 cm) was made after the abdomen was shaved. A pump was inserted into the peritoneal cavity. The incision was closed in layers. Each rat received 60,000 units of procaine penicillin sc for 3 days after the insertion of the pump. At the end of the life of the pump, a second pump was inserted as described earlier and the old (used) pump was removed. Rats were weighed periodically and plasma samples for glucose estimation obtained by tail bleeding. In one group of rats plasma glucose was measured every day at 8:00 AM, 4:00 PM, and midnight for several days. Subsequently, in all the insulin-treated groups rats were periodically weighed at 9:00 AM and blood samples were collected by tail bleeding for plasma glucose measurements during the active life of the minipumps. At the end of the experimental period, i.e., 60–80 days after the induction of diabetes, all rats in control, untreated diabetic, and insulin-treated diabetic groups were fasted for 18 hr. All rats were weighed and blood samples were collected by tail bleeding for plasma glucose and plasma glucagon determination. Plasma glucose was measured by the glucose oxidase method with a glucose autoanalyzer (Beckman Instruments). Plasma glucagon was determined by radioimmunoassay method as described by Faloona and Unger (9). Both standards and samples were extracted with ice-cold acetone as published by Von Schenck and Nilsson (10) to eliminate spurious increments in glucagon values caused by the unidentified "big plasma glucagon." In this assay rabbit anti-beef pork glucagon serum (30 K, specifically for pancreatic glucagon, purchased from Dr. Unger's Laboratory) was used in conjunction with crystalline porcine pancreatic glucagon (as standard, Eli Lilly & Co.) and ^{125}I-glucagon (New England Nuclear). Statistical significance was computed by Duncan's multiple range test.

Results and Discussion. One day after the injection of streptozotocin all rats had 4+ glycosuria measured with a test tape (Eli Lilly & Co.). Three rats were treated with insulin (16.84 ± 1.18 U/kg/day) infused constantly by osmotic minipumps. Plasma glucose levels were determined at 8-hourly cycles starting from 8:00 AM for 6 consecutive days. Table I depicts that the mean plasma glucose values in the diabetic rats fluctuated minimally throughout the day when treated with the osmotic minipumps (8:00 AM, 51.3 ± 16.2 mg%; 4:00 PM, 58.9 ± 22.8 mg%; and midnight, 64.8 ± 31.5 mg%). However, the dose used was too high as most of the time rats were hypoglycemic. Therefore, in subsequent studies diabetic rats were treated with different doses of insulin to determine the optimum dose.

The average daily dose of insulin required for maintenance of normoglycemia was 8.09

TABLE II. PLASMA GLUCOSE LEVELS ESTIMATED AT 9:00 AM FOR SEVERAL DAYS DURING THE ACTIVE LIFE OF THE MINIPUMPS IN DIABETIC RATS

Range of dose of insulin (U/kg/day)	Plasma glucose (mg%)
6.5 to 7.0 (2)	289.3 ± 175.4
7.0 to 7.5 (3)	226.7 ± 171.0
7.5 to 8.0 (4)	189.9 ± 152.1
8.0 to 9.0 (10)	145.7 ± 117.6

Note. Results are expressed as means ± SD. The rats were not fasted. The minipumps were inserted one day after the injection of streptozotocin. Plasma glucose levels were determined for several days during the active life of the minipumps. Numbers of pumps in parentheses. Mean plasma glucose computed as in Results and Discussion.

± 0.71 U/kg body weights. The mean plasma glucose levels before insertion of the pump were 349.9 ± 49.94 mg% and were accompanied by 4+ glucosuria. Plasma glucose levels were periodically measured (9:00 AM) during the active life of each pump and these values were computed to determine the mean plasma glucose concentrations during the active life of the pump. Table II depicts the mean plasma glucose levels studied for several days during the active life of the minipumps. The rats were grouped according to the different ranges of doses of insulin. The mean plasma glucose values declined progressively from 289.3 ± 175.4 to 145.7 ± 117.6 mg% for doses ranging from 6.5 to 7.0 to 8.0 to 9.0 U/kg/day. This suggests that insulin infusion had maintained near normal plasma glucose levels in the diabetic rats throughout the day when insulin dosage was between 8.0 and 9.0 U/kg/day.

Table III shows body weights, plasma glucose, and plasma glucagon levels in streptozotocin diabetic untreated, insulin-treated (8.0 to 9.0 U/kg/day), and age-matched control rats after 18-hr fasting. The mean body weights of the untreated diabetic rats (233.0 ± 26.87 g) were significantly lower than those of both insulin-treated (377.2 ± 42.4 g, $P < 0.01$) and control rats (414.86 ± 38.39 g, $P < 0.01$), which were essentially similar. The mean plasma glucose value for the untreated diabetic rats (545.44 ± 56.62 mg%) was significantly higher than that in controls (133.14 ± 11.98 mg%, $P < 0.01$) and insulin-treated diabetic rats (276.0 ± 105.2 mg%, $P < 0.01$). There was a significant difference ($P < 0.01$) between the mean plasma glucose levels between controls and insulin-treated diabetic rats. The plasma glucose concentrations were determined in all the groups a few days after the active life of the pumps was over, so this could explain the significant difference in mean plasma glucose values between controls and insulin-treated diabetic rats as well as the variability among individual insulin-treated diabetic rats. The mean plasma glucagon levels, as expected, were also significantly higher in untreated diabetic rats (99.72 ± 35.07 pg/ml) in comparison with those for controls (26.57 ± 11.06 pg/ml, $P < 0.01$) and insulin-treated diabetic rats (49.0 ± 28.28 pg/ml, $P < 0.01$). The mean plasma glucagon in insulin-treated diabetic rats seemed to be higher than that in controls but the difference was not statistically significant. Raskin and Unger (11) have reported that aggressively administered insulin significantly reduces the average fasting and fed state glucagon values in most Type I and

TABLE III. BODY WEIGHT, PLASMA GLUCOSE, AND PLASMA GLUCAGON VALUES IN CONTROL AND DIABETIC RATS WITH AND WITHOUT INSULIN TREATMENT DETERMINED AFTER 18-hr FAST

Groups	Body weight (g)	Plasma glucose (mg%)	Plasma glucagon (pg/ml)
Control (7)	414.86 ± 38.39	133.14 ± 11.98	26.57 ± 11.06
Diabetic (9) (untreated)	233.0 ± 26.87[a,b]	545.44 ± 56.62[a,b]	99.72 ± 35.07[a,b]
Diabetic (6) (insulin treated)	377.20 ± 42.40	276.0 ± 105.20[c]	49.0 ± 28.28

Note. All results are expressed as means ± SD. Number of observations in parentheses.
[a] $P < 0.01$ compared with control.
[b] $P < 0.01$ compared with insulin-treated diabetics.
[c] $P < 0.01$ compared with control.

Type II diabetics. Similarly, Kawamori *et al.* (12) also reported normalization of excessive glucagon response to arginine in both Type I and Type II diabetics with the aid of the artificial beta-cell system originally developed by Goriya *et al.* (13). Frankel *et al.* (14) also obtained improved control of blood glucose in diabetic Chinese hamsters when treated with insulin delivered via the 7-day Alzet minipump. However, Harshfield and Griffey (15) could not normalize the hyperglucagonemia after 2 weeks of insulin treatment (15.40 U/kg/day) with the osmotic minipump; albeit during the second week the plasma glucagon values were significantly lower than those in untreated diabetic rats. They may have normalized the plasma glucagon levels in insulin-treated diabetic rats, as in my study, if they prolonged treatment. VanderWeele *et al.* (16) also published their data stating that the minipumps delivering regular insulin (15 U/kg/day) for 7 days to diabetic rats lowered plasma glucose, eliminated glycosuria, and prompted a resumption in body weight gain. They did not measure plasma glucagon. The results of the present study on normalization of body weights, plasma glucose, and plasma glucagon values suggest that insulin infusion treatment via the minipump had maintained diabetic rats in good glycemic control for a prolonged period and so could be used for hormonal and metabolic studies of the role of good glycemic control in diabetics.

The studies cited in literature of experiments with osmotic minipumps (17–20) have employed varying doses of insulin and none has been continued for a prolonged period of about 60 to 80 days as in my study. I used doses varying from 6.50 to 9.50 U/kg/day. The best glycemic control was obtained when the dose ranged from 8.0 to 9.0 U/kg/day. Therefore, it could be concluded that in streptozotocin diabetic rats the normoglycemic and normoglucagonemic states could be maintained with insulin treatment via the Alzet osmotic minipump which releases its contents constantly for 14 days. The average dose required is between 8.0 and 9.0 U/kg/day and insulin solution should be prepared with L-glutamic acid (7 mg/ml) to ensure a smooth constant flow of insulin from the minipump.

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