One Month of Sustained Release of Insulin from a Polymer Implant

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SUMMARY

Rats made diabetic with streptozotocin received a single subcutaneous implant of an insulin polymer pellet that released insulin continuously at approximately 2 U/day. Continuous normoglycemia was achieved for up to 1 mo. Mean glucose level for treated animals was 113 mg/dl as compared with 398 mg/dl for untreated diabetic controls. Diurnal blood glucose values for treated animals ranged from 71 to 116 mg/dl. Polyuria and glycosuria were corrected by the presence of the insulin + polymer. Treated animals gained weight normally and reached a mean weight of 350 g, whereas untreated control animals lost weight, to a mean of 150 g. When insulin + polymer preparations were periodically implanted and removed at 7-day intervals, normoglycemia was only associated with the presence of implants. DIABETES 29:37–40, January 1980.

In a previous report,[1] we demonstrated that a variety of macromolecules could be continuously released in a biologically active form from pellets of vinyl acetate-ethylene copolymer. We now show that diabetic rats can be made normoglycemic for 1 mo after a single subcutaneous implant of such a polymer that releases insulin in a sustained manner.

MATERIALS AND METHODS

Animals. Twenty-three male cesarean-delivered rats (21 days old) (Charles River Breeding Laboratories), weighing from 150 to 250 g, were made diabetic with streptozotocin and were distributed into four experimental groups: (1) 15 animals received single insulin + polymer implants; (2) 1 animal received an insulin + polymer that was repeatedly implanted and removed every 7 days; (3) 2 untreated control animals received no polymers; (4) 2 untreated control animals received empty polymers. Animals received water and rat chow ad libitum. Body weights were measured every 8–10 days.

Induction of diabetes mellitus and determination of blood glucose levels. Each animal received an intravenous injection of 75 mg/kg of streptozotocin, suspended in 0.4 ml of 0.005 M citric acid at pH 4.5. The rats were left untreated for 6 days and were then entered into the experimental design. During the 6-day period, glucose determinations were made at days 1, 3, and 6 to insure a consistently high blood glucose value.

To measure glucose levels, 200 μl of blood was withdrawn from the tail (samples were generally taken in the morning or early afternoon, except for the diurnal study) and centrifuged at 15,000 × g. Twenty-microliter samples of plasma were analyzed on a YSI glucose analyzer. Glucose levels were determined about every 3 days.

Preparation and implantation of sterile polymer implants. A number of preliminary experiments were conducted to determine a polymer design that would release about 2 U of insulin a day in vitro. The insulin released in vitro was monitored spectrophotometrically at 220 nm. Insulin + polymer preparations were incubated in physiologic saline in glass tubes coated with Siliclad. The spectrophotometric measurements were corroborated by both an insulin radioimmunoassay[2] and injections of released insulin into diabetic rats. In both cases, it was found that all the released insulin was active.

The polymer implants were made using a modification[3] of our previously described procedure.[1] First, 100 mg of bovine pancreatic insulin (Sigma or Calbiochem, 25 IU/mg) was mixed with 0.4 cc of 10% (w/v) vinyl acetate-ethylene copolymer (Eluax 40, Dupont) in methylene chloride. The resulting suspension was vortexed and poured into circular glass molds, which were supercooled over dry ice. Molds containing insulin + polymer were then refrigerated overnight at −20°C. To evaporate methylene chloride, the molds were then placed under vacuum (600 mm Hg) for 24 h. The

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resulting discs (1.3 cm by 1 mm) were incubated in lactated Ringer's solution (Abbott) for 24 h and then implanted subcutaneously in the lower abdomen. Implant sites were closed with Michel's wound clamps.

Variation in diurnal serum glucose, urine glucose, and urine volume. Blood and urine samples from two treated animals and one control were analyzed with the YSI glucose analyzer every 2–5 h for 31 h. Urine was collected by housing the animals in metabolic cages.

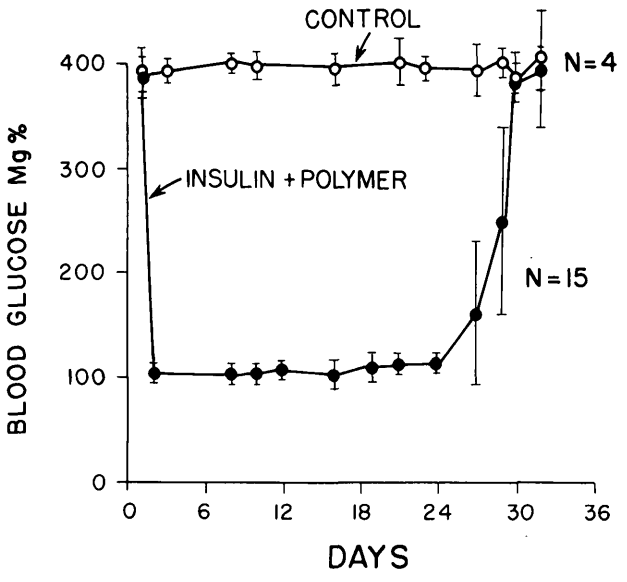
RESULTS

Glucose analysis. At 24 h after the administration of streptozotocin, glucose levels were elevated to 374–412 mg/dl. Within the first 24 h after implantation of insulin + polymer, glucose levels fell from 374–412 mg/dl to 87–127 mg/dl and remained stable for 26 days. During the 29-day experiment, the mean glucose level for all treated animals was 113 ± 29 mg/dl. The mean glucose level for controls was 398 ± 22 mg/dl. On the 27th day of the test, 27% of the treated animals had glucose levels between 252 and 295 mg/dl compared with the original values of between 382 and 411 mg/dl before treatment. On the 29th day, all animals were hyperglycemic (Figure 1). In contrast, all diabetic control animals displayed a mean value of 398 ± 22 mg/dl throughout the experiment.

Diurnal glucose measurements and urine analysis. Figure 2 illustrates the variation in blood glucose concentrations on the 16th day of treatment for two experimental animals and one control. During the 31 h of monitoring, glucose levels for treated animals ranged from 71 to 116 mg/dl, with a mean daily concentration of 95 ± 13 mg/dl. Mean glucose concentration for the control was 338 ± 61 mg/dl. Values ranged from 297 to 470 mg/dl.

Treated animals voided a total of 38 and 44 ml of urine in 31 h. Glucose was not detected in the urine of treated animals. In contrast, the untreated control voided 183 ml of urine during the 31 h and urine glucose concentrations exceeded 500 mg/dl.

FIGURE 1. Implantation of single insulin-containing polymers into diabetic rats. Control animals were diabetic rats receiving empty polymers or no polymers.



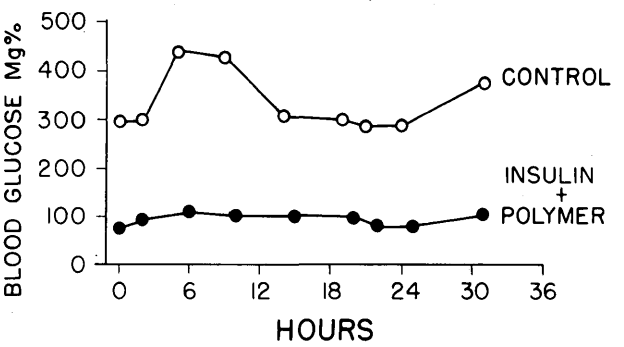


FIGURE 2. Determinations of blood glucose, made every few hours around the clock, for two treated animals and one control animal.

Periodic implantation and removal of the polymer. When a diabetic animal was treated with the insulin + polymer for 7 days, glucose levels ranged between 87 and 106 mg/dl. After the implant was removed, values rose to 378–440 mg/dl. After reimplantation, glucose fell to between 105 and 119 mg/dl (Figure 3).

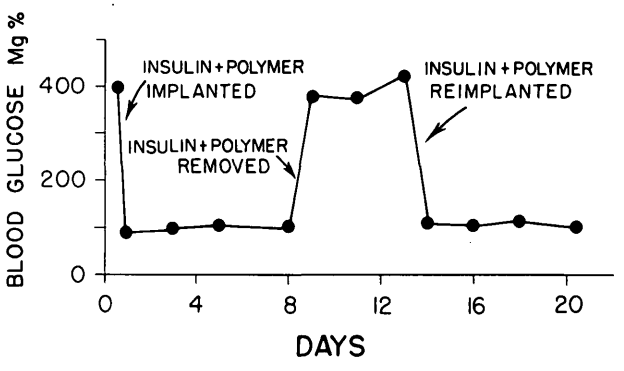
Polymer activity. After 31 days, all insulin + polymer preparations appeared to have stopped releasing insulin. They were removed, washed in saline, and implanted in untreated diabetic animals. No effect on hyperglycemia was observed. When a new, unexpended insulin + polymer was placed in a diabetic animal previously treated for 31 days, glucose levels were again lowered to 103 mg/dl.

Growth and development. Figure 4 shows the change in body weight for all experimental animals. The weight gain of controls was depressed as a result of the disease. Treated animals gained weight at the normal rate of approximately 40 g/wk. Some became slightly obese, to over 400 g. The mean body weight for treated animals at 28 days was 350 ± 55 g. By contrast, untreated controls lost weight or maintained their starting weight. By the end of the experiment, they averaged 105 ± 23 g.

DISCUSSION

These experiments show that a small subcutaneous implant of vinyl acetate-ethylene copolymer can release insulin in biologically active form for 29 days. Each implant released about 2 U of insulin a day throughout the 1-mo period. Diabetic rats carrying the implanted insulin + polymer main-

FIGURE 3. An experiment in which a diabetic rat was used as its own control. Without the insulin + polymer, blood glucose levels were about 400 mg/dl. Implantation and reimplantation of the same polymer caused glucose levels to be nearly 100 mg/dl.



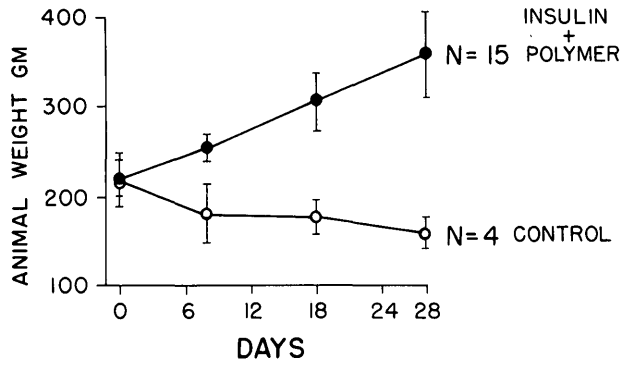


FIGURE 4. Weight determinations of control and treated animals.

tained normoglycemia and gained weight normally. This indicates that the rats adjusted their dietary intake to match insulin levels in their blood.

Blood glucose levels in the treated animals were not erratic, as shown by the glucose determinations made every 2–5 h (Figure 2). The polymer implants all stopped releasing insulin at the same time. This release time span was a function of such design factors as percent loading and particle size of the insulin + polymer configuration, as well as the external coating of the polymer.[1,3] In fact, the polymer design in the present study was not effectively optimized. Our calculations show that only about 3% of the insulin incorporated into the polymer escaped at the level of 2 U a day. The insulin was apparently not denatured, because we have taken insulin released from polymers in vitro at day 25, subjected it to both an insulin radioimmunoassay and injection into diabetic rats, and found that nearly 100% of the insulin released, as determined by protein measurement, was biologically active. Current experiments in our laboratory show that such simple adjustments as increasing the particle size of lyophilized proteins before incorporation into the polymers or increasing the drug loading can alter release rates by as much as 50 times.[3] In the case of insulin, particle size appears to be a critical factor, because the commercial insulin powder we used is less than 50 μm in diameter (which is much smaller than most protein powders) and release is slow, with much of the drug retained, unless extremely high loadings are employed. Future experimentation in our laboratory will be directed toward achieving an insulin-releasing system that more optimally utilizes the drug and releases insulin for far longer periods of time. Nevertheless, the present experiments are the first to demonstrate that even one month of sustained release of insulin can be accomplished.

No rats in this study spontaneously recovered from their diabetes. To further strengthen the experiments, diabetic rats were used as their own controls. Periodic implantation and removal of an insulin + polymer in the same animal correlated with normoglycemia or hyperglycemia. When an expended polymer was removed from a diabetic rat and implanted into a previously untreated diabetic rat, there was still no effect from the polymer. This indicated that the shutdown of insulin release was intrinsic to the polymer itself and not due to fibrous encapsulation or to a foreign body reaction around it. Furthermore, when a fresh insulin + polymer pellet was implanted into the first rat (i.e., the previously treated rat), glucose levels returned to normal. This indicated that the shutdown of insulin release from the expended polymer was unrelated to any type of "resistance" to insulin by the host animal.

The only previous report in which insulin released from long-term implants was studied was by Davis,[4] who used polyacrylamide as a vehicle. He measured weights of diabetic animals receiving such implants for 2–3 wk, but blood glucose was not measured. Also, polyacrylamide implants are inflammatory[1] and acrylamide monomers have been shown to be toxic to both animals and humans.[5] The ethylene-vinyl acetate copolymer used in our study causes no detectable tissue reaction.[1]

Several other novel approaches for maintaining normoglycemia in diabetic animals and patients have been investigated recently; these include pancreatic islet cell transplants, infusion pumps, servomechanisms, and glucose-sensing artificial pancreases.[6–13] The sustained release of low levels of insulin from a single polymer implant offers another potential therapeutic approach or adjunct. One could speculate that, with further development, these insulin + polymer implants might be used clinically in conjunction with dietary control or as an insulin depot linked to a regulatory feedback control with a glucose sensor.[14,15] It is also conceivable that insulin + polymers could maintain a constantly low level of insulin and that additional insulin release could be triggered by some external force, such as temperature. While any future clinical applications of these implants remain to be seen, their immediate practical use may be as a means of studying the kinetics of dietary intake and blood glucose level when insulin input is continuous.

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