Functional Evaluation of a Bioartificial Pancreas Using Isolated Islets Perifused with Blood Ultrafiltrate

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SUMMARY

A new bioartificial pancreas, in which rat islets within a Millipore chamber are perifused with blood ultrafiltrate (polyacrylonitrile membrane), has been developed and its function evaluated in vitro and in vivo. Use of ultrafiltration (instead of diffusion) permitted rapid transmission of changes in blood glucose to the islet compartment (less than 10 min). To determine whether ultrafiltrate of Krebs-Ringer bicarbonate buffer (KRBB) and blood could support normal islet B-cell function, insulin secretion of freshly isolated islets in response to a 20-mM glucose challenge was examined during perifusion with KRBB and then with ultrafiltrate produced either from KRBB itself or from blood of normal and streptozotocin-induced diabetic rats. In the latter case, perifusate was reinfused to the animals, whose plasma glucose and insulin were then measured.

Insulin responses to glucose were virtually identical when the same islets were perifused with KRBB and then with ultrafiltrate of KRBB (47 ± 12 versus 41 ± 9 ng/100 islets/24 min), or when islets were perifused with KRBB and then with ultrafiltrate produced from blood of normal rats (69 ± 6 versus 73 ± 11 ng/100 islets/24 min). When five diabetic rats were connected to the device containing 600 islets, their plasma glucose decreased from 19.8 ± 1.2 to 6.1 ± 0.5 mM ($P < 0.001$) within 120 min without overshoot hypoglycemia, and remained normal for up to 6 h. Plasma insulin increased from 2.0 ± 0.1 to 11.4 ± 1.4 ng/ml ($P < 0.02$) at 30 min and then decreased to 2.9 ± 0.6 ng/ml. After intravenous glucose administration (0.5 g/kg, N = 4), plasma insulin increased twofold within 15 min, and glucose disappearance rates were identical to those obtained in four normal rats (K = 2.13 ± 0.18 versus 2.23 ± 0.09, NS).

These results indicate that ultrafiltrate of KRBB and blood can acutely support normal islet B-cell function, and that ultrafiltration can provide satisfactory glucose-insulin kinetics for an implantable closed-loop insulin delivery system, in which nonsyngeneic islets may be protected against immune rejection. DIABETES *30*:296–301, April 1981.

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Received for publication 2 June 1980 and in revised form 12 November 1980.

Currently, nonsyngeneic transplantation of islets of Langerhans for the treatment of diabetes mellitus is limited by immune rejection.[1] To circumvent this problem, several systems in which islets are separated from blood of recipients by membranes permeable to glucose and small peptide hormones, but impermeable to antibodies and immunocytes, have been devised.[2–6] In all systems proposed to date, glucose and insulin cross the membrane mostly by diffusion,[7] a relatively slow process.[8] This may represent a potential obstacle for maintenance of euglycemia, since the pancreatic B-cell normally responds rapidly to changes in blood glucose concentration[9] and any delay in insulin secretion, such as that found in maturity-onset (type II) diabetes,[10] could lead to hyperglycemia. However, convection by the solvent drag effect is generally a more rapid process than diffusion; consequently, use of convection may provide more appropriate transfer of glucose through the membrane of a bioartificial pancreas. Moreover, its use has the added advantage that this kind of transfer (ultrafiltration) does not depend upon generation of a concentration gradient. Therefore, the present experiments were undertaken to determine whether ultrafiltrate generated by the convection process from physiologic buffer, as well as from circulating blood, could support normal B-cell function in a bioartificial pancreas.

MATERIALS AND METHODS

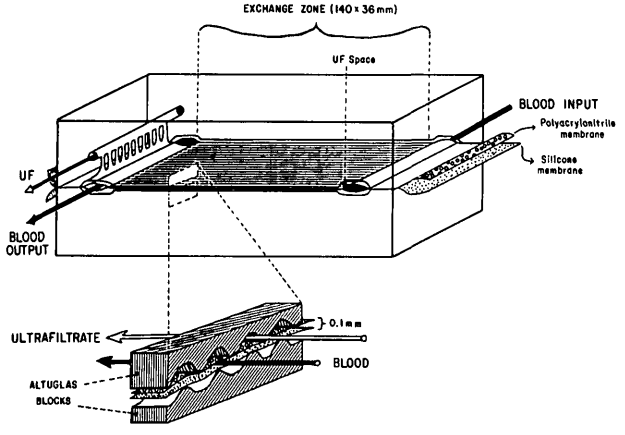
Ultrafiltrate (UF) from Krebs-Ringer bicarbonate buffer (KRBB) containing 0.3% bovine serum albumin (BSA, fraction V, Armour, Phoenix, Arizona) and from circulating rat blood was produced by an ultrafiltration cell (UFC) having the general features of a miniaturized artificial plate kid-

ney,[11] (Figure 1). The medium (KRBB or blood) was allowed to circulate between two sheet membranes (courtesy of Mr. Christen, Rhône-Poulenc, Paris, France), one made of silicone and the other of polyacrylonitrile (AN69, Rhône-Poulenc, Paris, France). The membranes were placed between two altuglas blocks that were tightened together by screws around the periphery. The polyacrylonitrile membrane was chosen because of its high hydraulic permeability (50×10^{-6} ml/min × cm² × mm Hg), its selectivity (mean 50% molecular weight cutoff: 11,500 daltons), and its previously demonstrated biocompatibility.[12] The exchange surface of the unit was 50 cm² (140 × 36 mm), and the volume between the two membranes was less than 1 ml.

For experiments using KRBB ultrafiltrate, KRBB was pumped to the UFC by a RP04AD pump (Hospal, Paris, France) at a rate of 5 ml/min. For experiments using rat blood ultrafiltrate, the UFC was connected to the animal via a vascular shunt, and blood pressure was used to pump blood to the UFC; for connection of the UFC, male Wistar rats (293 ± 7 g body wt, N = 20) were anesthetized (pentobartital, 50 mg/kg i.p.), and a homolateral carotid artery and jugular vein were each cannulated with short polyethylene (PE 90 Intramedic, Clay Adams, Parsippany, New Jersey) catheters and subsequently connected with silicone tubing (1 × 2 mm, Scurasil, Roger Bellon, Paris, France) filled with heparinized saline. Animals were then injected with heparin (50 U i.v., Abbott, North Chicago, Illinois). This dose was repeated every 2 h in the long-term experiments. The arterial line was connected to the input of the UFC, and the jugular line was connected to its output. UF was pulled from the cell by a pump (Minipuls, Gilson, France) and passed through a Millipore chamber containing isolated rat islets (see below and Figure 2) at a rate of 0.15 ml/min. The total dead space between the exchange membrane and the Millipore chamber was less than 1 ml.

Islet isolation and perifusion. Pancreatic islets were isolated from fed normal rats by the method of Lacy and Kostianovsky.[13] After digestion by collagenase (Worthington, Freehold, New Jersey), islets were hand-picked, washed twice in Hanks' solution, and then transferred into KRBB containing 2 mM glucose. They were then collected with a syringe and injected into the Millipore chamber (Swinnex 13, Millipore filter 10 μM, Millipore Corp., Bedford, Massachusetts) through a silicone T tubing. The Millipore chamber was placed in a thermostating jacket (37°C). In all studies, islets were initially perifused for 60 min with KRBB containing 2 mM glucose and 0.3% BSA equilibrated with O_2:CO_2 (95:5).

FIGURE 1. The ultrafiltration cell. Blood (black arrow) circulates between a supporting silicone membrane and a polyacrylonitrile membrane, across which ultrafiltrate is formed.



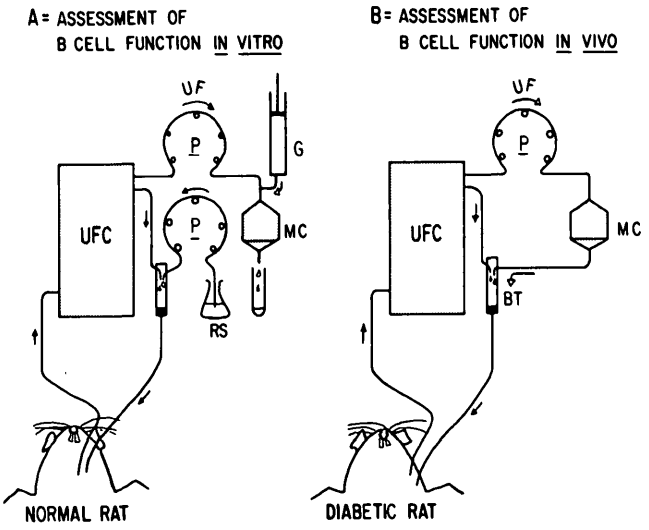


FIGURE 2. Islet perifusion with blood ultrafiltrate. (A) Assessment of B-cell function in vitro. Ultrafiltrate (UF), pulled out from the UFC by a pump (P), perifuses islets in a Millipore chamber (MC) whose effluent is sampled for glucose and insulin determination. The same pump is used for infusion of a replacement solution (RS). Glucose (G) is infused into the Millipore chamber containing the islets with a Harvard pump. (B) Assessment of B-cell function in vivo. Blood ultrafiltrate, having perifused islets, is reinfused to the animal whose blood is sampled by a femoral catheter (not represented). The junction site of blood and UF to be reinfused has a bubble trap function (BT).

Streptozotocin-induced diabetic rats. Fasting rats (297 ± 6 g body wt, N = 5) were injected with 40 mg/kg streptozotocin in citrate buffer at pH 4.2. Experiments were carried out at least 4 days later, and only glycosuric animals were used. No animal was ketonuric (Ketodiastix, Ames, Elkhart, Indiana).

EXPERIMENTAL DESIGN

Kinetics of glucose transfer from blood to the islet chamber. Normal fasting rats (N = 4) were connected to the UFC; UF at the entrance of the Millipore chamber containing no islets and blood were simultaneously sampled at 0, 2, 5, 10, 15, 30, and 60 min after i.v. glucose administration (1 g/kg body wt). Glucose disappearance rates[14] (K values) in blood and UF were calculated from 10-, 15-, and 30-min levels. Areas under the curve were calculated by integrating the incremental glucose concentrations above basal for each experiment using a Texas Instruments SR-60 calculator.

Composition of ultrafiltrate produced from blood of normal rats. Six fasting normal rats were connected to the UFC, and, after a 20-min equilibration period during which ultrafiltrate was reinfused to the animal, UF was sampled and aliquoted for glucose, sodium, potassium, calcium, total protein, and pH determination. Immediately thereafter, 6 ml arterial blood was rapidly collected from the carotid catheter, centrifuged, and the resultant plasma aliquoted for the same determinations.

Islet studies. B-cell responses to glucose were investigated in three sets of experiments.

Islet perifusion with KRBB and ultrafiltrate of KRBB. At the end of a 60-min pre-perifusion with KRBB containing 2 mM glucose, islets were perifused with KRBB containing 20 mM glucose for 20 min; islets were then again perifused with the initial KRBB containing 2 mM glucose for 20 min. Thereafter, this KRBB was passed through the UFC, and islets were perifused with its ultrafiltrate for 10 min; after this the islets were perifused with UF of KRBB containing 20 mM glucose for 20 min.

Islet perifusion with ultrafiltrate produced from blood of normal rats. Initially, islets were perifused as above in KRBB containing, successively, 2, 20, and 2 mM glucose, and then, after connection of the UFC to a fasting normal rat (325 ± 15 g body wt, N = 4), the islets were perifused with ultrafiltrate produced from blood of the animal, instead of ultrafiltrate from KRBB. After a 30-min exposure of islets to blood ultrafiltrate, a 245-mM glucose solution was infused through a side-arm into the input of the Millipore chamber (Figure 2A) at a rate of 0.01 ml/min with a Harvard pump for 20 min to assess islet B-cell function. UF effluent from the Millipore chamber was sampled for glucose and insulin determination. To avoid dehydration and electrolyte loss in rats due to hemofiltration, a replacement solution (NaCl, 146 mM; KCl, 4.4 mM; $CaCl_2$, 2.1 mM; KH_2PO_4, 1.5 mM; and $MgSO_4$, 1.2 mM adjusted to pH 7.6) was infused into the rats at the same flow rate as the UF flow rate (0.15 ml/min). Periodically, it was necessary to repeat pentobarbital injection to maintain the animals' anesthesia.

Islet perifusion with ultrafiltrate produced from blood of diabetic rats. Approximately 600 islets obtained from three normal fed rats were first perifused with KRBB containing 2, then 20, then 2 mM glucose as above; then the UFC was connected to a diabetic rat and the islets were perifused by ultrafiltrate produced from blood of the diabetic animal. The ultrafiltrate effluent from the Millipore chamber was infused into the animal at a silicone junction site. The configuration of this site was such that it acted as a bubble trap (Figure 2B). In these studies, rat blood was sampled by a femoral venous catheter (PE90, Intramedic) for glucose and insulin determination. Each blood sample was quantitatively compensated for with reinfusion of heparinized blood obtained from a normal fasting rat. Animals respired spontaneously under a O_2:CO_2 (95:5) flow and were warmed under a lamp (rectal temperature = 37°C) throughout all experiments.

In one experiment, the rat was disconnected from the device after glycemic normalization and maintained under the same conditions. Plasma glucose level was measured during the next 90 min. In some experiments, intravenous glucose tolerance tests (0.5 g/kg body wt) were performed in diabetic rats connected to the device. Similar tests were carried out in fed normal rats under similar conditions (oxygenation, thermostating, and compensation of blood loss), except that the rats were not connected to the device.

Sampling and assays. Effluent from the Millipore chamber was collected over 3-min intervals into pre-chilled tubes; aliquots were stored at −20°C until assayed for glucose and insulin. Glucose was measured with a YSI glucose analyzer (Yellow Springs, Ohio). Insulin was determined by radioimmunoassay using rat insulin as a standard.[15] Blood was collected in glass tubes and immediately centrifuged. An aliquot was stored at −20°C for assay of insulin. Blood and UF Na, K, Ca, total protein, and pH were determined by Autoanalyzer and blood gas analyzer.

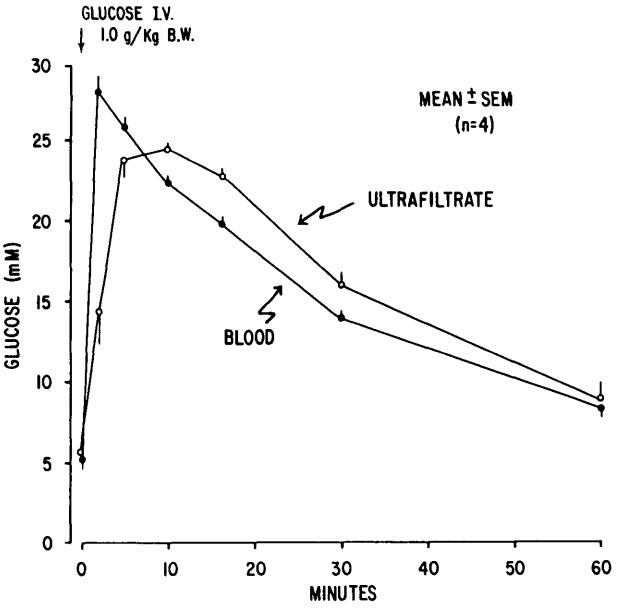


FIGURE 3. Kinetics of glucose transfer from blood to islet chamber. Glucose was assayed in simultaneously collected blood (closed circles) and ultrafiltrate (open circles) sampled after intravenous injection of glucose (1 g/kg) into normal rats connected to the device.

Presentation of results and statistical methods. All data in text and figures are given as the mean ± SEM, and their statistical significance was evaluated using paired and, when appropriate, unpaired, two-tailed *t* test.[16] Areas under the insulin curve were calculated from summation of each 3-min insulin output after subtracting the basal insulin output.

RESULTS

Kinetics of the glucose transfer through the membrane (Figure 3). Figure 3 shows the simultaneous blood and ultrafiltrate glucose concentrations after intravenous glucose (1.0 g/kg) administration into 4 fasting normal rats. The peak glucose concentration of the UF was slightly blunted (24.3 ± 0.5 versus 28.2 ± 0.8 mM, $P < 0.01$), and there was a 5–10-min lag between blood and UF concentration. However, both glucose disappearance rates (2.17 ± 0.26 versus 2.35 ± 0.14 %/min) and integrated areas under the curves (643 ± 41 versus 620 ± 41 mM/60 min) were virtually identical for UF and blood, respectively.

Composition of ultrafiltrate produced from blood of normal rats (Table 1). Table 1 gives the glucose Na^+, K^+, Ca^{2+}, and protein concentration, and the pH of blood and ultrafiltrate of blood in 6 normal rats. The only significant differences between blood and UF from blood were the lack of

TABLE 1
Na, K, Ca, total protein, glucose concentrations, and pH in plasma and blood ultrafiltrate (N = 6, mean ± SEM)

	Plasma	Blood ultrafiltrate
Na (meq/L)	148 ± 1	146 ± 1
K (meq/L)	3.6 ± 0.2	3.5 ± 0.1
Ca (meq/L)	5.0 ± 0.05	2.1 ± 0.1*
Proteins (g/L)	6.0 ± 0.1	Undetectable*
Glucose (mM)	7.6 ± 0.5	7.0 ± 0.2
pH	7.15 ± 0.05	7.61 ± 0.02*

* $P < 0.001$.

protein, the decreased Ca^{2+} concentrations, and the increased pH of blood ultrafiltrate.

Islet perifusion with KRBB and ultrafiltrate of KRBB (Figure 4). Glucose concentrations in effluent from the Millipore chamber were virtually identical in both perifusion conditions. In the KRBB perifusion, when the glucose concentration increased from 2.4 ± 0.3 to 18.4 ± 0.2 mM, insulin output increased from 1.6 ± 0.4 to 9.9 ± 1.3 ng/100 islets/3 min ($P < 0.02$), and then decreased to 4.3 ± 1.1 ng/100 islets/3 min when the glucose concentration decreased to 2.7 ± 0.1 mM. When islets were perifused with ultrafiltrate of KRBB and the glucose concentration increased from 2.5 ± 0.3 to 16.8 ± 0.1 mM, insulin output increased from 5.5 ± 0.6 ng/100 islets/3 min to 10.9 ± 1.1 ng/100 islets/3 min, $P < 0.005$); insulin output returned to the prestimulatory rates (4.5 ± 1.0 ng/100 islets/3 min) after the end of the stimulation period. The total amount of insulin released (area under the curve) was not significantly different when islets were perifused with KRBB (47 ± 12 ng/100 islets/24 min) and KRBB ultrafiltrate (41 ± 9 ng/100 islets/24 min).

Islet perifusion with KRBB, and ultrafiltrate produced from blood of normal rats (Figure 5). During the KRBB perifusion, when medium glucose concentration increased from 2.4 ± 0.2 to 20.5 ± 0.2 mM, insulin output increased from 1.1 ± 0.2 to 13.3 ± 1.4 ng/100 islets/3 min ($P < 0.005$); when the glucose concentration decreased to 2.5 ± 0.1 mM, insulin output returned to prestimulatory rates (1.3 ± 0.1 ng/100 islets/3 min). When the Millipore chamber was perifused with UF from blood of normal rats, the glucose concentration in the effluent of the Millipore chamber increased from 2.4 ± 0.1 to 7.5 ± 0.7 mM ($P < 0.005$) and remained stable at that concentration for the 20-min period. A significant release of insulin was observed during this period: insulin output increased from 1.8 ± 0.3 to 7.8 ± 0.7 ng/100 islets/3 min ($P < 0.005$) within the first 6 min. It then decreased to 5.1 ± 0.9 ng/100 islets/3 min over the next 6 min before increasing again to 8.9 ± 1.0 ng/100 islets/3 min. Subsequently, when glucose was infused into the Millipore chamber and the glucose concentration was increased to 21.2 ± 0.8 mM, insulin output increased from 7.3 ± 1.3 to 18.9 ± 0.8 ng/100 islets/3 min

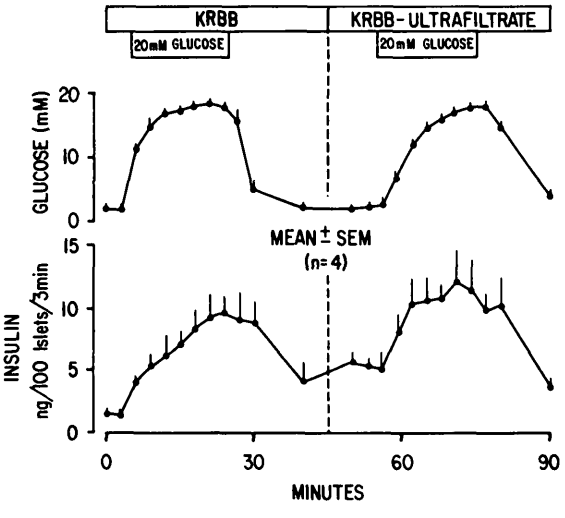


FIGURE 4. Islet perifusion with KRBB and ultrafiltrate of KRBB. Glucose (upper panel) and insulin (lower panel) in the effluent of the Millipore chamber are presented as mean ± SEM (N = 4).

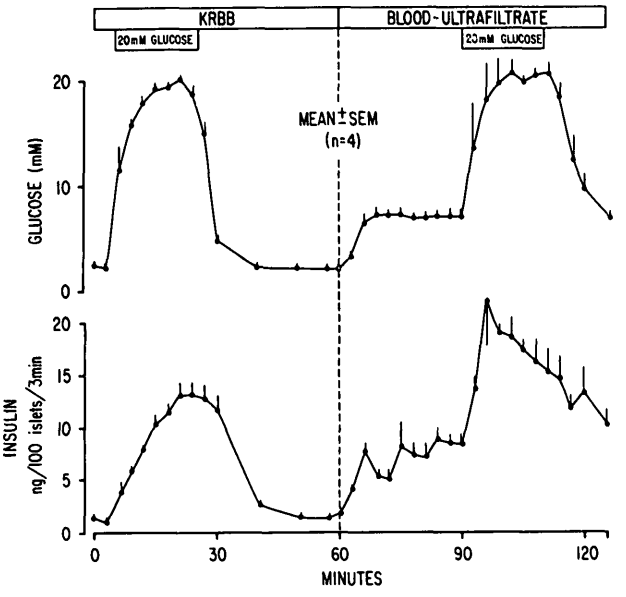


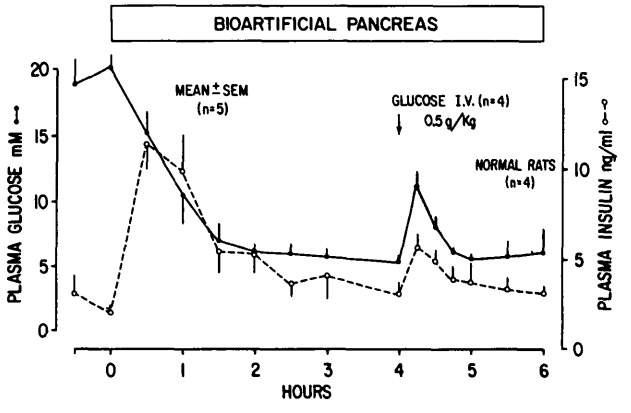
FIGURE 5. Islet perifusion with KRBB and blood ultrafiltrate (N = 4). Same presentation as in Figure 4.

($P < 0.01$); when the glucose infusion was terminated, insulin output subsequently returned to prestimulatory rates (10.0 ± 1.6 ng/100 islets/3 min). The total amount of insulin released (area under the curve) was similar in KRBB and blood UF perifusions (69 ± 6 versus 73 ± 11 ng/100 islets/24 min, respectively).

Islet perifusion with KRBB, and ultrafiltrate from blood of diabetic rats with reinjection of ultrafiltrate to the animals. In the first part of the experiment, 600 islets were perifused with KRBB and stimulated by 20 mM of glucose. Insulin output from the Millipore chamber increased from a 11 ± 2 ng/3 min basal output to 71 ± 7 ng/3 min ($P < 0.01$), and then decreased to 11 ± 1 ng/3 min after the end of the stimulation (N = 5). Immediately thereafter, the diabetic rats were connected to the UFC and the effluent of the Millipore chamber was reinfused into the animal. Glucose and insulin were assayed in femoral venous blood (Figure 6).

The initial plasma glucose concentration of diabetic animals before connection to the device was 19.8 ± 1.2 mM (N = 5); after connection of the animals to the UFC, plasma

FIGURE 6. Plasma glucose (closed circles) and insulin levels (open circles, dotted line) in streptozotocin-diabetic rats connected to the device. Four hours after connection, four animals were given an intravenous injection of glucose (0.5 g/kg). The shaded area represents plasma glucose levels (mean ± SEM) after intravenous injection of glucose (0.5 g/kg) in normal rats.



glucose decreased to 6.1 ± 0.5 mM ($P < 0.001$) within 120 min and remained at this level for 6 h. Plasma insulin increased from a basal level of 2.0 ± 0.1 to 11.4 ± 1.4 ng/ml ($P < 0.02$) at 30 min, and then decreased to approximately 4 ng/ml after 3 h. In an additional experiment, one rat was disconnected from the device at 120 min. Initial plasma glucose (19.7 mM) had decreased progressively to 4.9 mM at 90 min; after disconnection, plasma glucose increased from 5.9 mM to 9.0 mM within 30 min and reached 15.5 mM at 90 min after disconnection.

In four of these animals, intravenous glucose tolerance tests (0.5 g/kg) were performed 4 h after connection. The plasma glucose concentrations and the glucose disappearance rates observed were not significantly different from those in normal control animals ($K = 2.13 \pm 0.18$ versus 2.23 ± 0.09 %/min, NS). Plasma insulin increased twofold from a basal level of 2.9 ± 0.6 ng/ml to a peak value of 5.6 ± 0.8 ng/ml at 15 min, and then decreased toward basal levels. In normal fed control rats, plasma insulin increased from 4.9 ± 1.9 to 13.4 ± 6.9 ng/ml 15 min after glucose injection and then decreased to 3.7 ± 0.5 ng/ml after 60 min.

DISCUSSION

The present study was undertaken to determine whether ultrafiltrate would support normal islet B-cell function and, thus, whether the convection process could be used to achieve a rapid transmembrane passage of glucose for a bioartificial pancreas. This question derived from the theoretical consideration that diffusion of glucose and insulin through a membrane is relatively slow, and it has been suggested that use of diffusion in artificial devices could not provide an accurate control of plasma glucose concentration.[8] By contrast, transfer by convection, with the solvent drag effect, is generally more rapid. Moreover, this procedure has several advantages: the rapidity of glucose transfer would depend on the flow rate of ultrafiltrate and not on the generation of a concentration gradient (i.e., the presence of hyperglycemia), and all potential stimuli of insulin secretion could cross the membrane with the same rapidity provided that their molecular weights would be less than the exclusion limit of the membrane pores. Consequently, for the purposes of this study, an ultrafiltration cell suitable for experimentation on small animals was designed, and the kinetics of glucose transfer across its membrane and the B-cell function of islets perifused with ultrafiltrate produced by it were examined. After i.v. glucose administration to normal rats connected to the device, similar changes in glucose concentration in UF and blood were observed with a shift to the right of the UF curve of less than 10 min (Figure 3), indicating that this ultrafiltration cell was able to achieve an accurate minute-by-minute transmission of glucose level from blood to islet compartment.

In initial experiments to determine if ultrafiltrate per se could support normal B-cell function, insulin release from isolated islets of Langerhans perifused with ultrafiltrate from KRBB was compared with that from the same islets perifused with KRBB itself. Insulin responses to a glucose challenge were not significantly different with KRBB and KRBB-ultrafiltrate, indicating that ultrafiltrate of KRBB could substitute for KRBB and that proteins with a molecular weight greater than 40,000 daltons were not essential for normal acute release of insulin.

On the basis of these results, the effect of blood ultrafiltrate on B-cell function was next investigated. When islets perifused with blood ultrafiltrate were submitted to a glucose challenge, insulin responses were not significantly different from those observed when the same islets were subjected to a glucose challenge during perifusion with KRBB. Since glucose was infused directly into the Millipore chamber, this insulin release represented the secretion by the islets only and cannot be ascribed to that released from the pancreas of the connected rat. These results indicate that blood ultrafiltrate could acutely support normal B-cell function. Of interest is the observation that, when blood ultrafiltrate was allowed to perifuse islets after the initial KRBB perifusion, release of insulin (Figure 5) was observed. This phenomenon was not noted when islets were perifused with 2 mM glucose KRBB-ultrafiltrate, and could have been due to the increase in glucose concentration from 2.5 to 7.5 mM or perhaps to some other stimulant to B-cell secretion, such as amino acids. Here again, this could not be ascribed to endogenous insulin of the donor rat, since, in control experiments, when rats were submitted to hemofiltration without islets in the Millipore chamber, insulin was not detected in blood ultrafiltrate.

Additional experiments were undertaken to determine whether insulin released from islets perfused with blood ultrafiltrate could restore normoglycemia in streptozotocin-diabetic rats. Islets were perifused with ultrafiltrate of blood from streptozotocin-diabetic rats, and the effluent from the Millipore chamber containing the islets was reinfused into the diabetic animals. After connection of the animals to the device, plasma glucose decreased rapidly from approximately 20 mM (360 mg/dl) to normal levels within 90 min. This normalization of plasma glucose was more rapid than that in previous studies using the diffusion process.[3,6] For example, in a similar study using diffusion of blood glucose from diabetic rats through an artificial capillary system containing 1200 islets (twice more than in the present study), plasma glucose levels of the hyperglycemic diabetic rats were not normalized before 5 h. This delay could have been due to the lower exchange surface of the latter system or simply to the use of the diffusion process, which might have resulted in slower transfer of glucose to and insulin from the islet chamber.[6]

In contrast to other systems, the present device is based on a unidirectional ultrafiltrate flux and represents a closed-loop system with rapid kinetics for each of its three components: (1) the afferent transmission of changes in glucose concentration through the membrane was delayed by only 5 to 10 min (this lag may not be of sufficient magnitude to produce any significant alteration in glucose homeostasis); (2) the B-cell responses to both increases and decreases in glucose concentration of perifusion medium were immediate (Figure 4 and 5); and (3) the rapid delivery of secreted insulin to animals connected to the device was achieved by infusion and did not involve diffusion through a membrane. This latter point is particularly important since diffusion of insulin through membranes is slower than that of glucose.[7] These rapid kinetics were probably responsible for the apparent near normal "minute-by-minute" regulation of insulin secretion by blood glucose observed in the experiments involving diabetic rats. Indeed, when glycemia of diabetic animals decreased rapidly there was no overshoot hypoglyce-

mia, and stable normoglycemia could be maintained for up to 6 h. Moreover, plasma glucose responses after intravenous glucose challenge were normalized without secondary hypoglycemia, indicating that islets perifused with blood ultrafiltrate respond rapidly and appropriately to acute changes in glucose concentration without the apparent need for neural modulation. It could be observed that the increase in plasma insulin in diabetic rats submitted to a glucose challenge was relatively low with regard to that observed in normal rats, although the tolerance to glucose was normalized. This would suggest an increased sensitivity to insulin in the diabetic rats, perhaps due to their previous insulinopenic state.

In summary, the present studies demonstrate the short-term ability of blood ultrafiltrate to support normal islet B-cell function. Whether blood ultrafiltrate will permit long-term survival of islets with intact function awaits further investigation. There are some differences between the composition of ultrafiltrate and that of KRBB and blood (higher pH, absence of proteins) that might have adverse long-term effects on islet B-cell function. On the other hand, islets in vivo are not in contact with blood, but are perifused with an interstitial fluid; interstitial fluid in vivo, like ultrafiltrate produced from blood with our device, is formed in part by ultrafiltration of blood[17] and has a lower protein content and higher bicarbonate concentration than in blood. Further concerns are whether a system, such as that employed in the present study, has adequate biocompatibility as well as its need for chronic vascular access. Clotting has been a problem with other systems in previous studies.[2-6] Nevertheless, the ability of blood ultrafiltrate to support normal islet function should provide a model for further evaluation of a bioartificial pancreas in which islets could be protected by selective membranes against immune rejection; the use of ultrafiltration instead of diffusion could permit sufficient rapid transmission of metabolic signals from blood to islets to allow placing of islets at a distance from the ultrafiltration cell in a separate disposable chamber, which could be replaced if islet function became impaired.

ACKNOWLEDGMENTS

We are indebted to Dr. N. K. Man for his helpful advice concerning ultrafiltration. The excellent technical help of J. King, W. Blanchard, L. Hall, T. Rambis, and K. Greene, and the skillful editorial assistance of C. Wells is gratefully acknowledged.

This work was supported in part by Public Health Service International Research Fellowship 1-F05-TW02754-01 to Dr. Reach, by grants from the USPHS (AM20837), the Institut National de la Santé et de la Recherche Médicale (467778 and 497778), the Association Claude Bernard, and the Mayo Foundation.

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