Transplantation of Islet Allografts and Xenografts in Totally Pancreatectomized Diabetic Dogs Using the Hybrid Artificial Pancreas

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Previously the authors reported on a Hybrid Artificial Pancreas device that maintained patent vascular anastomoses in normal dogs and, when seeded with allogeneic canine islets, maintained normal fasting blood sugars (FBS) in diabetic pancreatectomized dogs. Eventual failure of these devices was believed to be related to loss of islet viability and/or insufficient islet mass. The current study evaluates the effect of increased islet mass produced by implantation of two islet-seeded devices in pancreatectomized dogs and compares the results with those from dogs that received a single device. Twelve of fifteen dogs receiving single devices showed initial function as determined by elimination or reduction of exogenous insulin requirement; four showed initial function and seven showed extended function (100 to 284 days). Excessive weight loss (more than 20%), despite normal FBS and insulin dependence, required that four animals in this latter group be killed. Devices seeded with xenogeneic islets have met with limited success. One dog that received two bovine islet-seeded devices achieved function for more than 100 days; the remaining bovine-seeded devices (n = 8) functioned for only 3 to 16 days. Porcine islet-seeded devices functioned for 14 and 80 days. Metabolic effects of the devices were assessed by intravenous glucose tolerance tests (IVGTT). Recipients of two devices seeded with allogeneic islets demonstrated improved IVGTT results when compared to those from pancreatectomized dogs and recipients of single devices but were abnormal when compared to intact animals. Histologic examination of device and autopsy material from all failed experiments was performed and showed no mononuclear cell infiltration of the islet chamber or vascular graft material, only a few incidence of device thrombosis, and varying degrees of islet viability as judged by morphologic and immunohistochemical evaluation. The authors believe they have demonstrated progress toward the development and clinical applicability of the Hybrid Artificial Pancreas.

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CLINICAL PROGRESS IN both renal and extrarenal whole-organ transplantation in the last decade has been phenomenal. Replacement of vascularized whole organs is common and generally considered the most effective treatment for a number of morbid and fatal diseases. This success is due, in part, to understanding the immunologic mechanisms of allograft rejection and to the prevention and treatment of rejection with increasingly effective immunosuppressive agents. Unfortunately chronic maintenance immunosuppression is associated with a number of complications: opportunistic infection, increased incidence of spontaneous neoplasms, failure to control rejection, metabolic alterations secondary to drugs, and direct drug toxicities. Nevertheless the benefits of successful whole-organ transplantation far outweigh the risks and complications of chronic immunosuppression in most situations.

Despite efforts to control blood glucose levels with diet, exercise, and exogenous insulin, patients with insulin-dependent diabetes mellitus continue to sustain secondary vascular, neurologic, and other complications. Although still controversial, the current weight of evidence suggests that this is due to failure to achieve normal physiologic control of blood sugar levels by these methods. Because of this whole-pancreas transplantation has been undertaken with increased frequency and improved success.[1] Excellent blood glucose control and insulin independence has been achieved after pancreas transplantation. Unfortunately, to date, reversal or stabilization of diabetic complications has been equivocal or unsubstantiated,[2-5] presumably because most pancreas transplants have been performed in patients with already-established late complications. Although patient and graft survival rates have improved steadily, whole-pancreas allotransplantation has

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the disadvantages of the risks of major surgery, the necessity for chronic immunosuppressive therapy, and the limited applicability because of shortage of donor organs. Although it is becoming apparent that pancreas transplantation should be done early to prevent development of complications, there is a natural reluctance to risk a major surgical transplant under significant immunosuppression very early in the course of diabetes when the disease is well controlled with insulin, the patient is free of complications, and when it is not known that such complications will occur in a given patient.

Islet transplantation has been studied extensively as an alternative to whole-pancreas transplantation. Islet transplantation has a number of practical and theoretical advantages. These include major surgery (and its potential complications) are avoided; multiple donors can be used as islet sources[6,7]; islets can be stored easily and frozen until used[8,9]; xenograft islets theoretically could be used[10]; and islets can be modified (immunomodulation) by culturing and *in vitro* other techniques to decrease immunogenecity.[11] The disadvantages of islet transplantation are that large numbers of human islet allografts are not available, large-scale isolation procedures are not well developed,[12] and islets are still of sufficient immunogenecity to require large amounts of immunosuppression to prevent rejection.[13] Nevertheless progress in clinical islet allografting was recently made.[11,14,15]

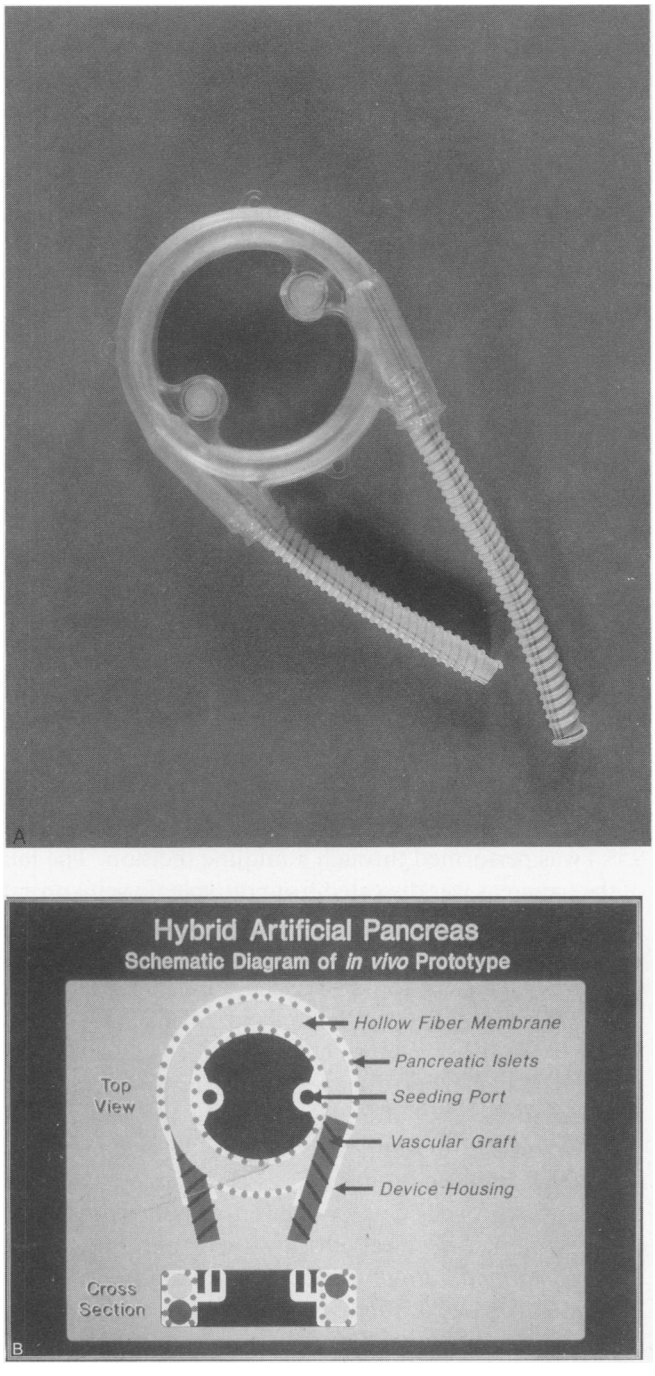
Because cellular transplants, such as islets, do not require large-vessel surgical anastomoses, they have the additional theoretical advantage of transplantation under conditions that produce a physical barrier between transplanted cells and the recipient's immune system, thus circumventing rejection and obviating the requirement of immunosuppression. In most barrier isolation systems, islets are separated from the host by some type of semipermeable membrane that allows molecules of defined size to pass through. Small molecules, including glucose, insulin, and other nutrients, are exchanged across these membranes, while effector lymphocytes, immunoglobulins, and other transplant rejection effector mechanisms are excluded. Two general kinds of immunoexclusion methods have been studied. In microencapsulation methods, single islets or groups of islets are enclosed or encapsulated by semipermeable membranes of structured organic components (such as alginate).[16,17] Microcapsules can be implanted into the peritoneal cavity or some other anatomic location. Nutrients pass across the membranes to nourish islets from the surrounding extracellular fluids and insulin secreted in response to glucose levels passes across into the extracellular fluid and is eventually absorbed into the circulation. These devices, in which the membrane is in direct contact with extracellular fluids, have been limited by fibrosis around the membrane, which eventually prevents adequate solute exchange. Progress in understanding and preventing fibrosis has been made.[18] A variation of this diffusion concept involves creation of larger diffusion chambers composed of semipermeable membranes holding a larger number of islets. In the second kind of immunoexclusion device, the semipermeable membrane is formed into a hollow tube, which is contained in a housing that is connected to a circulatory system of the host as an arteriovenous shunt. Islets are placed inside the device but outside the hollow tube membrane. Glucose, nutrients, and oxygen cross the membrane directly from the blood. Insulin produced by the islets diffuses directly into the blood stream. Rejection of islets is prevented because immune lymphocytes and immunoglobulins are excluded by the semipermeable membrane. In this type of exclusion device, the membrane is in direct contact only with blood and no other body tissues or fluids. Fibrosis of the membrane thus may be less likely to occur. Also, such macroencapsulation, intravascular exclusion devices depend on the nonthrombogenecity of the semipermeable membranes on contact with the blood stream.

We have previously reported initial studies using the Hybrid Artificial Pancreas device, which is suitable for implantation into larger animals.[19] In these initial studies we showed that the device could be implanted unseeded (without containing islets) into normal dogs and remain patent for almost 1 year and be well tolerated by the recipient animals. We also reported initial studies in which single devices containing canine allograft islets could maintain completely pancreatectomized dogs with normal fasting morning blood glucose levels with reduced or absent exogenous insulin requirements for varying periods of time without the need for immunosuppression. Recently we also reported the insulin output and response kinetics of seeded devices perfused *in vitro* with variable glucose concentrations.[20] Furthermore, because the current device configuration limits the amount of insulin output per device, efforts have been made to increase the insulin available *in vivo* for implanted diabetic recipients by implantation of two devices.[20] In this paper we report in detail the control of diabetes in pancreatectomized dogs by implantation of two devices seeded with canine allografts. We also report the results of initial experiments in which devices seeded with bovine and porcine islets were similarly transplanted to pancreatectomized diabetic dogs.

Materials and Methods

Hybrid Artificial Pancreas Device

The design of the Hybrid Artificial Pancreas used a modification of the technology that developed for a hollow fiber cell culture device. The prototype device consists of a single-coiled, hollow fiber membrane contained within a disk-shaped, acrylic housing (Fig. 1) that provides a



FIGS. 1A and B. (A) Photograph of a hybrid artificial pancreas device and (B) schematic representation of the device.

compartment for the islet cells. The specially prepared 5- to 6-mm internal diameter (ID) membrane was fabricated from an acrylic copolymer material using a modified solution spinning technique previously described.[21] The resulting ultrafiltration membrane has a nominal molecular weight cut-off of approximately 50,000 daltons (50 kd). An annular cavity surrounds the tubular membrane and defines the islet-containing chamber within the device. Access to this cavity can be achieved through two syringe ports capped with medical grade silicone. The Hybrid Artificial Pancreas device measured 9 cm in diameter, is 2 cm high, and weighs 50 g. The annular-shaped acrylic housing contained 30 to 35 cm of coiled, tubular membrane (ID, 5 to 6 mm) with a wall thickness of 120 to 140 nm. As configured the device provided approximately 60 cm^2 of membrane surface area and compartment volume around the membrane of 5 to 6 mL (Fig. 2). For these devices to be used for *in vivo* implantation, the venous and arterial ends of the hollow fiber membrane were connected to polytetrafluoroethylene (PTFE) vascular grafts with a matched inner diameter and an external coil wrap (IMPRA, Tempe, AZ). They were sealed into the housing using a medical grade epoxy. For those devices to be perfused *in vitro* (see below), the ends of the hollow fiber membrane were sealed directly within the acrylic tubing flanges. Both *in vivo* and *in vitro* devices were sterilized using ethylene oxide gas and aerated before use.

Islet Isolation and Device Seeding

Pancreata were surgically removed from dogs under general anesthesia with zero to minimal warm ischemia time. Islets were isolated using a collagenase digestion and discontinuous Ficoll density gradient method as described by Warnock and Rajotte.[22] Islets isolated from two to

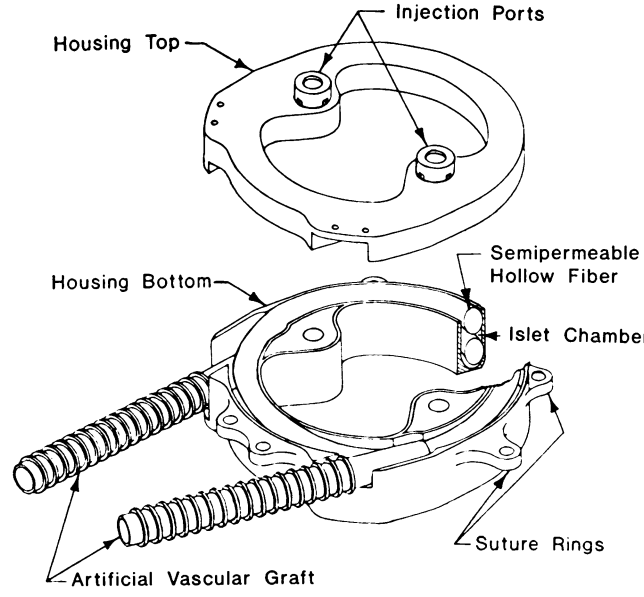


FIG. 2. Schematic diagram of the hybrid artificial pancreas device showing the relationships of the islets to membrane and blood stream.

three dogs were pooled before use. Islet purity exceeded 95% as determined by dithizone staining, amylase content, and histologic analysis. On the day of device implantation, isolated islets that had been in culture for 24 to 72 hours (see below) were collected by centrifugation. The islet pellet was resuspended in M199/EBB medium (Sigma Chemical Co., St. Louis, MO) supplemented with 5% fetal bovine serum, 40 IU/mL penicillin, 0.5 mol/L (molar) HEPES, and 2 mmol/L (millimolar) glutamine, and 1% agar. An aliquot of islets was taken for control culture dishes. The remaining islets were divided according to the number of devices being seeded. The islet suspension was injected into the device through the seeding port. The *in vivo* devices were transported on ice to the surgical site for implantation. Perfusion of *in vitro* devices was begun at the time *in vivo* devices were surgically implanted.

For those experiments in which xenograft islets were used, bovine pancreata were obtained from local abattoirs after animals were slaughtered in standard fashion. Pancreata were then removed with an extended warm ischemia time of 15 to 30 minutes. Porcine pancreatic glands were obtained by surgical removal using methods similar to those described for canine glands. Thereafter the islet isolation, purification, and device seeding procedures were essentially the same as with canine islets.

Culture of In Vitro *Control Devices*

For each experiment a corresponding *in vitro* control device was seeded at the same time and under the same conditions as the *in vivo* device and was placed in perfusion culture at 37°C in a humidified atmosphere of 5% CO_2 in air, as recently reported.[20] The *in vitro* devices were perfused with M199/EBSS medium containing 200 mg/dL glucose. The culture medium circulated through the device at a flow rate of 100 mL/minute using a peristaltic pump. The medium was changed three times per week. A sample was taken at each change for determination of immunoreactive insulin concentrations and stored at −20°C until assayed.

Measurement of Insulin Release

Determination of insulin levels in the *in vitro* perfusion studies was done using a previously reported radioimmunoassy protocol.[20] Free antigen was separated from the antigen–antibody complex by precipitation with IgGsorb (*Staphylococcus aureus* protein A, The Enzyme Center, Boston, MA). The limit of assay sensitivity was 25 U (1 ng)/mL.

Animals

Female mongrel dogs weighing 15 to 20 kg were purchased from Biomedical Association, Inc., Friedensbury, PA. In some experiments female beagles weighing 10 to 15 kg also were used. All dogs were examined by a veterinarian to ensure their health and were housed in compliance with United States Department of Agriculture (Animal Welfare Act) Regulations Part III at the Animal Resources Center facility of Harvard Medical School. In compliance with humane care considerations of the Animal Care Committee of Harvard Medical School, no animal could undergo more than three surgical procedures and animals that lost 20% or more of preoperative body weight were killed.

Surgical Procedures

Anesthesia. Baseline body weight, blood chemistry, and hematologic profiles were obtained before surgery. Animals were fasted overnight before surgery but had free access to water. Atropine (0.01 mg/kg) and, if required, acepromazine maleate (0.5 mg/kg), was administered intramuscularly 30 to 45 minutes before surgery. Anesthesia was induced with intravenous 4% Bio-tal (thiamylal sodium 0.5 mL/kg) injection and maintained with 1% to 2% halothane in 100% oxygen administered *via* an endotracheal tube. Lactated Ringer's solution (50 mL/kg) was given intravenously during surgery.

Pancreatectomy. Total pancreatectomy (more than 95%) was performed through a midline incision. The tail of the pancreas was dissected from the splenic vein, portal vein, and surrounding mesentery. The duodenal portion of the pancreas was separated from the duodenal wall. Care was taken not to damage the duodenal artery and vein that are situated parallel to the duodenal arch. Small vessels were either cautery coagulated or ligated and divided. The pancreatic duct was identified approximately 5 cm below the gastroduodenal junction and dissected in preparation for division. The head of the pancreas was dissected from the surrounding vessels and mesentery and the gland removed. On completion of pancreatectomy, the mesenteric defect was closed with 4-0 chromic catgut.

Device implantation. Device implantation was performed 2 to 3 weeks after pancreatectomy, during which time diabetes was treated by daily exogenous insulin. Laporatomy was performed through a vertical midline incision and the iliac vessels were exposed. The arterial limb of the device was anastomosed end to side to the left common iliac artery with continuous 6-0 Prolene and the venous limb was similarly anastomosed end to side to the right common iliac vein. During implantation no heparin was used except for flushing of the vessels before anastomoses. On completion of the anastomoses, the intestines was covered with the omentum and the device was placed between the omentum and the anterior abdominal wall. The device was fixed to the abdominal wall with inter-

rupted 2-0 Ethilon sutures (Ethicon, Somerville, NJ). The abdominal wall muscles and the skin wound were then closed with continuous 2-0 and 3-0 Ethilon, respectively. For those dogs in which two devices were implanted, the same techniques were used except that the arterial and venous limbs of the second device were anastomosed to the right common iliac artery and left common iliac vein, respectively.

Postoperative Care

Immediately after surgery, dogs were covered with blankets until they were fully awake. Acepromazine maleate and/or pentazocine lactate (Talwin; Winthrop Laboratories, New York, NY) was administered for pain relief if necessary. Water was given *ad libitum* but food was withheld until the second postoperative day. Amoxicillin (50 mg) was given for 3 days starting from the day of surgery. After operation multivitamin tablets (Vita-Min Palatabs, ESSAR Corp., Fort Dodge, IA; 6 tablets a day) and, in the case of pancreatectomized dogs, pancreatic enzyme tablets (Viokase-E, A.H. Robins Co., Richmond, VA; 2 tablets a day or equivalent as Viokase powder) were administered every day mixed with food. Aspirin (75 mg) was administered every day to animals in which devices were implanted. Vascular patency of the device was determined daily by the presence of a bruit(s) over the device(s). In the absence of a bruit on 2 consecutive days, the seeded device(s) was removed under general anesthesia using sterile procedures. Dogs from whom seeded devices were removed were subsequently killed when blood glucose concentrations returned to preimplantation levels.

Metabolic Studies and Postoperative Insulin Requirements

In pancreatectomized dogs with or without seeded devices, fasting morning blood glucose levels were determined using Chemstrip tapes as well as a Beckman glucose analyzer (Beckman Instruments, Brea, CA). Glucose values determined by chemstrip tapes correlated well with those obtained using the glucose analyzer (Spearman correlation, $R = 0.98$ with $n = 100$). Subcutaneous porcine lente insulin was administered once a day to maintain blood glucose levels between 150 and 250 mg/dL. Animals were weighed two to three times a week after pancreatectomy and seeded device implantation. Because of the tendency for hypoglycemia to occur in pancreatectomized dogs with excess exogenous insulin, no attempt was made to keep blood glucose levels in the 100 to 150 mg/dL range.

Intravenous glucose tolerance tests were performed between pancreatectomy and device implantation and periodically following device implantation on all dogs with functioning devices. A blood sample was taken for a baseline glucose level and then 50% dextrose (0.5 mg/kg) was infused intravenously. In pancreatectomized dogs, 0.25 mg/kg of dextrose was used. Further blood samples for glucose determination were taken 5, 10, 20, 30, 40, 50, 60, 90, and 120 minutes after injection. Postprandial glucose tolerance was determined by measuring blood glucose concentrations serially before and after standard meal feeding.

Histologic Studies

A complete gross and microscopic examination was performed on each removed device. All specimens were fixed in 10% buffered formalin and processed by standard techniques. Tissue sections were stained with hematoxylin and eosin. Appropriate sections of vascular grafts were studied to document anastomosis-related fibrous hyperplasia. Viability of islets was estimated by examining the H&E-stained islets under ×160 magnification. Islets were examined for the presence of central necrosis, generalized loss of nuclei or pyknotic nuclei, and reduction of cell size due to necrosis. An estimate of the percentage of viable islets was made using the extent of the presence of these changes in the individual islets. Also sections containing islets were stained with an immunoperoidase method to demonstrate stored insulin within the beta cells.[20] Complete autopsies were performed on all dogs in these experiments, except for those animals that received unseeded devices and those whose seeded devices are still functioning.

Results

Long-term Follow-up of Implanted (Unseeded) Devices in Normal Dogs

In our initial report,[19] two series of normal dogs were implanted with single devices that were not seeded with islets (unseeded devices). Table 1 presents updated results on these dogs, which have now been followed for an additional 8 months. In the first series, the arterial limb of the device was anastomosed end to end to the common iliac artery and the venous limb end to side to the common iliac vein. One dog maintained device patency for 560 days and another for 663 days. In the second series of dogs, the anastomoses were performed end to side (except

TABLE 1. *Summary of Unseeded Device Implantation*

Group	n	Device Patency (days after implantation)
I	12	4, 8, 18, 16, 26, 28, 50, 63, 170, 270, 560, >663
II	8	267, >514, >521, >530, >530, >537, >537, >544

Group I: End-to-end arterial anastomosis; no aspirin.
Group II: End-to-side arterial anastomosis; low-dose aspirin.

for two dogs) and all dogs received daily aspirin and perioperative antibiotics. Currently seven of eight of these normal dogs have patent devices implanted for more than 500 days confirmed by audible bruits and doppler ultrasonography. In both series of dogs, no significant weight loss or health abnormalities have been observed through the present time.

Metabolic Effects of Pancreatectomy

Since our initial report[19] in which 26 dogs had been completely pancreatectomized in preparation for device implantation, an additional 41 total pancreatectomies have been performed. All but one of these 60 dogs became hyperglycemic (more than 300 mg/dL) within 24 hours. The single dog that survived and was normoglycemic had a normal IVGTT after surgery; presumably fasting normoglycemia was maintained by residual pancreatic tissue. The failure to induce diabetes by our method of total pancreatectomy was less than 1.5%. Insulin dose required to maintain blood glucose concentrations in the 150 to 250 mg/dL range during the period between pancreatectomy and placement of seeded devices averaged from 18 to 32 units/day. As mentioned above blood glucose values were maintained at relatively high levels (approximately 200 mg/dL) because of the difficulty in maintaining constant normal glucose levels (100 to 125 mg/dL) in pancreatectomized dogs. In addition, despite the use of exogenous insulin, pancreatic enzymes and multiple vitamins, many of the dogs lost approximately 10% of their prepancreatectomy body weight in the 2 to 4 weeks before placement of seeded devices.

Effect of Single Devices Seeded with Canine Islet Allografts

Table 2 lists the postoperative insulin requirements of 15 pancreatectomized dogs implanted with single devices seeded with varying numbers (12,000 to 22,000 islets/kg) of canine islet allografts (13 previously implanted and reported,[19] and an additional two dogs subsequently transplanted). The average daily insulin requirement for each week after operation is listed for the first 14 weeks. Devices were defined as nonfunctioning on the postoperative day that exogenous insulin requirements reached 50% or more of the preoperative exogenous insulin dose. Devices in three dogs (PS11, PS12, PS16) failed to alter significantly the fasting blood glucose levels and were considered to be failed (F) despite successful initial surgery. The remaining 12 dogs had varying degrees of reduced insulin requirements for 14 to 280 days. Dogs PS19 and PS20 showed only minimal function for 1 to 2 weeks, failure being attributed to loss of islet function (device patent) in dog PS19 and infection in dog PS20. Five dogs demonstrated function for 42 to 76 days (PS7, PS8, PS18, PS21, PS25) and five dogs had function for 103 to 280 days (PS9, PS10, PS15, PS17, PS27). Note should be made that four dogs (PS7, PS10, PS15, PS27) had no insulin requirements for long periods during the first 14 weeks after transplantation. Stable glucose levels of 100 to 200 g/dL were achieved in some dogs immediately after implantation and in others only after 2 or 3 weeks. Of the three dogs with no significant function, failure was due to collapse of membrane and vascular graft (PS11), thrombosis (infection) (PS12), and islet failure (PS16). Of the 12 dogs that showed any significant post-transplant function, loss of device function

TABLE 2. *Insulin Requirement After Single Seeded Allograft Devide Implantation*

	Mean Insulin Requirement (unit/day)															
		Postimplantation Week														
	Pre-Device −1 Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Function (days)	Cause of Failure
PS7	16.9	0	0	0	0	0	0	0	0.6	0	0	1.4				76	Clotting
PS8	19.1	2.6	0	0	3.0	8.0	10.0									36	Islet
PS9	24.9	6.0	8.9	2.0	3.7	3.1	4.7	5.4	14.0	16.0	10.0	3.1	8.0	8.0	8.5	141	Islet
PS10	10.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	280	Unk
PS11	34.3	14.0	29.0	34.0												F	Device
PS12	22.5	9.4	18.6	22.0												F	Clotting
PS15	18.3	7.4	7.0	4.0	0	0	1.4	4.9	4.3	4.0	1.7	0	0	0	0	103	Clotting
PS16	14.0	9.4	20.3	22.0	17.3											F	Islet
PS17	28.3	5.7	9.4	10.0	10.6	12.9	14.0	12.3	10.0	17.7	18.0	16.6	18.0	16.6		64	Islet
PS18	24.3	9.1	12.6	10.9	10.3	14.0	19.0									31	Islet
PS19	18.0	2.9	12.3													9	Device
PS20	19.7	7.4	13.1	13.3												4	Infect
PS21	19.4	10.6	19.4	10.6	4.3	5.7	15.6	16.3	18.3	22.9	21.5					3	Islet
PS25	23.1	4.3	14.6	17.1	16.3	12.6	11.1									8	Islet
PS27	18.6	0	0	0	6.9	0	0	0	0	6.0	6.0	6.0	0	0	0	139	Islet

F, no function.
ISLET, failure of glucose control attributed to loss of islet function; anastomosis patent through postoperative course.

was attributed to late thrombosis in two (PS7 and PS15), membrane collapse (PS19), infection (PS20), and loss of islet function (viability) with patent devices (PS8, PS9, PS17, PS18, PS21, PS25, PS27). Dog PS10 functioned for 280 days and was found dead in his cage; autopsy failed to identify an obvious cause of death, although the animal had sustained significant (15%) weight loss.

We have reported previously that in animals in which single-device implantation was successful (as measured by reduced insulin requirements), the function of the device may be considered partial or complete. Figure 3 is taken from our previous publication[19] and illustrates the representative postimplantation course of animals with partial function. In dog PS15 up to 24 units/day of insulin were required before implantation to maintain fasting blood glucose levels at approximately 300 mg/dL. Blood glucose concentrations gradually returned to normal (100 mg/dL) after device implantation followed by relatively stable levels at approximately 200 mg/dL. Accordingly minimal doses of insulin (0 to 6 units/day) were required during this period. The dog was killed on day 103 after implantation because of loss of bruit associated with an abrupt increase in blood glucose levels. Histologic examination revealed thrombosis at the venous anastomosis

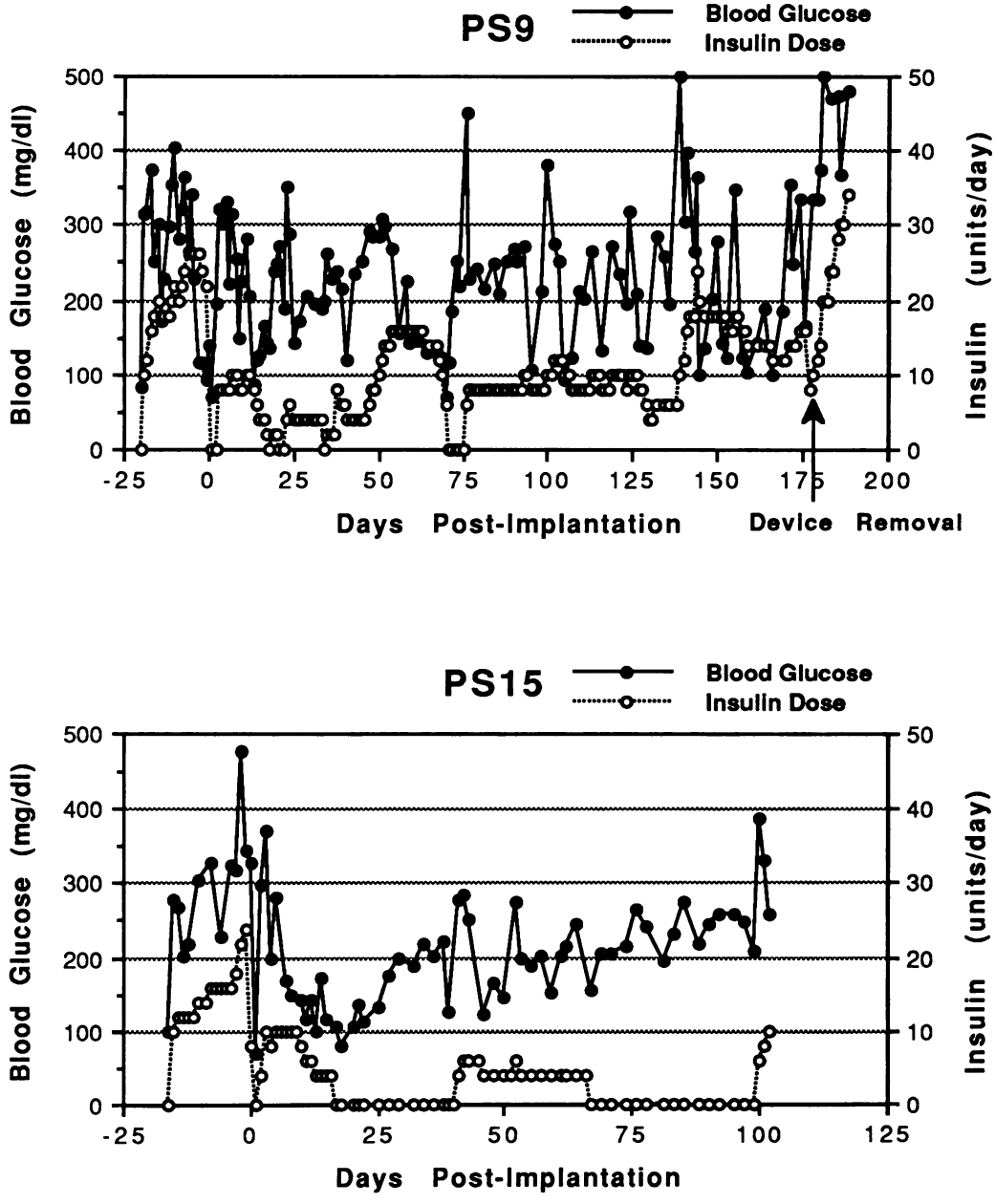


FIG. 3. Fasting blood glucose levels and exogenous insulin requirements in two dogs following implantation of single devices (allograft seeded) that show partial function. Dogs (PS15 and PS9) were pancreatectomized on days 16 and 20, respectively, relative to device implantation on day 0. Dog PS15 was killed on day 103 after transplantation because of loss of bruit. Dog PS9 had the device removed on day 177 after transplantation because of loss of function (day 175).

secondary to stenosis resulting from fibrous hyperplasia. Dog PS9 required much less insulin during the postimplantation period than during the 3 weeks before device implantation (up to 26 units/day); nevertheless it was extremely difficult to stabilize blood glucose levels in this severely diabetic dog. With removal of the device, however, the insulin dose increased from 16 to more than 32 units/day, confirming the finding that the implanted device had maintained some function, although it was not adequate to restore normoglycemia. Histologic examination of the removed device showed no cellular infiltration anywhere in or around the islets. Approximately 17% of islets were viable and contained insulin as evidenced by immunoperoxidase staining.[19]

Figure 4 taken from our previous report[19] illustrates the postimplantation courses of two dogs (PS10 and PS7) that showed long-term full function of the device. In dog PS10 blood glucose levels were as high as 350 mg/dL and up to 18 units/day of insulin was required before implantation. After implantation fasting blood glucose levels immediately returned to normal (100 to 125 mg/dL) and no insulin was required after the third day after implantation. However the fasting blood glucose levels slowly began to increase from 100 to 140 mg/dL to 150 to 250

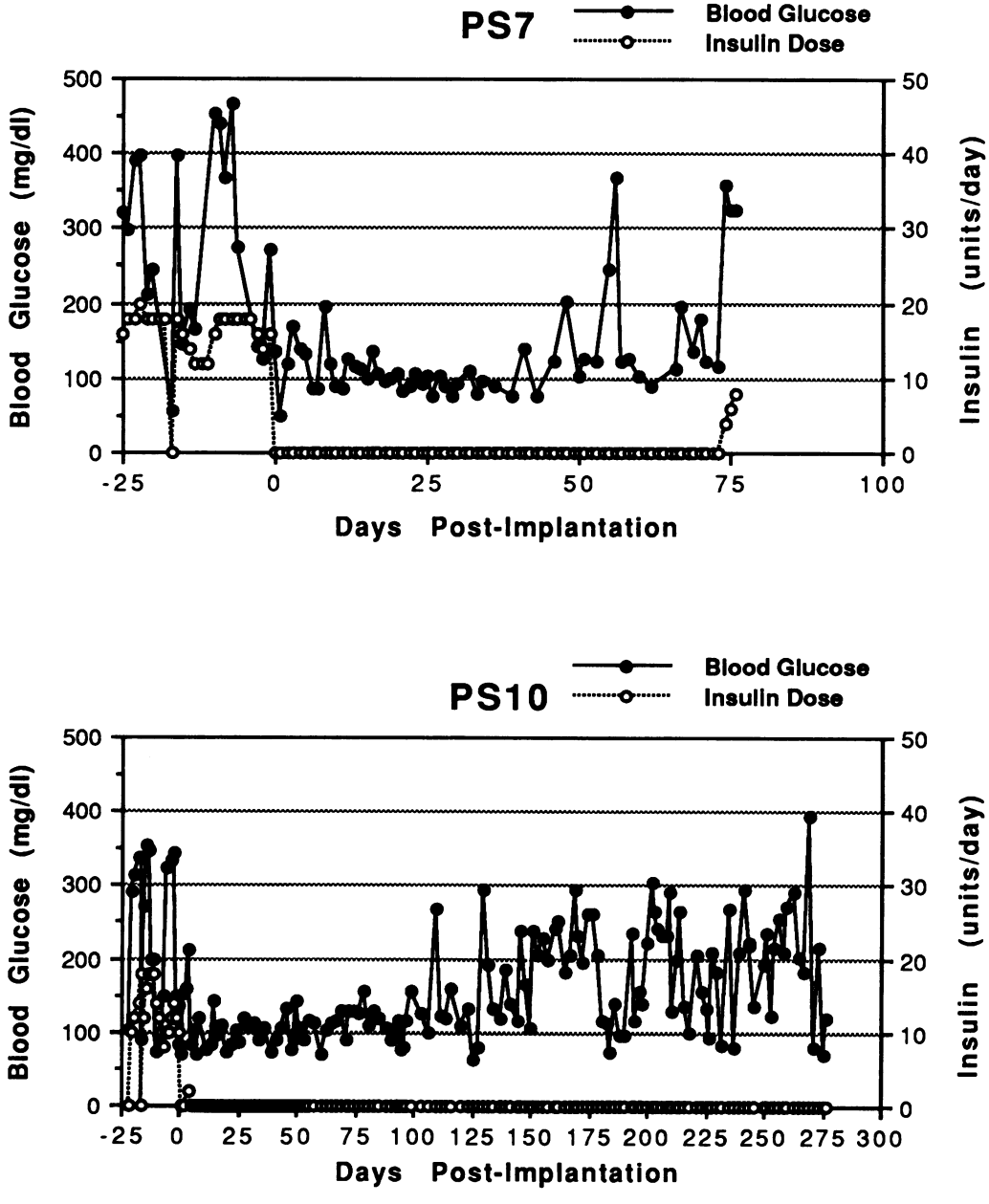


FIG. 4. Fasting blood glucose levels and exogenous insulin requirements in two dogs after single device implantation (allograft seeded) that show no further requirements for exogenous insulin. Dogs PS10 and PS7 were pancreatectomized on days 24 and -30, respectively, relative to device implantation on day 0. Dog PS7 had the device removed on day 76 after implantation because of functional deterioration subsequently found to be secondary to vascular thrombosis.

mg/dL after 125 days after implantation. The dog continued to do quite well with blood glucose values in the 150 to 250 mg/dL range without exogenous insulin for the next 150 days. This dog died (cause unknown) on postoperative day 280 off insulin with a patent functioning device. Dog PS7 required 17 units of insulin per day after pancreatectomy with poor fasting blood glucose control. After transplantation there was excellent glucose control (100 to 125 mg/dL) with no insulin requirements. The device was removed from dog PS57 on day 76 because of functional deterioration (thrombosis).

Effect of Double Devices Seeded with Canine Islet Allografts

In recent studies[20] we demonstrated that the output of the seeded devices as currently configured is limited by the maximum number of islets that can be placed in the device volume. Pending redesign of the hybrid pancreas device, experiments were done to increase the output of insulin available to pancreatectomized dogs by implanting two seeded devices (Table 3). All 13 dogs implanted with double devices showed some degree of device function. Four dogs had function for 100 to 284 days (PS22, PS23, PS26, PS36) and three dogs are still functioning more than 78 days (PS56), more than 162 days (PS50), and more than 125 days (IP-1) after transplantation. The remaining six dogs had function from 26 to 72 days. One dog (PS23) died of postoperative small bowel obstruction with a functioning device with patent anastomosis. Four dogs (PS28, PS30, PS36, PS49) were killed with functioning devices because of excessive weight loss despite the fact that they were off exogenous insulin at the time they were killed. All four dogs were found to have patent anastomosis (dog PS28 had one device anastomosis occluded). By our laboratory rules, if any animal loses up to 20% body weight the animal is killed on the basis of humane care. One dog (PS48) lost function on day 72 from clinically occluded devices (loss of bruit) confirmed at autopsy. Four dogs (PS22, PS24, PS26, PS35) lost function with clinically patent devices (audible bruits). Both devices at autopsy were patent in dogs PS22, PS24, and PS26 and the cause of failure of these devices was attributed to loss of islet function. Dog 35 had one (left) device patent and cause of failure was also attributed to loss of islet function. Thus implantation of two devices was more effective in controlling diabetes in totally pancreatectomized dogs. All dogs showed some function and 10 of 13 dogs were completely free of exogenous insulin for a significant period of time.

Figure 5 illustrates the first 200 days after transplantation course of dog PS22, which required approximately 18 units of insulin/day before double-device implantation. Note that for the first 100 to 150 days the fasting morning glucose levels were well controlled and were stabilized in the range of 100 mg/dL. During the next 50 to 75 days, the dog required no insulin, but the initial low glucose levels gradually increased. After 200 days the animal intermittently required 4 to 6 units of insulin to maintain the blood glucose level under 200 mg/dL. By day 284 this dog required up to 10 units of regular insulin per day and the devices were declared nonfunctioning. The devices were recovered and vascular anastomoses were patent.

TABLE 3. *Insulin Requirement After Double Seeded Allograft Device Implantation*

	Mean Insulin Requirement (unit/day)																
		Postimplantation Week															
	Pre-Device −1 Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Function (days)	Cause of Failure
PS22	18.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	263	Islet
PS23	20.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	209	Ileus
PS24	26.6	12.6	16.3	12.3	7.4	4.3	0	12.3	7.7	8.6	14.0	17.4	20.0	22.5		64	Islet
PS26	27.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	169	Islet
PS28	22.9	5.7	2.9	3.1	0	0	0	0								51	BW↓
PS30	16.3	0	0	0	0											28	BW↓
PS35	29.7	12.9	16.3	10.0	14.3	20.0	17.1	19.3	20.0	22.9	27.7	21.7	24.0			28	Islet
PS36	32.3	0	0	0	0	0	0	0	0	0	1.1	6.6	5.4	5.1	6.0	99	BW↓
PS48	19.9	1.1	1.7	2.9	1.7	2.9	0	1.7	8.0	80	7.7	10.9	12.6	14.3		90	Clotting
PS49	20.9	0	1.1	0	0											26	BW↓
PS50	22.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	>162	
PS56	36.9	6.0	6.0	6.0	5.1	0	0	0	0	0	0	0	0	0	0	>78	
IP1	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	>125	

Islet, failure of glucose control attributed to loss of islet function; anastomosis patent through postoperative course and at postmortem examination.

BW, dog killed because of more than 20% loss of body weight with functioning device; anastomosis patent at postmortem examination; systemic bacterial sepsis present.

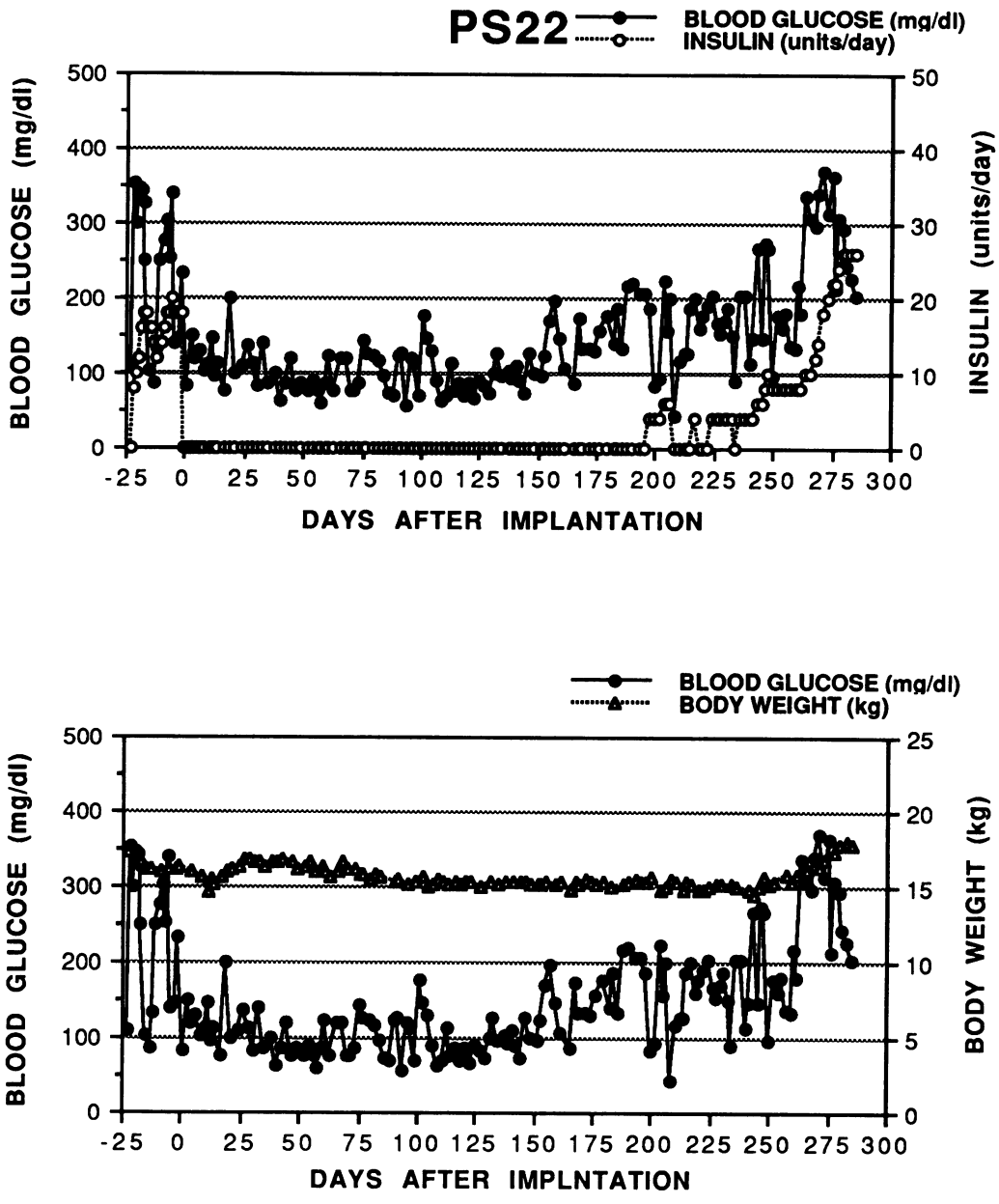


FIG. 5. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-de-vice implantation (allograft seeded) in dog PS22 (see text).

Less then 20% of the islets in both devices (see below) were found to be viable. Note should be made that the animal maintained his weight throughout the course of the experiment. Figure 6 illustrates the case dog PS23, which required approximately 20 units of insulin per day before implant. Again excellent, tight glucose control was maintained for the first 75 days with a blood glucose level at 100 mg/dL or less. At approximately 100 days after operative, tight glucose control was lost with varying glucose levels at or less than 200 mg/dL, although no insulin was required. On day 207 the animal became distended and eventually died on day 209 from small bowel obstruction secondary to adhesions to one of the devices. Weight was maintained normally throughout the postoperative course. On postmortem examination both devices were patent. Histologic examination revealed less than 20% of islets were viable. Figure 7 shows the postoperative course of dog PS50. This animal required 22 units of insulin per day before transplantation. Excellent glucose control was maintained for the first 40 to 50 days, followed by some deterioration for the succeeding 50 days, and spontaneous improvement for the next 60 days. This dog has not required insulin for more than 150 days after double-device implantation. Body weight is well maintained.

Figure 8 shows the postimplantation course of dog PS56 after double-device implantation. This dog initially required some insulin immediately after operation. How-

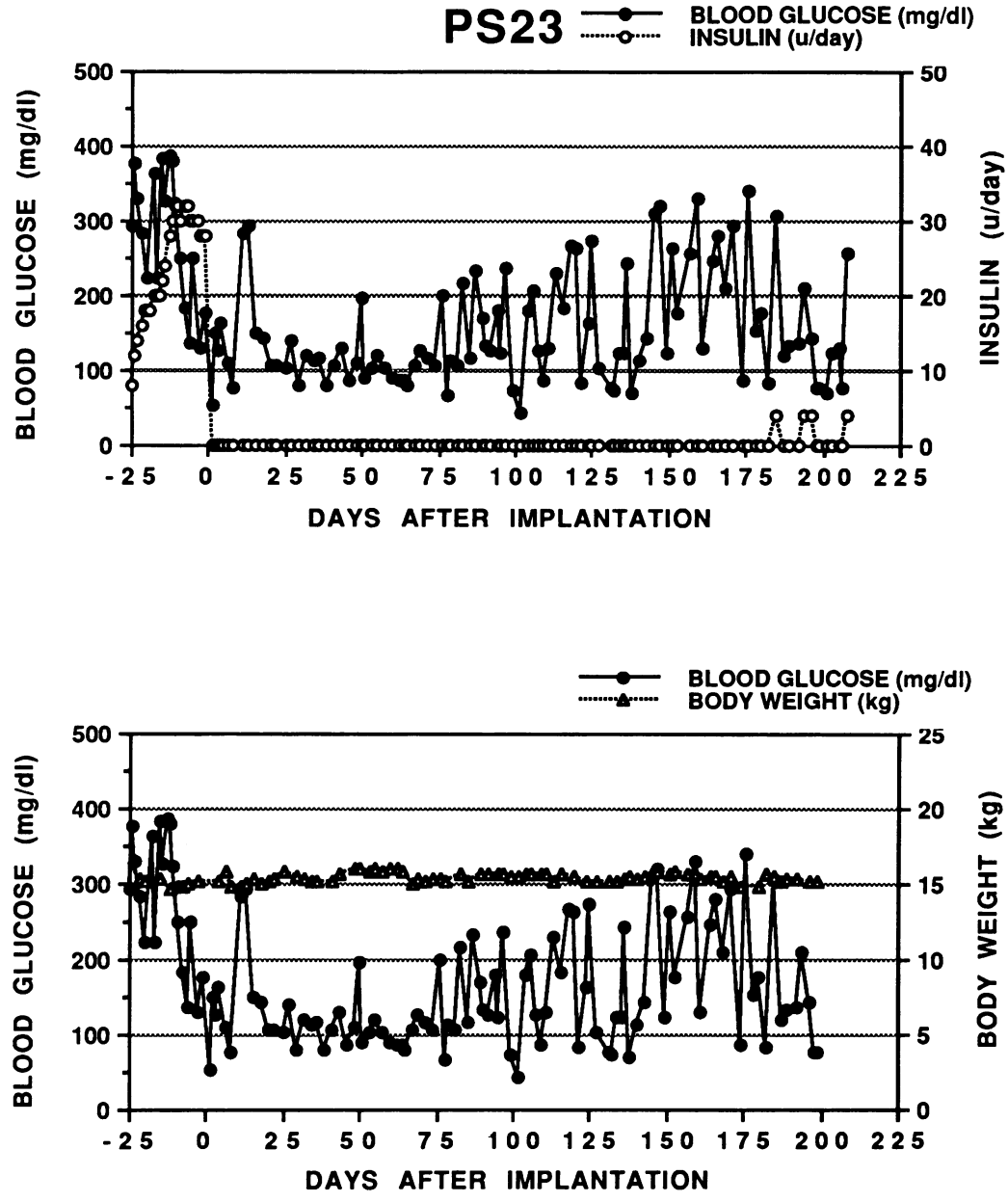


FIG. 6. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-device implantation (allograft seeded) in dog PS23 (see text).

ever, after approximately 3 weeks, blood glucose levels decreased and no exogenous insulin was required. This dog has not required insulin for 78 days after device implantation.

Xenograft-seeded Double Devices

Nine dogs have each received two implanted devices seeded with bovine islets and two dogs (PS43 and PS63) received devices seeded with porcine xenograft islets (10,000 to 15,000 islets/kg per device). Thus far we have had limited success (Table 4). Of nine dogs given bovine seeded devices, one had significant function at 106 days (PS29) while the other eight have essentially failed in that function (as defined as less than 50% of preimplantation insulin requirement) was only achieved for 3 to 16 days. Two dogs (PS42 and PS63) were implanted with porcine islets and functioned for 80 and 14 days, respectively.

Dog PS32 showed variable function for the first 3 weeks but lost audible bruit on day 21 and had occluded anastomoses at autopsy with 0% islet viability in both devices. PS32 had no real function and at autopsy had patent anastomoses but poor islet viability. Dog PS38 also had no real function and at autopsy showed thrombosed arterial and venous anastomoses with extensive infection in the device and zero islet viability in both devices. PS40 presumably had function for 2 to 3 weeks. At autopsy the right device was patent but the left was occluded with no islet viability. Dog PS46 functioned for about 2 weeks

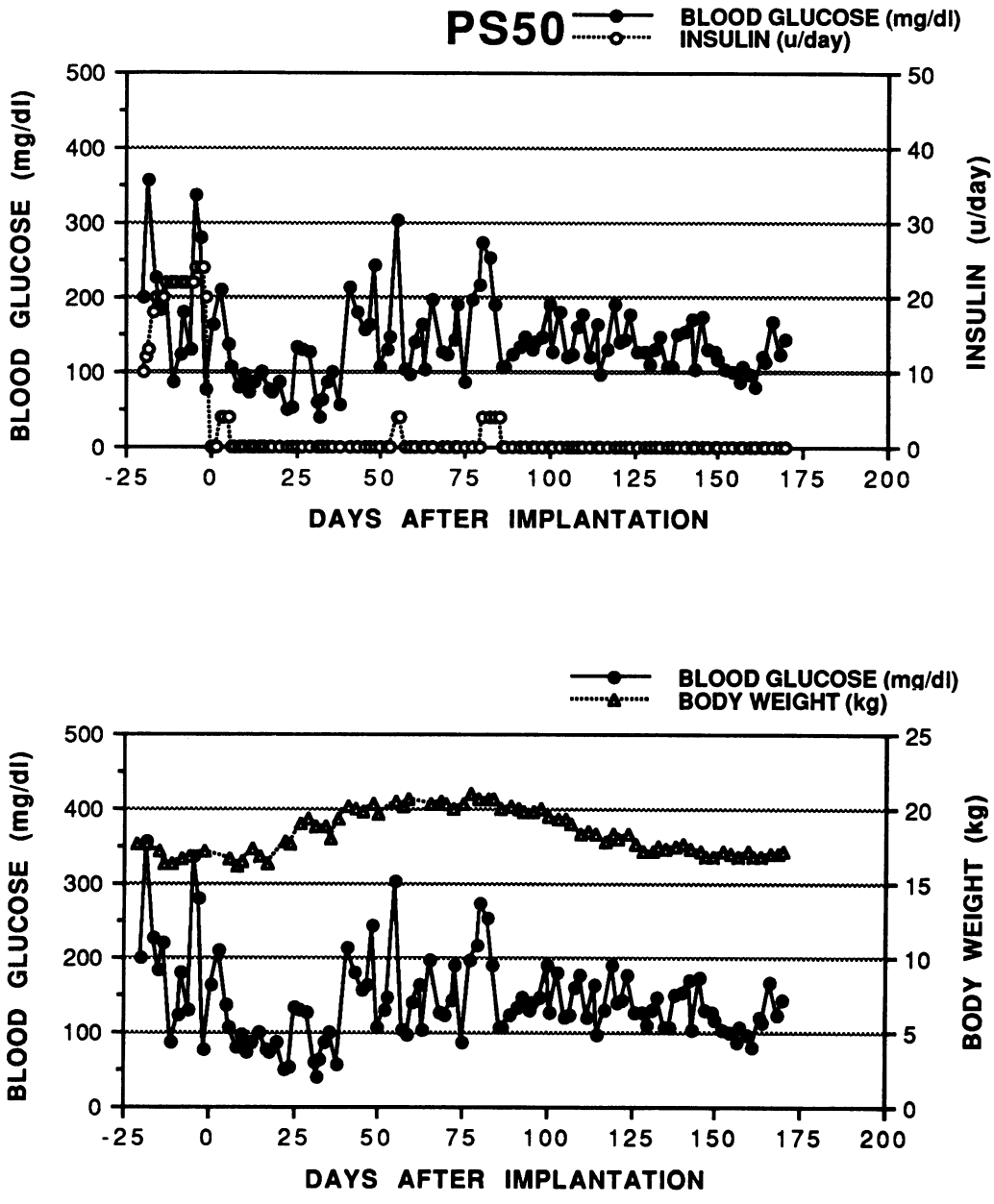


FIG. 7. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-device implantation (allograft seeded) in dog PS50 (see text).

and then lost function. At autopsy both devices were occluded and there was no islet viability.

Dog PS51 had occluded devices and no viability with extensive bacterial sepsis. Dog PS52 had patent devices but no islet viability in both devices as well as bacterial infection of both grafts and systemic sepsis. Dog PS53 had patent devices at autopsy but no islet viability in both devices and failure was attributed to lack of islet function. Dog PS63 had excellent function for 2 weeks but developed signs of pneumonia and had to be killed (autopsy results not available at this time).

Figure 9 illustrates the course of dog PS29, an animal that required 14 units of insulin per day before double-device xenograft implantation. For the first week after transplantation, no insulin was required followed by small requirements during the second and third weeks. The insulin requirement was then zero for approximately the next 50 days, with excellent fasting glucose control (less than 100 mg/dL). After 11 weeks insulin was again required with poor glucose control. After 100 days the insulin requirement exceeded 50% of the pretransplant dose and the device was considered nonfunctional. The weight of the dog was well maintained throughout this study. The animal was found dead in his cage on day 100 and autopsy failed to reveal a cause of death. Histologic examination of the device showed islet viability of less than

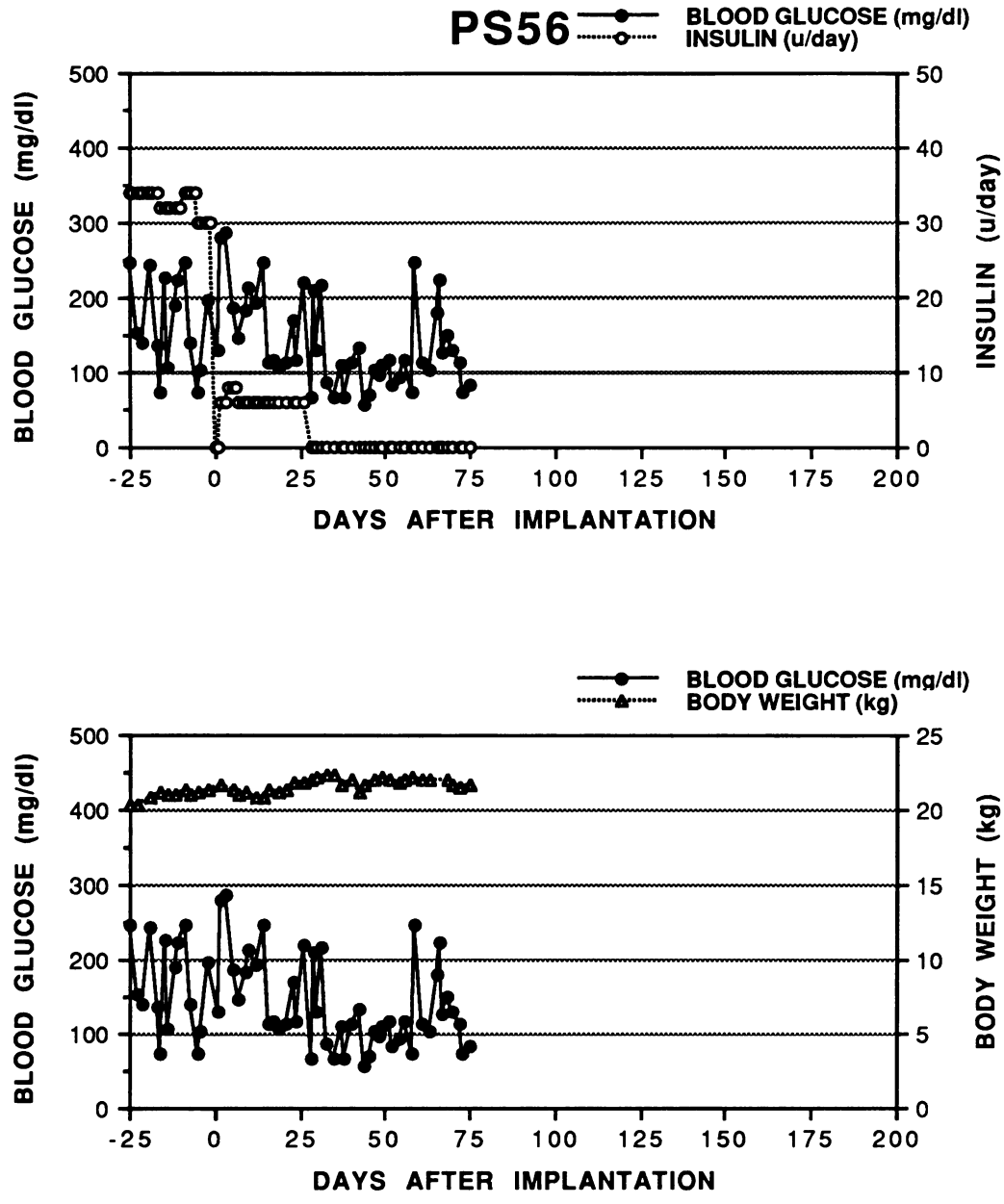


FIG. 8. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after device implantation (xenograft seeded) in dog PS56.

20%, although vascular anastomoses were patent. Because the insulin requirement was more than 50% before the animal's death and both devices were patent at autopsy, loss of islet function was the designated cause of failure. Figure 10 shows the postimplantation course of dog PS43. This animal required approximately 24 units of insulin per day before transplantation. For the first 10 weeks after transplantation, small amounts of insulin were required and the glucose control was acceptable but erratic. From days 75 to 100 after operation, insulin requirements increased to more than 50% of preoperative levels and glucose control deteriorated. The device was declared nonfunctional at day 80 (when more than 50% preoperative insulin was required). The device was recovered on day 142 and no viable islets were found to be present, despite the fact that the vessels were patent.

Metabolic Studies on Seeded Device Implanted Dogs

In our previous studies[19] of single-seeded devices of canine islet allografts in pancreatectomized diabetic dogs, data suggested that although both postprandial blood glucose levels and intravenous glucose tolerance tests were somewhat improved, they remained abnormal. Figure 11 is taken from a previous publication.[19] In both dogs (PS10 and PS7) whose cases are illustrated, the IVGTT improved

TABLE 4. *Insulin Requirement After Xenograft (Bovine and Porcine) Double Device Implantation*

	Mean Insulin Requirement (unit/day)														
		Postimplantation Week													
	Pre-Device −1 Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Function (days)
PS29	14.0	0	3.4	2.6	0	0	0	0	0	0	0	0	4.3	7.4	6.3	106
PS32	18.0	8.3	12.3	6.0												4
PS37	22.9	10.9	21.7	23.1	15.1	14.6	19.1	15.7	19.4	26.9	22.0	26.9	25.3	26.9	25.3	3
PS38	38.6	15.8														4
PS40	35.3	11.1	16.3	18.9	22.0	26.3	26.0	28.0	32.0							16
PS46	24.9	8.0	16.0	18.6	24.9	20.3	22.3	16.3	19.7	20.0	13.7	17.7				14
PS51	19.0	3.0														3
PS52	27.4	5.4	14.3	28.0												11
PS53	30.9	14.6	28.4													5
PS43	23.7	5.1	3.4	6.6	6.6	5.4	2.9	6.9	10.6	7.7	6.9	10.0	12.0	18.0	26.3	80
PS63	23.1	5.5	4.9													14

but never to normal. Also the abnormal postimplantation IVGTT stabilized for a period and then deteriorated. Because of difficulties of getting accurate postprandial studies in dogs (food must be given by gavage), only IVGTT studies have been performed in dogs that received double devices. Figure 12 illustrates the IVGTT in dog PS50, which has not required insulin for more than 160 days. On day 27 after operation, the IVGTT was abnormal but surprisingly improved. More than 120 days later, the IVGTT was still remarkably improved without further deterioration (as shown with dogs PS10 and PS57). Figure 13 shows a dog (IP-1) that had a double device implanted and after 2 weeks had a total pancreatectomy. After pancreatectomy the animal required little or no insulin. The animal has not required insulin for more than 100 days after pancreatectomy with well-maintained weight. Figure 14 shows that the IVGTT in this animal is also abnormal but significantly superior to that which one could expect in a completely pancreatectomized dog, as illustrated by the postpancreatectomy IVGTT (preimplantation) of dog PS50. Similarly dog PS56, which was free of insulin on day 70 after implantation, has an abnormal but significantly improved IVGTT at day 64. Although it is difficult to quantify, it is our impression that IVGTT studies in double-device allograft seeded dogs are more improved than in single-device animals and the IVGTT remains more stabilized.

Analysis of In Vitro *Insulin Output of Devices and Correlation with* In Vivo *Function as Determined by Reduction in Exogenous Insulin Requirement*

We recently reported the *in vitro* output of Hybrid Artificial Pancreas devices seeded with canine islet allografts. The devices were shown to maintain insulin output in the presence of a constant stimulatory glucose level (200 mg/dL). In perfusion studies the device could support islet function for more than 9 months, although the level of insulin output did show a decrease during the time of the culture. Data reported[20] from 18 devices maintained on culture for periods of 60 to 156 days (80 ± 5, mean + SEM) showed that insulin secretion peaked within the first 2 months of culture and then decreased slowly. Perfusion studies also demonstrated that islets responded to an increase in glucose concentration from 100 to 300 mg/dL with an increase in insulin secretion.

Table 5 lists the *in vitro* insulin output of canine allograft seeded devices at various times during perfusion. Because insulin output peaked in the 2 months after initiation of perfusion, correlation was made with average daily outputs during the first 30 to 60 days only.

It is important to re-emphasize that for each dog in the double-device allograft group (shown in Table 3) a device was seeded with a similar number of islets from the same islet preparation. This device was perfused *in vitro* beginning the same day the other two devices were implanted surgically. To estimate the amount of insulin that might be secreted *in vitro* by the two implanted devices, the output of the single-perfused device was simply doubled. Dogs PS22, PS23, PS30, PS48, PS49, and PS50 required little or no insulin after implantation through the first 30 to 60 days and the estimated *in vivo* output of two devices (double the *in vitro* output of a single device) correlated well with the preoperative insulin requirement. Data from dog PS28 also correlated remarkably well. Dog PS24 required almost 14 units per day and thus correlated with a estimated output of two devices of 11 units and a postoperative insulin requirement of 26.6, *i.e.*, estimated *in vivo* output plus postoperative exogenous insulin equalled the preoperative insulin dose. On the other hand, dogs PS26, PS36, and PS56 showed less correlation and the preoperative requirements were larger than estimated *in vivo*

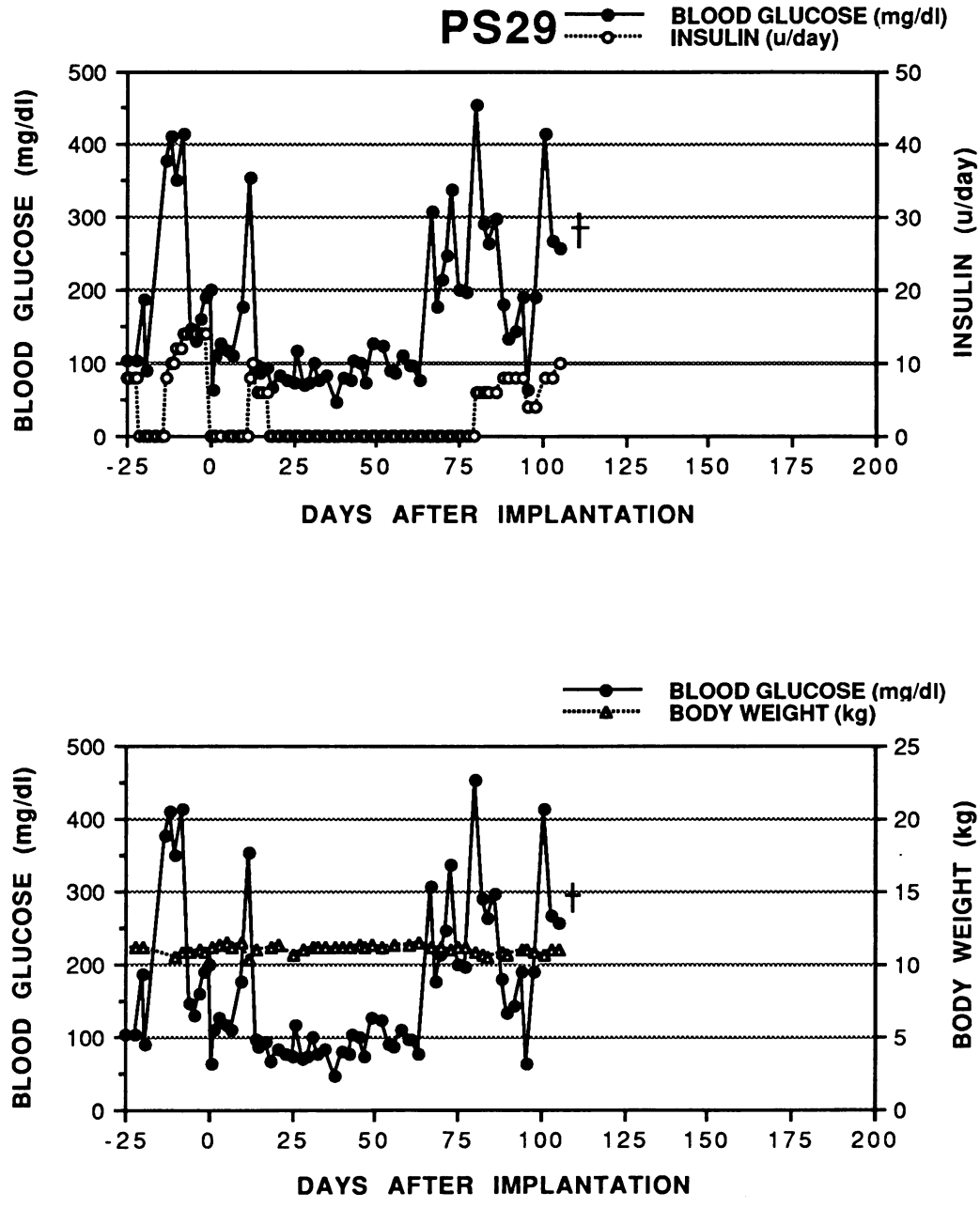


FIG. 9. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-seeded device implantation (xenograft seeded) in dog PS29.

output of two devices (PS26 and PS36) and exogenous insulin requirements were significantly less than would have been expected.

Table 6 presents a similar analysis for the xenograft animals. It is of interest that the two long-term xenograft dogs (PS29 and PS43) had good correlation between the estimated *in vitro* insulin output and the preoperative insulin requirement. Data was not available for dogs (PS38, PS51, PS52, and PS63). Few conclusions can be made from the data from the other dogs because their survival was so short relative to length of time of *in vitro* perfusion.

Analyses of Device Patency, Histologic Islet Viability, and Causes of Failure After Double-Device (Allograft) Implantations

Table 7 presents a summary of the length of function, anastomotic patency, estimate of percentage of islet viability on histologic analysis, and cause of failure for all dogs receiving double device (allograft) implantations. Dogs that lost function had devices surgically removed and subjected to histologic analyses. Dogs were subsequently killed and a complete autopsy was performed on

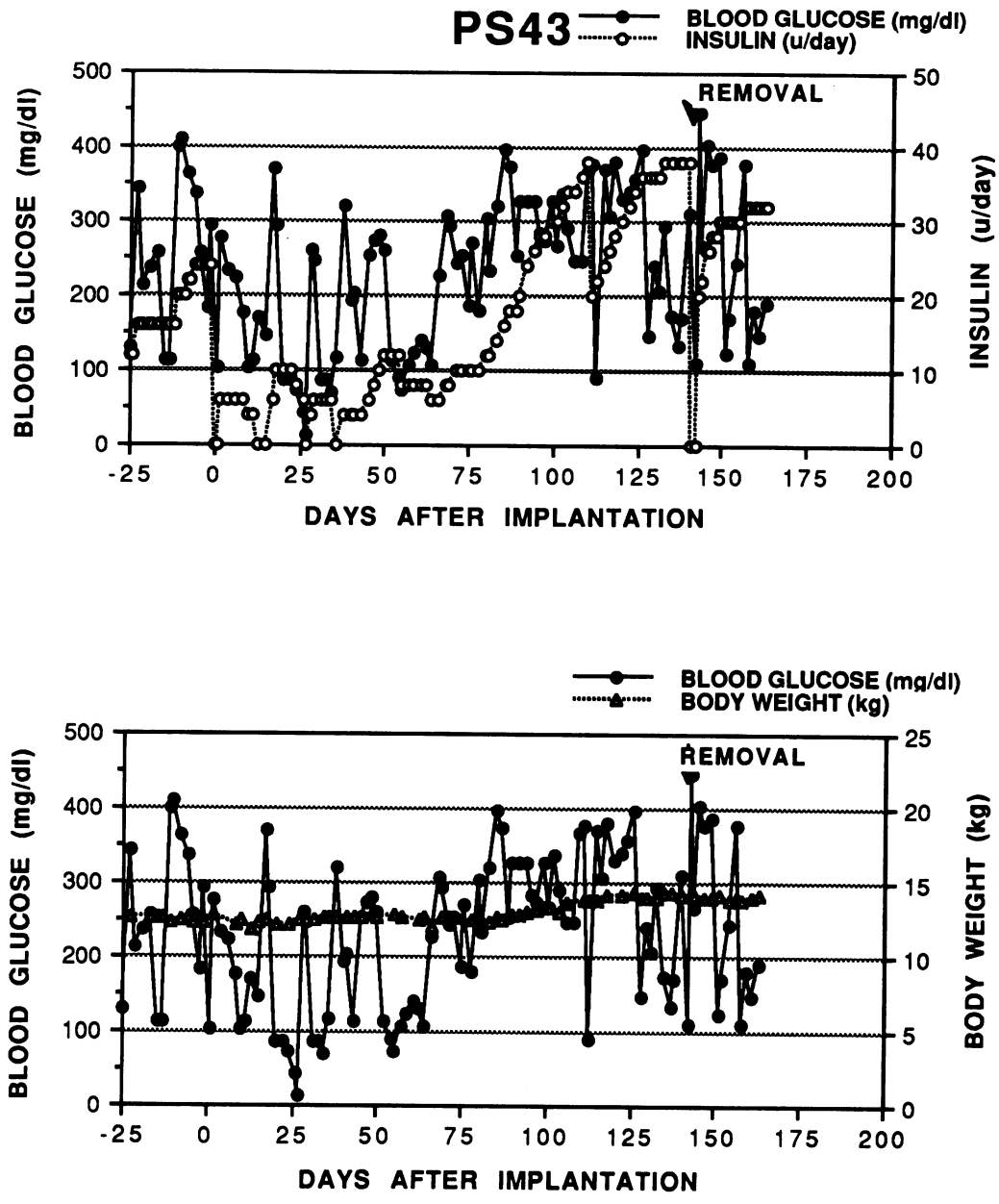


FIG. 10. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-seeded device implantation (xenograft seeded) in dog PS43.

each one after sufficient time passed to determine if additional exogenous insulin requirements developed after device removal. Some dogs were killed because they lost up to 20% of their body weight, even though the devices were functioning (dogs PS28, PS30, PS36, PS49). Histologic estimates of islet viability were performed by first examining all islets in several representative sections for characteristics of viability (see Materials and Methods). The number of islets showing 0% viability, 25% viability, 50% viability, 100% viability, and so on was determined and a composite score for total islet viability for the entire device was given.

It is clear that device thrombosis is not a major cause of device failure. Of 20 devices examined in this series of double-device implants, only three were thrombosed. An additional three dogs (PS50, PS56, 1P1) have devices still functioning (audible bruits and good glucose control) so it is likely that their device anastomoses are patent. Therefore it is likely that 22 of 26 devices in this series have remained patent during the study period (85% patency). Four dogs (PS28, PS30, PS36, PS49) were killed because of 20% loss of body weight with functioning devices. These dogs had essentially all patent anastomoses, except for the left device in PS28. It is of interest that these dogs had devices that generally contained more viable islets than the other dogs who had been functioning longer but that had subsequently lost function. The dogs that lost function with patent anastomoses (PS22, PS24, PS26, PS35) and the dog who died with a functioning device(s) (PS23) had low levels of viable islets.

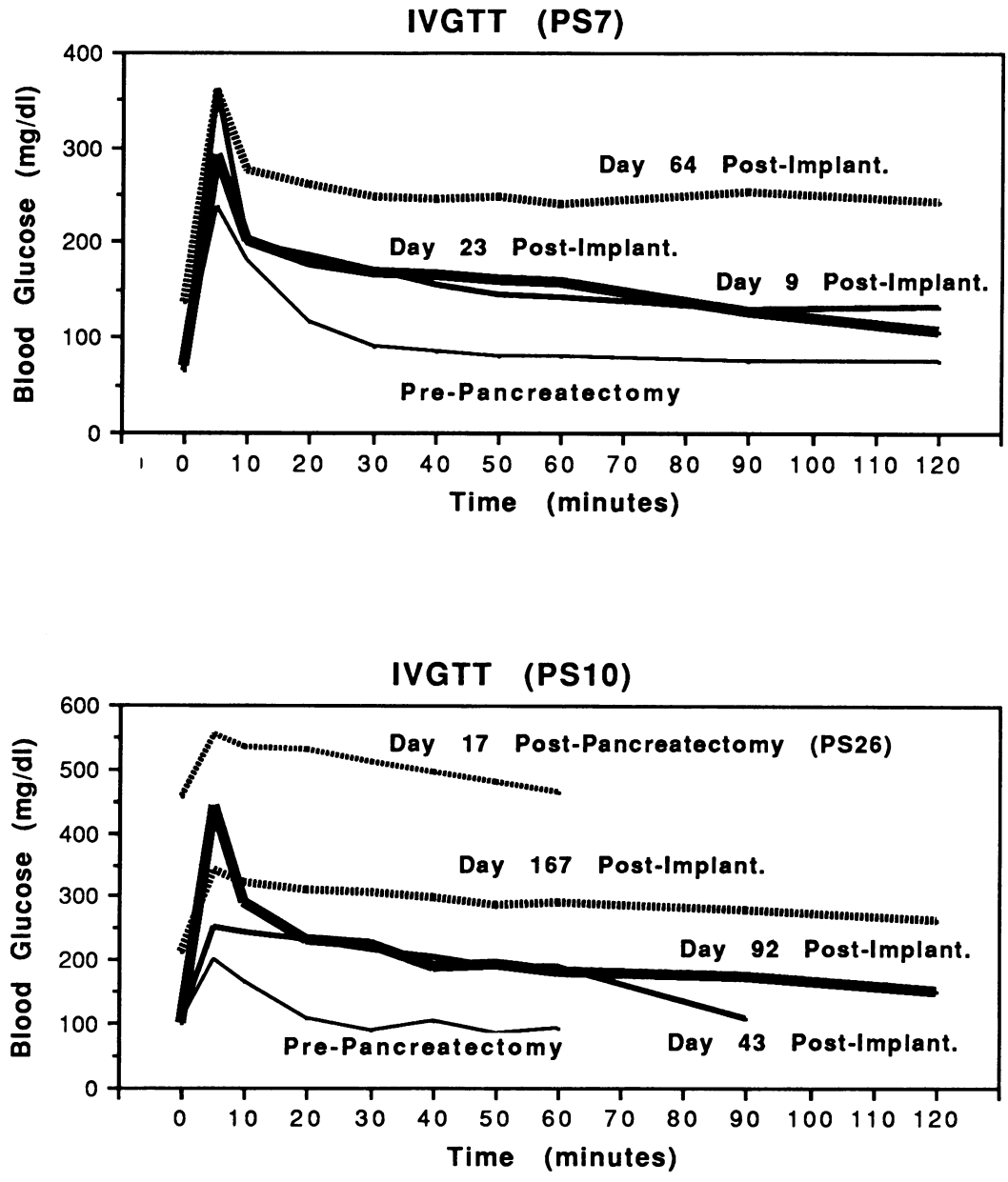


FIG. 11. Intravenous glucose tolerance test in dogs PS10 and PS7. An IVGTT of pancreatectomized dog (PS26) before device implantation is shown with IVGTTs of PS10. Deterioration of IVGTTs in both dogs is clearly shown with successive IVGTTs performed serially after implantation.

Table 8 presents a similar analysis of the small group of xenograft dogs. Thrombosis of devices was present in four dogs (PS32, PS38, PS40, PS51), three of which had very significant systemic infection. Islet viability was poor in all dogs, although dogs (PS29 and PS43) with the longest function still had some viable islets when they were killed.

Histologic Examination of Devices

Histologic examination of devices recovered at various times after implantation has revealed several important points. First the devices with intact membranes never showed any type of cellular infiltration within the chamber. Rather islets are found dispersed throughout the chamber with varying degrees of viability. Figure 15 shows a cluster of islets in the chamber of dog PS19 with excellent viability and insulin content. Note that no inflammatory cells or cells associated with immunologic rejection are apparent. Thus histologic evidence for a cell-mediated rejection or an antibody-mediated rejection is not readily identified. Figure 16 shows a group of canine islet allografts that are of poor viability (Fig. 16A), and a closer magnification of a different section (Fig. 16B) shows islets of excellent viability next to vacuolated areas in the surrounding device matrix that contained islets that have become necrotic. Figure 16 also shows that the semipermeable membrane surface facing the blood stream (nonislet side) has only a thin layer of fibrin deposit but no fibrosis. No cellular elements are discernible in the layer of fibrin deposition. Figure 17 shows a section for a xe-

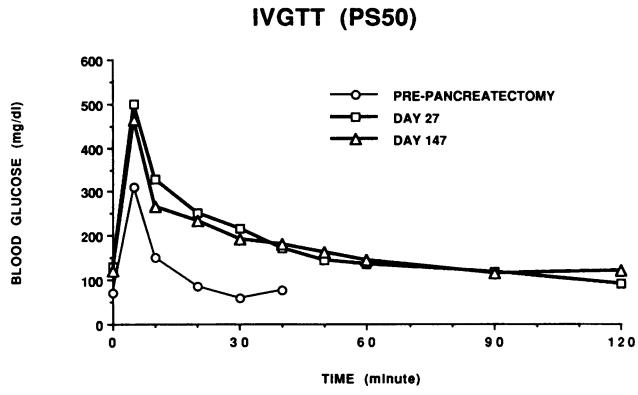


FIG. 12. Intravenous glucose tolerance tests in dog PS50, which has not required insulin for more than 160 days. The IVGTT, although abnormal compared to the prepancreatectomy studies, is significantly improved on day 27 after implantation and that improvement was maintained through day 147 after implantation.

nograft dog. These sections are of interest in that they show no inflammatory cell infiltration even around the viable islets close to the semipermeable membrane.

Discussion

The Hybrid Artificial Pancreas incorporates a single, large-bore, hollow fiber membrane that is connected to commercially available vascular PTFE grafts of the same diameter (5 to 6 mm, ID). The technical details of the development of this prototype design have been described and discussed in detail.[19] The current experiments have been devised to test further the performance and efficacy of the artificial pancreas as an immunoexclusion device for islet transplantation.

The experiments in which unseeded devices were surgically implanted and followed for up to 20 months clearly

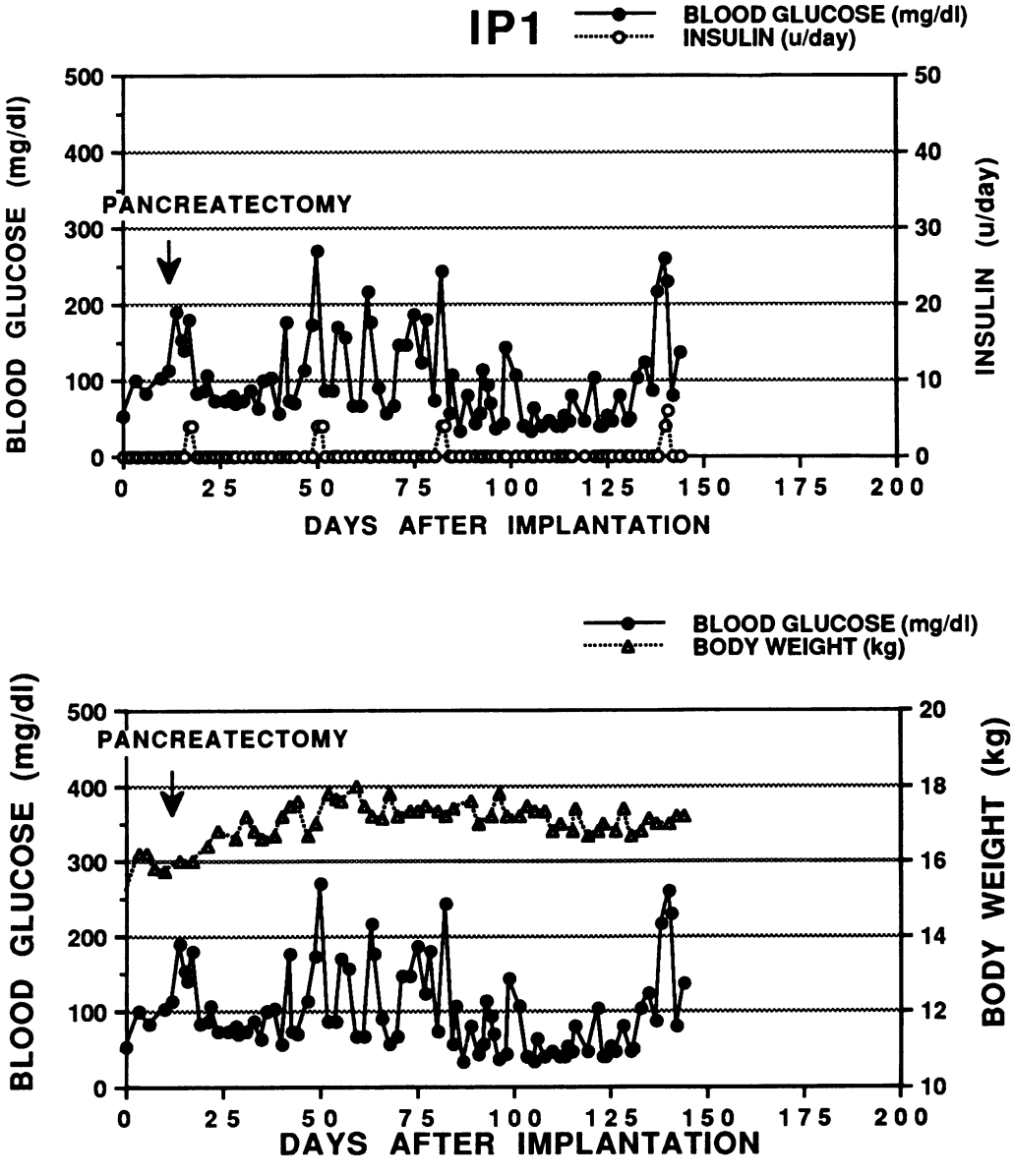


FIG. 13. Fasting blood glucose levels and exogenous insulin requirements (upper panel) and body weights (lower panel) after double-seeded device implantation (allograft seeded) in dog IP-1, which has not required insulin for 100 days (see text).

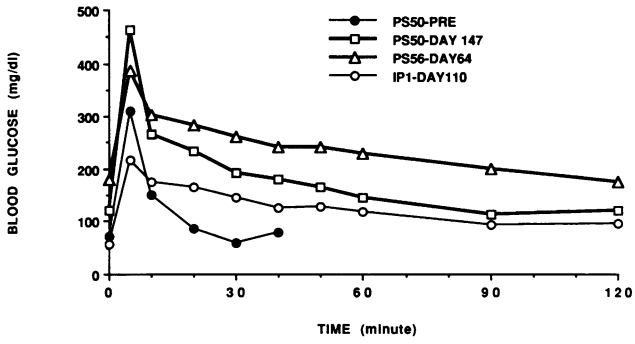


FIG. 14. Intravenous glucose tolerance tests in dogs PS50, 56, and IP-1 at various times after implantation of double devices (allograft seeded).

TABLE 6. *Correlation of Insulin Requirements After Double Xenograft Seeded Device Implantation and* In Vitro *Insulin Output*

Dog	Preimplantation Daily Insulin Req.	Average Daily Insulin Req.	Up to Day	*In Vitro* Output: Single Device	*In Vitro* Output: Double Device
PS29	14.0	0.7	60	8.7	17.4
PS32	18.0	8.9	20	4.7	9.4
PS37	22.9	18.5	60	7.8	15.6
PS38	38.6	Data not available			
PS40	35.3	17.1	30	4.7	9.4
PS43	23.7	5.6	60	8.4	16.8
PS51	19.0	Data not available			
PS52	27.4	Data not available			
PS53	30.9	21.5	30	5.1	10.2

demonstrate the potential long-term patency of the current device. The devices of 10 of 12 of the dogs in group I (unseeded device) eventually occluded. Their implantations may have failed because they were performed without benefit of aspirin therapy, an end-to-end anastomosis was used, and a certain amount of technical learning with the device was necessary. Devices remained patent in seven of eight group II unseeded dogs more than 500 days after end-to-end anastomoses and administration of low-dose aspirin therapy. Clearly the semipermeable membrane is not thrombogenic in this setting. Also any defect in the semipermeable membrane would cause bleeding into the chamber with collapse of the membrane leading to the hollow fiber membrane tube and graft thrombosis. That these devices remain patent attests to the fact that the membrane can withstand arterial pressure for prolonged periods without losing its physical integrity. Furthermore the long-term unseeded device dogs remain healthy thus far; they clearly tolerated the long-term arteriovenous shunt without cardiac or other difficulty. Finally it should be noted that a 5- to 6-mm PTFE graft was used on these prototype devices, a size that is commonly used clinically in humans. These grafts stayed open in 15- to 20-kg dogs. Thus it is highly probable that long-term patency in humans will be no problem.

The model of total pancreatectomy to induce diabetes used in these experiments is a difficult one but a highly effective one. The fact that less than 1.5% of dogs subjected to surgical total pancreatectomy fail to become diabetic attests to its efficacy. Also no dogs (66 of 67) that became hyperglycemic after pancreatectomy became spontaneously normoglycemic. The model does have some drawbacks in that despite nutritional supplements, some animals sustain weight loss. Also a number of animals lost significant amounts of body weight after device implantation and the experiments had to be terminated despite the fact that the devices were functioning normally. The diminished nutritional reserve of some dogs certainly may have contributed to susceptibility to infection, which also necessitated killing of several dogs with functioning devices.

Experiments with allograft seeded devices clearly emphasize that the Hybrid Artificial Pancreas can function

TABLE 5. *Correlation of Insulin Requirements After Double Allograft Seeded Device Implantation and* In Vitro *Insulin Output*

Dog	Preimplantation Daily Insulin Req.	Average Daily Insulin Req.	Up to Day	*In Vitro* Output: Single Device	*In Vitro* Output: Double Device
PS22	18.3	0.0	60	7.2	14.4
PS23	20.6	0.0	60	14.0	28.0
PS24	26.6	13.7	60	5.5	11.0
PS26	27.7	0.0	60	8.3	16.6
PS28	22.9	2.3	30	14.0	28.0
PS30	16.3	0.0	30	6.9	13.8
PS35	29.7	Data not available			
		0.0			
PS36	32.3	1.8	30	8.6	17.2
PS48	19.9	0.3	30	10.8	21.6
PS49	20.9	0.0	30	8.0	16.0
PS50	22.1	0.0	60	13.1	26.2
PS56	36.9	5.8	30	4.4	8.8

TABLE 7. *Anaysis of Device Patency, Islet Viability, and Causes of Failure After Double Device (Allograft) Implantation*

Dog	Function (day)	Device Patency: R	Device Patency: L	Islet Viability (%): R	Islet Viability (%): L	Cause of Failure
PS22	263	+	+	15	25	Islet
PS23	209	+	+	10	15	Ileus
PS24	64	+	+	10	10	Islet
PS26	169	+	+	25	15	Islet
PS28	51	+	−	40	0	↓BW (sepsis)
PS30	29	+	+	70	30	↓BW (sepsis)
PS35	29	−	+	0	NA	Islet
PS36	99	+	+	<15	NA	↓BW (parasites)
PS48	72	−	−	0	0	Thrombosis
PS49	26	+	+	25	15	↓BW (sepsis)
PS50	>162					
PS56	>79					
IP1	−125					

TABLE 8. *Anaysis of Device Patency, Islet Viability, and Causes of Failure After Double Device (Xenograft) Implantation*

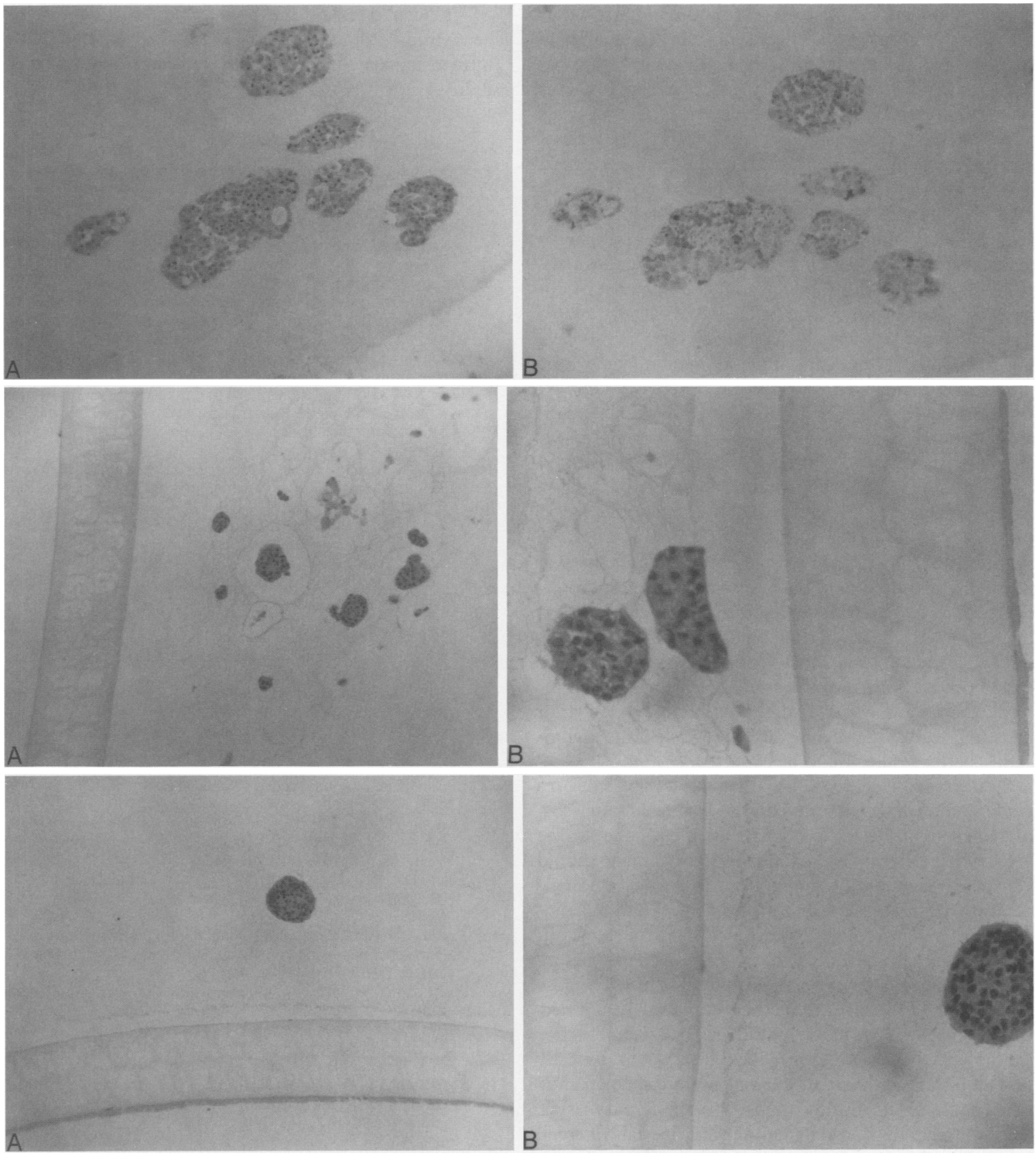
Dog	Function (day)	Device Patency R	Device Patency L	Islet Viability (%) R	Islet Viability (%) L	Cause of Failure
PS29	106	+	+	25	10	Islet
PS32	4	−	−	0	0	Thrombosis
PS37	3	+	+	20	20	Islet
PS38	4	−	−	0	0	Infection (thrombosis)
PS40	16	+	−	NA	0	Thrombosis
PS46	14	+	+	0	0	Islet
PS43	80	+	+	12	7	Islet
PS51	3	−	−	0	0	Infection (thrombosis)
PS52	11	+	+	0	0	Islet, infection
PS53	5	−	+	0	0	Islet
PS63	14	+	+	NA	NA	↓BW

in large animal species to facilitate blood glucose control. The extraordinary survival of totally pancreatectomized dogs with good blood glucose control without administration of insulin for extended periods after transplantation of islet allografts in this device is clearly encouraging. Canine islet allografts transplanted without immunosuppression or with large doses of multiple immunosuppressive drugs are invariably rejected in a few to several days.[13] Not only have islets in the Hybrid Artificial Pancreas survived without immunosuppression but they functioned for the longest periods thus far achieved in islet allotransplantation in large animal models. Four of fifteen single-seeded devices functioned more than 90 days with significant reduction of exogenous insulin requirements after implantation compared to 7 of 13 double-device dogs. Seven of fifteen single-device dogs showed little or no function while essentially all the double-device dogs had definite function. The most probable explanation for this is that single devices did not provide sufficient insulin output to alter glucose control measurably. Analysis of the *in vitro* insulin output by perfused devices in previous studies[20] and in these current experiments suggest that the average daily insulin output for one device is approximately 10 units of insulin or less. These findings support the inadequacy of single-device *in vivo* insulin output to control blood glucose in most dogs with this severe model of diabetes. It should be noted that the one single-device dog that lived more than 280 days (PS10) had preimplantation insulin requirements of approximately 11 units per day.

The current studies have permitted an extended analysis of the reasons for failure of the device to control blood glucose levels in these diabetic animals. It is noteworthy that failure in the single-device animal was attributed to clotting in only 3 of 15 dogs and device failure in only 2 of 15 dogs. As best as could be determined, an important cause for failure was related to loss of islet function and/or viability. Thrombosis was a cause of failure in only 1 of 13 dogs in the double-device allograft group and there were no device failures. Again the major cause for loss of device effectiveness was islet function loss.

In the single-device allograft studies,[19] IVGTT tests in dogs with functioning devices are improved over prepancreatectomy levels but still are not normal. Also postprandial blood glucose levels in such dogs are also significantly elevated for several hours. The abnormal IVGTT is probably not attributable to a slow response of the device to the increased glucose because of the large islet chamber volume (approximately 5 to 6 mL). *In vitro* studies of insulin secretory dynamics suggest that insulin output into the perfusate begins to increase 15 to 20 minutes after glucose stimulation. The problem appears to lie in the inadequate overall insulin secretion of these devices to manage hyperglycemic stress. One obvious cause is that in a single device, the device can be seeded with only a limited number of islets that produce insufficient amounts of insulin in response to carbohydrate stress. Alternatively the microenvironment surrounding the islets may still not be optimal and could contribute to loss of function and viability. In either case it is likely that insulin secretion is already close to maximal with these devices and excess carbohydrate loading thereafter results is sustained hyperglycemia. Evidence presented in these double-device allograft experiments support this conclusion. Double-device dogs have better long-term control of fasting blood glucose and a higher percentage of these dogs show significant function. Also IVGTT studies in these dogs appear to be more improved compared to postpancreatectomy tests and remain improved for longer times.

The studies of serial fasting blood glucose levels in dogs with two functioning devices demonstrated an excellent control of blood glucose for approximately 100 days. Not only was no insulin required but the blood glucose levels clustered around 100 mg/dL. Thereafter blood glucose control became less tight, with glucose levels increasing to the 150 to 250 mg/dL range but with little or no insulin required. Eventually most of these dogs appear to lose islet function and the device fails. This loss of islet function can be secondary to insufficient islet mass persisting in the device perhaps from loss of viability secondary to postprandial hyperglycemia and/or initial injury to the islets in their preparation and extraction. Loss of islet viability demonstrated by histologic analysis of the removed devices could also be due to inadequate nutrition within the microenvironment surrounding the islets. Loss of islet viability occurs in microencapsulated devices[17] secondary to pericapsule fibrosis. Fibrosis does not seem to occur on the blood stream side of the semipermeable membrane in the Hybrid Artificial Pancreas device. Clearly more sophisticated and detailed examination of the semipermeable membranes of removed devices is required, including electron microscopy studies, and so on. Also development of anti-insulin antibodies could impair function of device-



FIGS. 15A and B. (A) Histologic section of a device removed from dog PS19 showing a cluster of viable islets with no surrounding cell infiltration. H&E stain (×160). (B) The same section stained for insulin (×160).

FIGS. 16A and B. (A) Section of a device taken from dog PS25 showing islets of poor viability. The semipermeable membrane is superior (H&E, ×160). (B) Higher-power magnification of an area of device from dog PS25 showing viable islets next to a vacuolated area in the device matrix in which the islets have become necrotic and lyzed (H&E, ×400).

FIGS. 17A and B. Section of a device taken from a xenograft dog that had short-term partial function; the device was removed at day 18. The islet is viable and there is no surrounding cellular infiltrate, even though the islet is very close to the membrane. (A) H&E (×160); (B) (H & E, ×400).

produced insulin, although this is doubtful in the allograft situation. Another possibility is that insulin output is really adequate but some type of peripheral insulin resistance develops. Finally the possibility exists that loss of islet viability and function may be due to some type of rejection response. Against conventional rejection is that lack of demonstrated cellular infiltrate at any time in the removed devices or histologic examination. Destruction by some type of immunoglobulin and/or complement mechanism is doubtful but immunohistochemical studies and analysis of removed devices is clearly indicated. Similarly a lymphokine-mediated destruction should be ruled out by appropriate studies. Thus far detailed studies on the host immune response (except for histologic examination) have not been done in dogs with functioning or failed devices.

The studies in which xenograft islets were transplanted without immunosuppression in the hybrid artificial pancreas were not as successful as the allograft studies. Nevertheless the survival of the two xenograft transplanted dogs without immunosuppression represents the longest large animal islet xenograft survival thus far accomplished in any large animal model with or without immunosuppression. Indeed large animal islet xenograft survival is usually measured in hours even with immunosuppression.[23] The xenograft device studies were clearly limited by loss of islet viability and infection. Bovine xenografts were harvested at local abbatoirs under less-than-optimal conditions with significant warm ischemia times. Our future experiments will use surgically harvested xenograft pancreata with little or no warm ischemia time. Significantly better results are to be expected.

Subtherapeutic levels of insulin output by single devices is a limitation of the Hybrid Artificial Pancreas.[20] Redesign of the prototype device to increase the membrane surface area and the size of the available islet chamber should permit increased insulin output from single devices. Single devices with higher insulin outputs should also have increased insulin output with carbohydrate stress and improved postprandial glucose control. Nevertheless failure to achieve adequate control of postprandial blood glucose levels during times of carbohydrate stress would not necessarily preclude use of the hybrid artificial pancreas. For example the device could also be used to provide a steady state of insulin output, which would require supplemental exogenous insulin during times of carbohydrate stress. Thus the overall management of the difficult patient could be made easier. Long-term islet viability and function remains an important problem but should be improved by less traumatic isolation techniques and by changes in the microenvironment of the islet chamber. Another possible means of managing loss of islet viability is the capacity to reseed a functioning device that has lost function. It is planned that the device will be implanted in an anatomic location where access to the reseeding parts would easily be accessible through a local anesthetic procedure. Reseeding experiments have been started in dogs.

Significant progress has been achieved in developing the Hybrid Artificial Pancreas. Clinical application in the future using xenograft islets is a reasonable goal and possibility.

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