application, a 50-μl portion of the buffer solution was removed and assayed for cyclic AMP content by the method of A. G. Gilman [*Proc. Natl. Acad. Sci. U.S.A.* **67**, 305 (1970)], as modified by B. L. Brown *et al.* [*Biochem. J.* **121**, 561 (1971)].
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Artificial Pancreas Using Living Beta Cells: Effects on Glucose Homeostasis in Diabetic Rats

Abstract. *An artificial pancreas consisting of beta cells cultured on synthetic semipermeable hollow fibers was tested in rats with alloxan-induced diabetes. When implanted ex vivo as arteriovenous shunts in the circulatory system these devices lowered concentrations of plasma glucose from 533 to between 110 and 130 milligrams per 100 milliliters, increased concentrations of plasma insulin, and restored intravenous glucose tolerance tests essentially to normal.*

Transplantation of insulin-producing tissue constitutes a potentially important approach to the treatment of diabetics (*1*). Several studies have demonstrated that transplantation of the whole pancreas with vascular anastomosis in insulin-dependent diabetic patients restores glucose homeostasis to normal without further need for injection of exogenous insulin (*2*). Unfortunately, the morbidity and mortality associated with this procedure have been unacceptably high (*3*). Major difficulties have included (i) requirements for continuous immunosuppressive therapy after transplantation, (ii) problems associated with procurement of viable human donor pancreatic tissue, and (iii) the extensive surgery involved in implanting the donor pancreas, including the need to provide drainage for acinar enzymes. Although the use of isolated islets in place of the whole pancreas would simplify the surgical procedures (*4*), the problem of isolating large numbers of viable human islets from cadaver organs and the requirement for immunosuppressive therapy would still remain.

Various types of cells (*5*), including beta cells isolated from neonatal rat pancreas (*6*), have previously been successfully cultured for several weeks on the outside surfaces of bundles of synthetic capillaries perfused with tissue culture medium. Release of insulin into the perfusate, measured over 2-day intervals, remained relatively constant during this period and responded appropriately to changes in glucose concentration in the perfusion medium (*6*). More recent studies dealing with short-term insulin secretory dynamics showed that after an increase in the glucose concentration of the perfusate from 5.5 to 16.5 m*M* an increase in insulin levels in the effluent medium from the device could be detected within 5 minutes (*7*). The capillaries (Amicon XM-50 acrylic copolymer) used in these experiments had a nominal molecular weight cut-off of 50,000 and, therefore, were permeable to insulin but essentially impermeable to antibodies and lymphocytes. The walls of these fibers, therefore, act as a barrier against immune rejection, as is the case for chambers constructed from Millipore membranes that possess considerably larger pores (*8*).

The present experiments were undertaken to determine whether these devices would restore plasma glucose concentrations to normal in rats with allox-

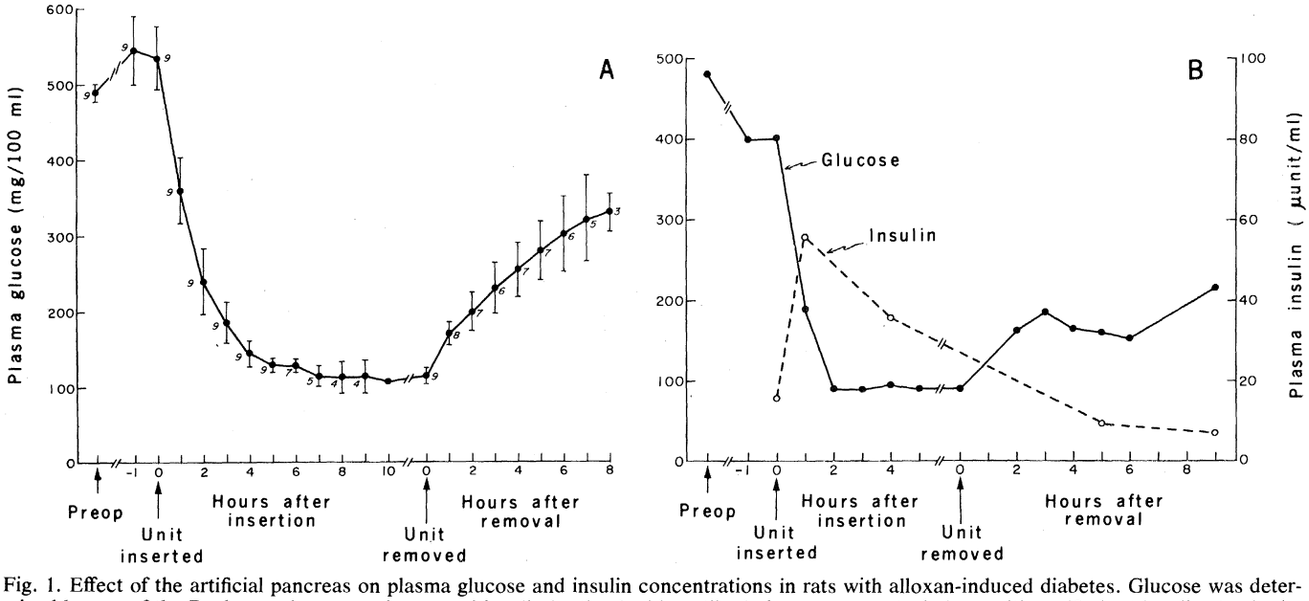

Fig. 1. Effect of the artificial pancreas on plasma glucose and insulin concentrations in rats with alloxan-induced diabetes. Glucose was determined by use of the Beckman glucose analyzer, and insulin by the double antibody immunoassay technique with purified rat insulin standards. Blood samples were obtained from the arterial side of the iliac artery-to-vein Silastic shunt. The preoperative sample was drawn prior to administration of anesthesia. After insertion of the iliac artery-to-vein shunt, samples were obtained at hourly intervals until concentrations of plasma glucose were relatively constant. The pancreatic device was then inserted into the shunt and left in place until the plasma glucose stabilized within the range of 100 to 150 mg per 100 ml. Devices were then removed and blood samples were again obtained at hourly intervals to follow the subsequent rise in plasma glucose and fall in circulating insulin levels. (A) Arterial plasma glucose levels (mean ± S.E.M.). The small numerals indicate the number of values used to compute each point. (B) Arterial plasma glucose and insulin concentrations in a representative diabetic animal. After insertion of the device in the shunt, there was a concomitant rise in plasma insulin and fall in plasma glucose. After the device was removed insulin levels fell while the plasma glucose rose.

an-induced diabetes. For this purpose, Silastic iliac artery-to-vein shunts were inserted in noninbred male albino rats (body weight, 300 to 400 g; Charles River) (*9*). Animals were rendered diabetic (routine plasma glucose consistently > 400 mg/100 ml) by the intravenous injection of alloxan (32 mg per kilogram of body weight) 4 to 14 days prior to surgery. The Silastic catheters were tunneled subcutaneously and brought out through the skin of the back within a short length of protective Tygon tubing. Animals were conscious and could eat and drink freely during the course of the experiments.

Devices were constructed by sealing the ends of bundles of 100 capillary fibers (Amicon XM-50, 11 cm long) into cylindrical glass or plastic jackets (inner diameter, 0.5 cm) with medical grade Silastic or epoxy cement. Cells were seeded onto the outside surfaces of these fiber bundles through a port in the wall of this surrounding jacket. Six of these devices, each seeded with islet cells from 180 neonatal rats, were maintained by perfusing the fibers with tissue culture medium for periods of 1 to 4 weeks, as previously described (*6*).

The effects of five of these devices were then examined in a total of nine alloxan diabetic rats. The sixth device was tested in two nondiabetic rats to ensure that the devices possessed sufficient feedback controls to prevent production of sustained hypoglycemia. When devices were used sequentially in more than one animal, the capillaries were flushed briefly with isotonic saline before transfer to the new host. In all nine diabetics studied (Fig. 1A) a decline in the concentration of plasma glucose was noted within 30 minutes after inserting the unit into the arteriovenous shunt. After 2 hours, concentrations of plasma glucose had fallen by 50 percent. The mean concentration of glucose eventually stabilized in the range of 110 to 130 mg per 100 ml. This value compared favorably to the normal plasma glucose of 138 ± 4 mg per 100 ml (mean ± standard error of the mean) for 15 untreated rats. After removal of the devices from the shunt, the plasma glucose began to rise within the first hour and eventually reached diabetic levels. This rise in plasma glucose was thought to be important since surgical trauma, repeated blood sampling, and reduced food intake occasionally impaired the ability of the diabetic animals to increase the concentration of plasma glucose. One of the devices was tested sequentially in three different diabetic animals for 10 hours each

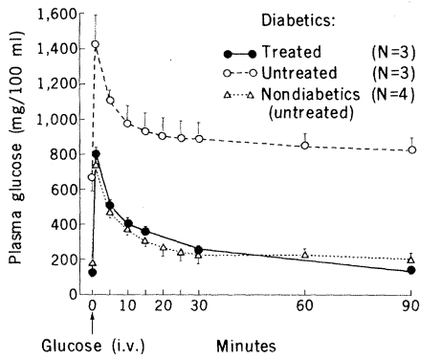

Fig. 2. Intravenous glucose tolerance tests in diabetic rats with the artificial pancreas inserted ex vivo in the arteriovenous shunt. After the plasma glucose had stabilized near normal, D-glucose, 1.0 g per kilogram of body weight, was injected into the venous side of the Silastic shunt. Blood samples for determination of plasma glucose were then obtained from the arterial side of the shunt at the indicated times. Results for untreated diabetics and nondiabetic control rats with shunts were included for comparison. The calculated glucose disappearance (*K*) rates (mean ± S.E.M.) for the three groups were: treated diabetics, 2.16 ± 0.22; untreated diabetics, 0.82 ± 0.15; and nondiabetic controls, 3.07 ± 0.62. Although the mean *K* rate for the treated diabetics was slightly less than normal, the difference was not statistically significant ($P > .25$). In contrast, the *K* rate for the treated diabetics was significantly greater ($P < .01$) than for the untreated diabetic group. Abbreviation: *i.v.*, intravenous.

(total of 30 hours) without apparent impairment of its ability to regulate the concentration of plasma glucose. Results in the two nondiabetic control animals showed a decrease in the mean plasma glucose to 75 to 83 mg per 100 ml within the first 4 hours after insertion of the device in the shunt, followed by a gradual return to normal concentrations.

Determination of the concentration of circulating plasma immunoreactive insulin (IRI) in representative animals during the course of the experiment revealed a significant rise after insertion of the device in the shunts. As plasma glucose concentrations were brought within the normal range by the devices, there was a concomitant fall in plasma IRI levels (Fig. 1B).

In order to evaluate further the ability of these devices to control rapid rises in plasma glucose concentrations, intravenous glucose tolerance tests were performed in representative animals at a time when these concentrations had been stabilized in the normal range by the devices. Results (Fig. 2) of these tests were similar to those observed in nondiabetic animals with iliac arteriovenous shunts. As anticipated, similar studies in untreated alloxan diabetic animals revealed sustained elevations of plasma glucose after intravenous injection of glucose, with significantly decreased glucose disappearance (*K*) rates (*10*) compared to both treated diabetics and to normal animals.

These data indicate that devices consisting of islet cells cultured on the outside surfaces of synthetic semipermeable hollow fibers can maintain relatively normal glucose homeostasis when implanted as arteriovenous shunts in the vascular system of diabetic animals. This is perhaps to be anticipated in view of previous studies based on the use of insulin-producing tissue in conjunction with these hollow fibers (*6*, *11*). Since the walls of these fibers should serve as an effective barrier to immune rejection of xenografted cells, serious consideration must be given to the eventual use of implantable artificial organs of this type for the treatment of human diabetes and possibly other diseases.

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9. Animals were anesthetized by intraperitoneal injection of sodium pentobarbital, 0.3 ml per animal. The iliac vessels were then cannulated with medical grade Silastic tubing (inside diameter, 0.16 cm; outside diameter, 0.24 cm). Each catheter was tipped with a short length of stiffer polyethylene (PE-100) tubing to facilitate cannulation. With a unit in place, estimated flow through these shunts was at least 2 ml per minute. To prevent clotting, animals were heparinized by injecting 25 units of heparin intravenously 1 hour postoperatively, and then every 2 hours. Following insertion in the shunt, the pancreatic devices were maintained at 37°C by means of a warm flow of air (Sage Air Curtain).
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Ovarian Hormone: Lack of Effect on Reproductive Structures of Female Asian Musk Shrews

Abstract. *Uterine and vaginal weights and histologies are not altered by ovariectomy or estrogen treatment in the Asian musk shrew (*Suncus murinus*). In addition, ovariectomized shrews mate. Thus, the role of ovarian hormones in the control of the reproductive status of this species does not conform to the accepted mammalian pattern.*

It is generally assumed that ovarian estrogens are required for the development and support of the sex accessories of female mammals. This generalization has been derived from observations of a limited number of species. However, the importance of estrogen in the reproduction of female elephants and Asian musk shrews has been questioned. In elephants, Plotka *et al.* (*1*) failed to demonstrate a distinct relation between the reproductive state (immature, mature, and pregnant) and the very low concentrations of estradiol in the plasma. Similarly, the concentration of plasma estradiol in adult musk shrews is below detection by radioimmunoassay (*2*). These observations suggest that the target tissue receptor mechanisms are extremely sensitive to low estradiol titers or that the sex accessories such as the uterus and vagina in these forms are not dependent on estradiol.

Since insectivores are generally considered the most primitive living eutherians (*3*), and the effects of sex steroids have not been studied in them, we have examined the sex accessories in tropical Asian shrews (*Suncus murinus*) subjected to gonadectomy and hormone replacement therapy. The results of this study indicate that the uterus and vagina in this species are apparently not dependent on ovarian hormones.

The history and maintenance of the *Suncus* colony have been described (*4*). Healthy mature animals not previously used for experimental purposes were used throughout (*5*). The effects of ovariectomy on uterine and vaginal histologies are shown in Table 1. Microscopic examination revealed no significant differences between the sex accessories from intact and ovariectomized shrews. Uterine and vaginal weights were also not dependent on ovarian hormones (Table 2). Wet and dry weights of these organs did not decline following ovariectomy and multiple injections of estradiol (*6*) failed to increase these weights in gonadectomized shrews. Changes in vaginal cytology of rodents are commonly employed in the bioassay of estrogenic hormones. Table 2 shows the effects of ovariectomy and estrogen treatment on the vaginal smear in *Suncus*. Nucleated epithelial cells predominated in the vaginal smear of intact shrews (*7*), and this smear was not altered by long-term ovariectomy or injection of massive amounts of the potent synthetic estrogen, diethylstilbestrol.

Since female shrews showed no anatomic dependence on ovarian hormones (Tables 1 and 2) it was of interest to examine the effects of orchidectomy and androgen treatment (*8*) in male shrews (Table 2). Long-term castration resulted in significant reductions in the weights of the prostate, ampulla, and epididymis. In addition, the weights of these organs were increased by testosterone treatment. It has also been shown that the plasma testosterone level in male musk shrews is similar to that observed in other mammalian species (*9*). Thus, maintenance of male sex accessories in *Suncus murinus* conforms to the pattern described for other mammals.

To evaluate the possibility that the apparent lack of ability of the shrew uterus and vagina to respond to estrogenic hormones (Table 2) is due to a corresponding absence of estrogen receptors, various tissues from females were incubated with [^{3}H]estradiol (*10*). After incubation, the amount of [^{3}H]estradiol bound to the nuclear fraction was determined (Fig. 1). The nuclear uptake of [^{3}H]estradiol by corresponding tissues of rats is shown in Fig. 1 for comparison [see also (*11*)]. The uterus and vagina of the rat incorporate more [^{3}H]estradiol than does muscle or kidney. This preferential accumulation is due to the presence of estrogen receptors which are confined to estrogen target tissues (*12*). The shrew uterus and vagina also retain more [^{3}H]estradiol than muscle or kidney, an indication that these reproductive tissues also have specific estrogen receptors.

Uterine and vaginal atrophy following ovariectomy during the reproductive period has been observed in most mammalian orders and has generally been assumed to be a universal phenomenon in mammals. Involution is characterized by

Table 1. Histology of the uterus and vagina in normal and ovariectomized shrews. Shrews were ovariectomized for a period of 1 month. Results are expressed as means ± standard error of the mean of three determinations.

Treatment	Diameter of endometrial gland (μ)	Height of uterine epithelial cells (μ)	Thickness of vaginal epithelium (μ)
Nonovariectomized	55.0 ± 4.5	44.3 ± 4.9	28.7 ± 2.3
Ovariectomized	52.7 ± 2.7	47.2 ± 2.8	30.7 ± 2.5