gradient. We believe that divalent cations catalyze the process by increasing both the probability of vesicle-membrane contact and the duration of that contact, thus allowing time for the osmotic swelling to occur. For phospholipids, millimolar amounts of any divalent cation are required, whereas the presence of calcium-binding protein in the planar membrane lowers this requirement to the micromolar range of Ca^{2+}, specifically. Still lower Ca^{2+} concentrations might produce fusion if calcium-binding protein were present in both planar and vesicular membranes. Limitations in the amount of protein available to us thus far have precluded experiments along this line.

The calcium-binding protein used here was obtained from synaptic membranes, but it is not clear at present whether it either promotes transmitter release at synapses or is particularly unique in its properties. Conceivably, many such binding proteins exist in neuronal or other membranes and function in related or different capacities. Its molecular weight of ~ 16,000, four calcium-binding sites, and K_m for Ca^{2+} of ~ 15 μM are strikingly similar to the properties of calmodulin (*19*); further work is required to determine if the two molecules are related.

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14. If small vesicles have a membrane of finite surface energy, the law of Young and Laplace [see (*15*)] requires that a hydrostatic pressure difference exist across the vesicle membrane; in order for the chemical potential of water within the vesicle to equal that of water outside the vesicle, the contents of the vesicle must be hyperosmotic. In this circumstance fusion is possible by the same mechanism described in the text without an osmotic gradient across the planar membrane (*6*).
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16. We do not discuss here our speculations concerning the molecular rearrangements of phospholipid molecules during incorporation of vesicle membrane into planar membrane, nor why vesicle bursting occurs preferentially at the region of contact with the planar membrane.
17. If the planar membrane is also slightly permeable to the osmoticant (as is the case for glycerol), it can be shown both theoretically and experimentally that fusion proceeds less effectively than if it were not, and if the vesicular and planar membranes are too permeable, fusion does not proceed at all.
18. Glucose was effective in the experiments described in Fig. 1 because the channels (VDAC) in the vesicular membrane are permeable to it.
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Microencapsulated Islets as Bioartificial Endocrine Pancreas

Abstract. *Single implantation of microencapsulated islets into rats with streptozotocin-induced diabetes corrected the diabetic state for 2 to 3 weeks. The microencapsulated islets remained morphologically and functionally intact throughout long-term culture studies lasting over 15 weeks.*

Many techniques have been used to circumvent the problem of immune rejection, a major difficulty in the transplantation of pancreatic islets, or B cell tissue. Lacy *et al.* (*1*) reported that allografts survived 100 days in rats if the islets were cultured for 7 days at 24°C before they were transplanted and the rats received a single injection of antiserum to lymphocytes immediately before the transplantation. Sutherland and coworkers (*2*) achieved varying degrees of success with the use of dispersed neonatal pancreatic tissue which contained few or no exocrine cells as a result of prior treatment of the donor rats with DL-ethionine. Mullen *et al.* (*3*) avoided transplantation rejection by using fetal pancreas. Other investigators (*4-10*) have used diffusion chambers and hollow fiber units to act as mechanical barriers between islets (or B cells) and the host's immune cells and molecules and to achieve normalization of glucose homeostasis in experimental diabetic animals.

We have obtained prolonged survival of islets in vitro and in vivo by using a novel microencapsulation procedure

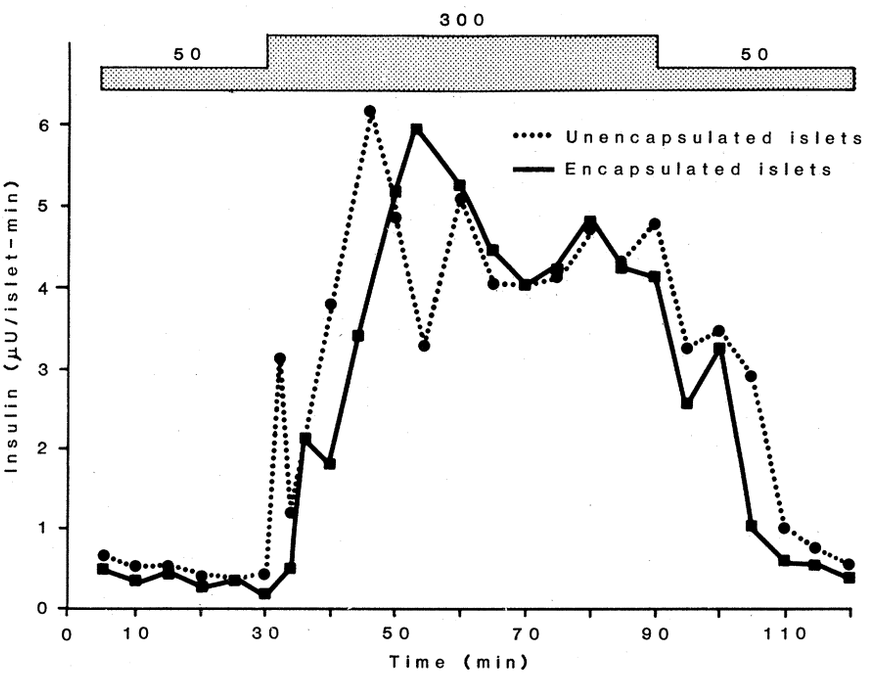

Fig. 1. Insulin release patterns of encapsulated and unencapsulated islets (~ 1 week in culture) in perifusion study. Both groups showed comparable response to high and low glucose concentrations. Microencapsulated islets appeared to show a slight delay in response initially (see text). The shaded area indicates the level and duration of glucose content (numbers are expressed in milligrams per 100 ml) in the perifusing culture medium.

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which completely encloses viable islets within a semipermeable membrane. The microcapsular membrane, composed of cross-linked alginate, a nontoxic polysaccharide, is permeable to small molecules such as glucose or insulin but totally impermeable to large molecules such as immunoglobulins or albumin.

Current conventional collagenase digestion techniques of islet isolation (*10, 11*) were used to obtain islets from adult Wistar rats. The islets were handpicked or separated by Ficoll gradient techniques, cultured for 1 to 7 days at 37°C, and pooled. They were microencapsulated by a process reported previously (*12*). Unwashed cultured islets were suspended uniformly in a 0.6 to 0.8 percent sodium alginate solution in physiological saline. Droplets containing islets were produced by syringe pump extrusion with the droplet-forming technique adapted from Ennis' droplet-forming apparatus, as modified by Sparks *et al.* (*13, 14*). These islet-containing droplets were dropped into 1.5 percent calcium chloride solution, which caused them to gel, and were then decanted and further treated in a polylysine solution (0.02 percent; molecular weight, 35,000; Sigma) for 3 to 5 minutes. The polylysine alginate microcapsules were washed once with 1 percent calcium chloride solution, resuspended in an aqueous polyethyleneimine solution (0.2 percent; molecular weight, 40,000 to 60,000; Polysciences) for 2 to 4 minutes, and then washed again first with calcium chloride and then with saline. Finally, they were suspended in an isotonic sodium citrate solution, *p*H 7.4, for about 5 minutes in order to liquefy the alginate gel inside the capsule. The microencapsulated islets, after a final washing in either saline or phosphate-buffered saline, were cultured at 37°C for at least 3 to 4 days before use. The culture medium used was CMRL 1969 (Connaught Laboratories) containing 9 percent bovine fetal serum, 20 μg of gentamycin per milliliter, 10m*M* Hepes buffer [4-(2-hydroxyethyl)-1-piperazineethanesulfonate], and 100 to 200 mg of glucose per deciliter. All the ingredients were sterilized either by membrane filtration or, in the case of the alginate, by heating at 100°C for 10 to 15 minutes.

The viability and integrity of microencapsulated and control (unencapsulated) islets were assessed by (i) perifusion experiments (*15*); (ii) histochemical staining of paraffin sections with aldehyde fuchsin; (iii) immunoperoxidase staining (*16*) for the four pancreatic hormones; and (iv) direct light microscope examination of islets within the culture flask. In the perifusion experiments dual chambers were used, one for the encapsulated and the other for the unencapsulated islet controls.

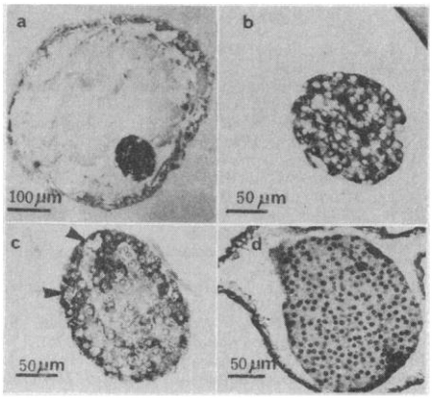

Fig. 2. Microencapsulated islets stained histochemically or immunohistochemically. (a) Islet within capsule showing well-granulated B cells after 12 days in culture. Aldehyde fuchsin-stained (×110). (b) Higher magnification of similar islet, microencapsulated and cultured for 13 weeks; aldehyde fuchsin (×210). (c) Microencapsulated islet cultured for 12 days and stained by an immunoperoxidase technique, showing various states of granulated B cells (dark insulin stains) and unstained non-B cells (see arrowheads) (×210). (d) Microencapsulated islet cultured for 12 days and stained by a similar technique, showing maintained integrity of A cells (dark glucagon stains) (×190).

Insulin output was determined by the radioimmunoassay of Hales and Randle (*17*). In general, the insulin secretion from the encapsulated islets into the perifusate was comparable with that from the control islets. When the glucose concentration was raised from 50 to 300 mg per deciliter, there was a biphasic response of insulin release from both groups of islets. However, the encapsulated islets gave a delayed response. This delay could be attributed to the time required for the passage of insulin across the capsular membrane into the medium (Fig. 1). The increase in quantity of insulin in the presence of high glucose concentration clearly demonstrated that the viability and function of the cells were retained throughout the process of microencapsulation. A normal degree of beta granulation was seen in microencapsulated islets that were cultured for as long as 13 weeks (Fig. 2).

Further evidence of viability of long-term cultured microencapsulated islets was provided by the immunoperoxidase-stained sections of 13-week-old microencapsulated islets. The presence and locations of the four pancreatic hormones indicated a relatively normal-looking (results for D and PP cells not shown) islet cell population and distribution (Fig. 2).

Isotransplantation experiments were performed to determine whether the encapsulated islets could survive and function. Wistar Lewis rats (Charles River Breeding Company) were rendered diabetic by an intravenous injection of streptozotocin (65 mg per kilogram of body weight). The results on implantation of microencapsulated islets (from Wistar rats) along with suitable controls showed that the unencapsulated islets survived for 6 to 8 days as anticipated. The five recipients of microencapsulated islets were normoglycemic for almost 3

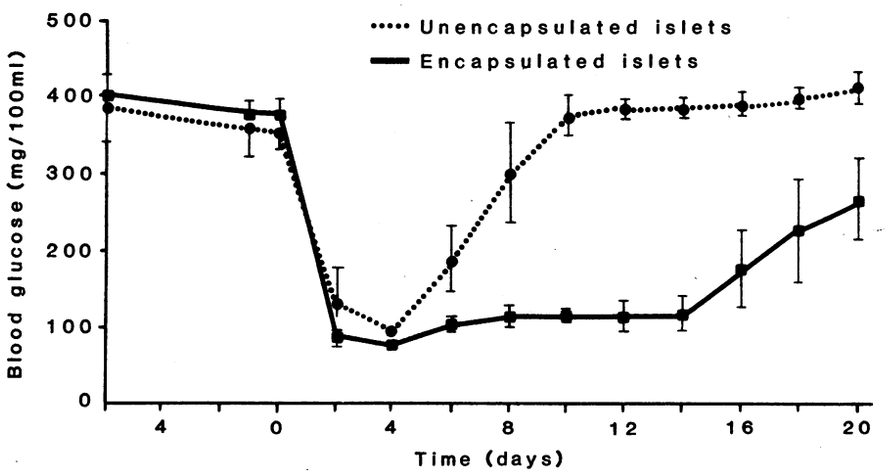

Fig. 3. Fasting blood glucose concentrations of diabetic rats before and after intraperitoneal transplantation of unencapsulated and microencapsulated islets. A single dose of about 2×10^3 to 3×10^3 microencapsulated islets from Wistar rats was injected into each of five diabetic Wistar Lewis rats 2 weeks after the animals had become diabetic. As controls, five diabetic rats received a transplantation of approximately the same number of unencapsulated islets as the experimental rats. In both situations the blood glucose was returned to normal and remained low for 4 days. In the rats that received the unencapsulated islets the blood glucose increased by 6 days and by 10 days had risen to the concentration before treatment. In the rats that received the microencapsulated islets the blood glucose was significantly lower than the controls for 20 days when the experiment was terminated.

weeks (Fig. 3); the symptoms of diabetes such as polyuria and polydypsia were markedly reduced.

These findings indicate that islet isograft survival and amelioration of the diabetic state can be obtained by intraperitoneal implantation of microencapsulated islets into rats with chemically induced diabetes. The microencapsulated islets remained morphologically and functionally intact for as long as 4 months in culture in vitro at 37°C. The microencapsulation technique described herein offers certain advantages over other transplantation methods. For example, the maintenance of viable islets or dispersed endocrine tissues in culture for long periods can contribute materially to the collection of sufficient islets for implantation. It also avoids the problems associated with diffusion chambers and hollow fiber units. These problems include (i) the small surface area of the devices, which necessitates the use of large units that may be too bulky and complex; (ii) the surgery required for implantation or shunt connection with risks of clotting or infection; and (iii) the relatively high diffusion resistance inherent in the filter membranes and hollow fibers, which results in long transit time (*18*).

Microencapsulation also provides more options for the method and site of implantation. Unlike the bulky hollow fiber units and diffusion chambers, microcapsules are small enough to pass through a gauge 18 or 19 hypodermic needle. Therefore, several options are available for method of implantation. The preferred manner of introduction of the microcapsules, for example, through surgical incision openings at one or multiple sites or through direct hypodermic injections, remains to be determined. Currently, placement of islets or dispersed pancreatic tissues in the liver by way of the portal vein is highly favored. Sutherland *et al.* (*2*) have also shown that injection into the splenic pulp is effective. Other studies have indicated that the intramuscular route may be as effective as the intraperitoneal route (*19*). Further studies should provide significant information in all of these areas and may lead to the use of islet transplantation in the treatment of human diabetes.

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Inhibition of Biological Activity of Mouse β-Nerve Growth Factor by Monoclonal Antibody

Abstract. *A continuous hybrid cell line was derived that secretes monoclonal antibody capable of inhibiting the biological activity of mouse β-nerve growth factor (β-NGF). Results obtained with monovalent fragments indicate that the monoclonal antibody inhibits activity by interfering with the direct interaction between β-NGF and the cell membrane receptor rather than by precipitating the dimeric form of β-NGF. This monoclonal antibody binds to an antigenic determinant common to mouse β-NGF, snake venom (*Naja naja*) β-NGF, and human β-NGF. These antibodies should provide specific molecular probes for a variety of studies of nerve growth factor including its tissue distribution and mechanism of action.*

Considerable data concerning the structure and function of nerve growth factor (NGF) have been obtained with the use of specific antiserums to mouse β-nerve growth factor (β-NGF). Developmental immunosympathectomy (immunological interruption of sympathetic nerve pathways) can be effected in various mammals by treating them after birth with rabbit antiserum specific for mouse β-NGF (*1*). Immunological comparisons among mammalian NGF's indicate that although some antigenic determinants are evolutionarily conserved, many are not (*2*). Precise immunochemical characterization of these determinants is not possible with polyclonal antibody reagents. Specific radioimmunoassays have been developed by using affinity purified antibodies that are sensitive to about 0.25 ng of β-NGF per milliliter (*3*, *4*). The limits of sensitivity and specificity have been reached in methods in which conventional antibody reagents are used, and it has been suggested that more sensitive assays will require the preparation of monoclonal antibodies (*5*). In this report we describe the derivation of a hybridoma that secretes monoclonal antibody capable of inhibiting the biological activity of mouse β-NGF.

From the submaxillary glands of adult male CF1 mice we isolated 7*S* NGF and separated the β subunit by carboxymethyl cellulose ion-exchange chromatography (*6*). Human β-NGF was isolated from term placenta according to the method of Mobley *et al.* (*7*). The purity of β subunit preparations was assessed by polyacrylamide gel electrophoresis at *p*H 10.3 (*8*) and by isoelectric focusing in a *p*H gradient (*p*H 3 to 10) in 7.5 percent acrylamide gels (*9*). A 5-week-old male Lewis rat was immunologically primed with an intraperitoneal injection of 500-μg of glutaraldehyde-polymerized β-NGF emulsified in complete Freund's adjuvant. Three weeks later the same rat was injected intraperitoneally with 300 μg of polymerized β-NGF in incomplete Freund's adjuvant.

Four days after the secondary immunization, spleen cells were obtained from the rat and suspended in RPMI 1640 medium. These cells were mixed with P3 × 63Ag8 mouse plasmacytoma cells at a ratio of 5:1 in a small petri dish and centrifuged at 1700 rev/min for 5 minutes (*10*). The fusing agent, PEG-1000 (polyethylene glycol; Baker Chemical Co.) dissolved 35 percent, weight to volume, in Dulbecco's modified Eagle's medium (DMEM) at 37°C, was carefully added to the resulting monolayer of cells over a 1-minute period. One minute later DMEM was slowly added and the supernatant

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