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Injectable Microencapsulated Islet Cells as a Bioartificial Pancreas

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ABSTRACT

Rat islets encapsulated in semipermeable membranes remained viable in culture for 4 months. Multiple allotransplants of islets encapsulated in alginate–polylysine–polyethyleneimine membranes restored normoglycemia in recipient diabetic rats for most of a 90-day experimental period. Each individual transplant restored normal fasting plasma glucose levels for 15–20 d. The failure of the encapsulated islets was caused by an inflammatory response induced by polyethyleneimine. In contrast a single transplant of islets encapsulated in a biocompatible alginate–polylysine–alginate membrane restored normoglycemia in recipient animals for up to 10 months. Capsules with intact membranes and containing viable islets were recovered from the abdominal cavity 5 months post-transplantation. SEM studies on capsule membranes revealed essentially smooth surfaces. Differences between wet and dry wall thicknesses indicated that the membrane is a hydrogel, 4.00 ± 0.28 μm thick in an aqueous environment.

The clinical potential of transplanting cells encapsulated in biocompatible semipermeable hydrogel membranes is demonstrated by this study.

Index Entries: Microencapsulation, of islet cells; islet transplantation, microencapsulation in; bioartificial pancreas; biocompatible microencapsulated islets; alginate, in encapsulated islet support; poly-L-lysine, in encapsulated islet support; polyethyleneimine, in encapsulated islet support; islets, microencapsulated; pancreas, microencapsulated islets as an artificial.

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INTRODUCTION

The major obstacle to islet transplantation as a treatment for diabetes is rejection of the transplanted tissue by immune rejection. One approach to overcome this problem is the introduction of a semipermeable physical barrier between the transplanted islets and the host's immune system. A limited amount of success has been achieved in experimental animals using hollow fiber devices (*1–3*). Microencapsulation of islets within semipermeable membranes is an attractive alternative. Microbial cells, yeast, chloroplasts, plant chloroplasts, mitochondria, and enzymes have been immobilized or entrapped using calcium alginate gels (*4–6*).

Lim and Sun (*7*) successfully microencapsulated islets using alginate gel, poly-L-lysine, and polyethyleneimine. They demonstrated also that the encapsulated islets could restore normoglycemia in diabetic rats when injected into the peritoneal cavity.

This paper describes long-term in vitro and in vivo studies with islets encapsulated using two different membrane formulations and demonstrates that transplanted islets encapsulated in a biocompatible membrane can survive for up to 10 months.

MATERIALS AND METHODS

Isolation of Islets

Islets were isolated from out-bred Wistar rats (body weight, 200–250 g) using collagenase digestion techniques (*8,9*). They were hand-picked and cultured for 1–3 d at 37°C in tissue culture medium CMRL-1969® supplemented with 7.5% bovine fetal serum and 300 mg/dL glucose (*10*).

Microencapsulation of Islets in Alginate–Polylysine–Polyethyleneimine Membranes

The method used was reported previously by Lim and Sun (*7*). Islets were suspended in 0.8% sodium alginate (Sigma, St. Louis) and 0.85% NaCl at a concentration of 1×10^3 islets/mL. Islet-containing droplets formed by syringe pump extrusion gelled upon collection in 1.5% $CaCl_2$. These gels were washed with 25 mL each of 0.1% CHES [2-(*N*-cyclohexylamino)ethane-sulfonic acid] and 1.0% $CaCl_2$ and then suspended in 25 mL of a 0.02% poly-L-lysine (Sigma, mw 25,000–70,000, PLL) solution for 6 min. After washing with 1.0% $CaCl_2$, the capsules were coated with polyethyleneimine (PEI) by suspension in a 0.2% solution of PEI (Polysciences, mw 40,000–60,000) for 4 min. Following washing steps with $CaCl_2$ (1%) and NaCl (0.9%), the capsules were suspended in isotonic sodium citrate for 6 min to liquify the unbound alginate within the capsules.

Microencapsulation of Islets in Alginate–Polylysine–Alginate Membranes

To produce more biocompatible and durable microcapsules, some critical changes were made in the microencapsulation procedure originally reported by Lim and Sun (7). The outer polyethyleneimine membrane was omitted and replaced by a more biocompatible outer alginate membrane. The strength of the polylysine membrane was enhanced by increasing its thickness.

Islet Culture

Both microencapsulated and unencapsulated islets were cultured at 37°C in Medium CMRL-1969 supplemented with 7.5% fetal bovine serum and 300 mg/dL glucose. During long-term cultures the medium was replaced weekly and the glucose concentration alternated between 50 and 300 mg/dL.

Electron Microscopy Studies

For electron microscopy, encapsulated rat islets were fixed in 2.5% glutaraldehyde, post-fixed in 1% OsO_4, dehydrated and embedded in an Epon-Araldite mixture. Ultrathin sections were cut and stained with uranyl acetate and lead citrate.

Histochemical Studies

Encapsulated islets and tissue segments were fixed in Bouin's solution and processed for light microscopy. Sections were stained with aldehyde thionin, hematoxylin, and eosin.

Allotransplantation Experiments

Diabetes was induced in out-bred Wistar rats by iv injection of streptozotocin (65 mg/kg body weight). Blood samples for fasting plasma glucose concentration were collected by bleeding from the orbital sinus. A Beckman Glucose Analyzer was used for glucose measurement. Diabetes was confirmed by the development of persisting fasting plasma glucose levels in excess of 350 mg/dL. Microencapsulated islets, suspended in saline, were implanted in the peritoneal cavity of the diabetic rats using a cannula attached to a 10-cc syringe.

Surface Finish Evaluation of Capsules by Scanning Electron Microscopy (SEM)

Microcapsules were placed directly on an aluminum SEM stub and dried in a vacuum oven at 60°C for 1.5 h. The samples were coated with approximately 250 Å of gold and inserted into the SEM. Both the interior

and exterior surfaces of the microcapsules were examined. The SEM was also utilized to make microcapsule wall thickness measurements.

Wall Thickness and Uniformity of Microcapsules

Microcapsules were placed on aluminized, front-surface mirrors and dried at room temperature for 24 h. The dry-wall thickness was measured by interferometry. The wet-wall thickness and wall uniformity of the microcapsules was measured by the image-shearing method. Four wall-thickness measurements were made at the equator of each capsule.

RESULTS

Electron Microscopy

An electron microscopy study of encapsulated islets revealed that the structural integrity of the cells was maintained. The typical granulation of both α-cells (Fig. 1a) and β-cells (Fig. 1b) was clearly seen in encapsulated islets which were maintained in culture for 90 d.

Histology of Microencapsulated Islets

Islets encapsulated in the alginate–polylysine–polyethyleneimine membrane were cultured for 90 d before being processed for light microscopy. The islets remained relatively intact and showed uniform and even staining with aldehyde thionin throughout all islet cross-sections (Fig. 2a). Islets encapsulated in the alginate–polylysine–alginate membrane showed good β-granulation until at least 135 d (Fig. 2b).

Allotransplantation Experiments with Alg–PLL–PEI Capsules

Two rats with streptozotocin-induced diabetes received multiple intraperitoneal transplants of approximately 3000 encapsulated islets as described in Methods. The blood glucose levels, urine volumes, and body weights were monitored at regular intervals. The initial transplants lowered the blood glucose concentration from 375 mg/dL to control values (75–150 mg/dL) within 2 d. When the animals returned to the diabetic state, a second transplant of encapsulated islets was performed and the blood glucose levels were again lowered to within the control range. This pattern was repeated with three further transplants. On each occasion, blood glucose concentrations remained below diabetic levels for 15–20 d (Table 1). A control group of diabetic rats receiving no transplants or transplants of empty capsules had blood glucose levels higher than 350 mg/dL throughout this study. Fluctuations in blood glucose concentration of the animals receiving transplants were reflected by alterations in the daily urine output, the volume decreasing with a lower-

ing of the blood glucose concentration and increasing as the glucose level increased.

The body weight of animals receiving islet transplants increased from 230 and 260 g to 285 and 320 g, respectively. In contrast, the body weights of the control diabetic animals did not change significantly (± 10 g) over the same period of time.

As is usual in streptozotocin-induced diabetes, the control diabetic animals all developed eye cataracts within 12 weeks of the onset of diabetes. In contrast, the eyes of the two animals receiving multiple islet implants remained cataract-free.

The animals were sacrificed 20 d after the final transplants. No free capsules were found in the abdominal cavity. Sections of liver, pancreas, kidney, and the peritoneum were taken for histology studies. Foreign body granulosa were observed on all tissues. The capsular membranes were completely surrounded by giant cells and fibrous tissue (Fig. 3). No islets were found in any of the sections examined. Implanation of empty capsules in control animals resulted in a similar foreign body reaction within 10–20 d.

Allotransplantation Experiments with Alginate–Polylysine–Alginate Capsules

A series of five diabetic rats received intraperitoneal allografts of 4.5×10^3 islets encapsulated in alginate–polylysine–alginate membranes. Normoglycemia (fasting plasma glucose < 200 mg/dL) was restored within 2 d and persisted for up to 10 months (Fig. 4). Of the five animals receiving transplants, four remained normoglycemic for > 77 d and three of these for > 120 d. One animal is still normoglycemic 150 d post-transplant, a second animal still has fasting plasma glucose levels < 200 mg/dL 310 d post transplantation. Capsules were recovered from the peritoneal cavity of one recipient 156 d post-transplantation. The capsules had some host cells attached to the outside surface of the membrane but were still intact. When maintained in culture these encapsulated islets secreted low levels of insulin into the culture medium.

All recipient animals gained weight steadily during their normoglycemic period. The mean weight gain was 116 ± 16.5 g (mean ± SEM). None of the animals developed eye cataracts.

Surface Finish, Wall Thickness, and Uniformity of Alginate–Polylysine–Alginate Capsule Membranes

Surface finish evaluation by scanning electron microscopy (SEM) revealed essentially smooth interior and exterior capsular surfaces. Although some samples were observed to have "pebble-like" surface features about 0.1 μm in size and 0.15 μm apart (Fig. 5a), this could have been caused by the electron beam of the SEM. Utilizing the SEM, dry

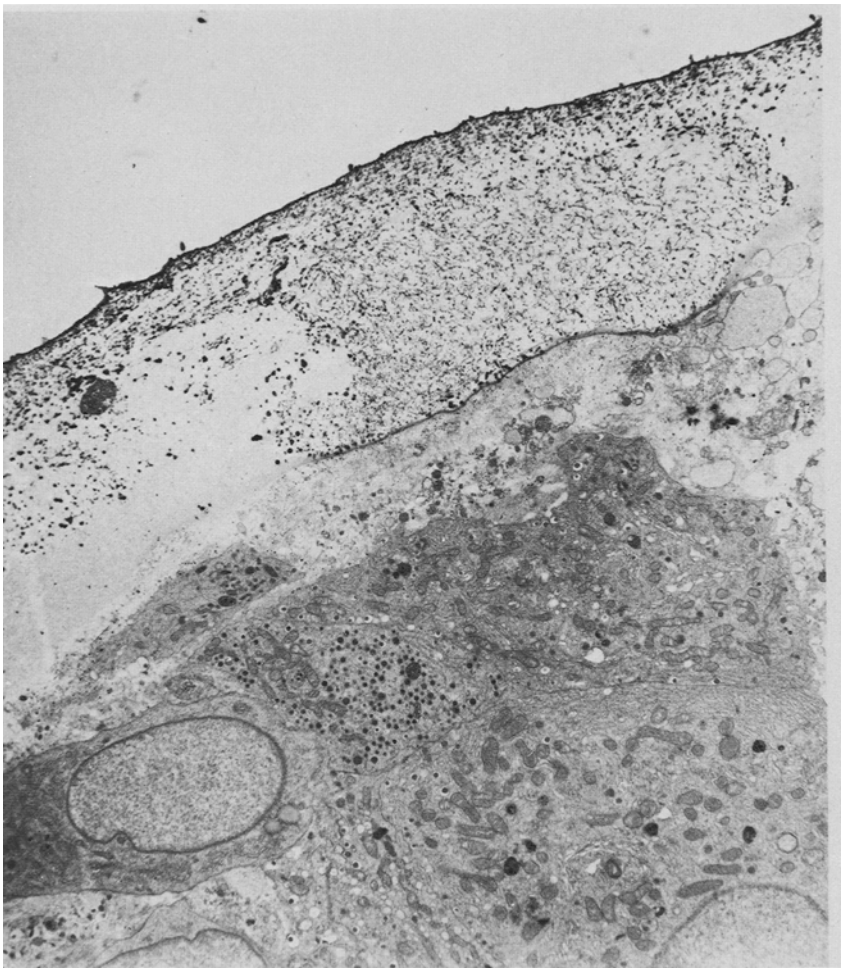

Fig. 1. Electron micrographs of sections of an encapsulated islet that had been maintained in culture at 37°C for 90 d: (a) section showing α-cell with glucagen granules (×9000). (b) section showing β-cell with insulin granules (×14,000).

capsule walls thicknesses ranging from 0.2 to 0.7 μm were observed (Fig. 5b), although it was not always clear whether or not a single wall was being measured. Using interferometry, the two-wall thickness of dried microcapsules was determined to be 0.57 μm ± (mean ± 0.11 μm SD; $N = 10$). Interferometric pictures demonstrated that there was very little variation in the two-wall thickness of individual dried microcapsules.

The "wet" wall thickness and wall variation of 10 randomly selected capsules, determined by the image shearing method, was found to be 4.00 ± 0.28 μm(± SD). The average capsule wall variation, obtained by averaging the 10 standard deviations obtained from the four thickness measurements of each microcapsule, was determined to be 0.32 μm. Only small differences were observed from sample to sample.

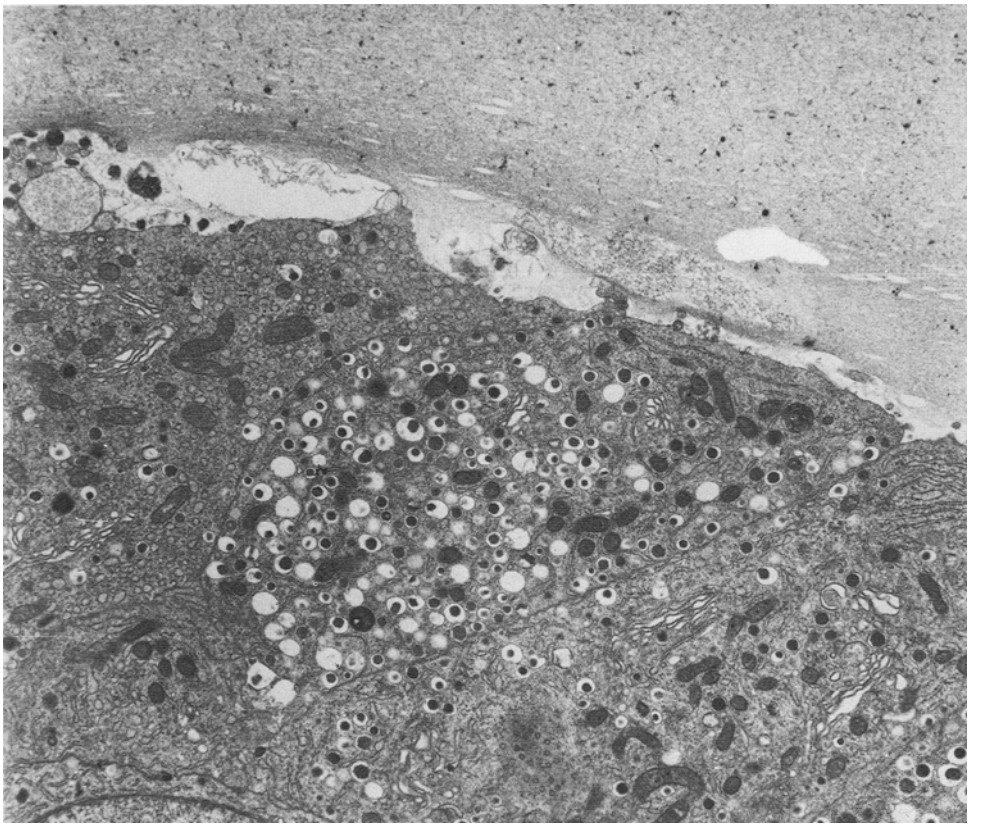

Fig. 1B.

DISCUSSION

The medical applications of immobilized enzymes have been established by Chang et al. (*11–13*), who also first proposed the possible application of microencapsulation for islet cells (*14*). Lim and Sun (*7*) demonstrated that rat islet cells, encapsulated in alginate–polylysine–polyethyleneimine membranes, could be maintained in culture for several months and when transplanted intraperitoneally into diabetic rats, restored normoglycemia for 15–20 d. The histology results in the Lim and Sun study and those described above showed that encapsulated islets could be maintained in a viable state in culture for at least 4 months and still retain a normal degree of β-granulation. The electron microscopy studies illustrated that the structural integrity of the α- and β-cells were retained during long-term culture. It has previously been reported by our laboratory that encapsulated islets continued to secrete insulin into the culture medium in response to a glucose challenge during a 2-month incubation period (*15*). Working with erythrocytes Pilwat et al. (*16*) also demonstrated that cells entrapped in a crosslinked alginate ma-

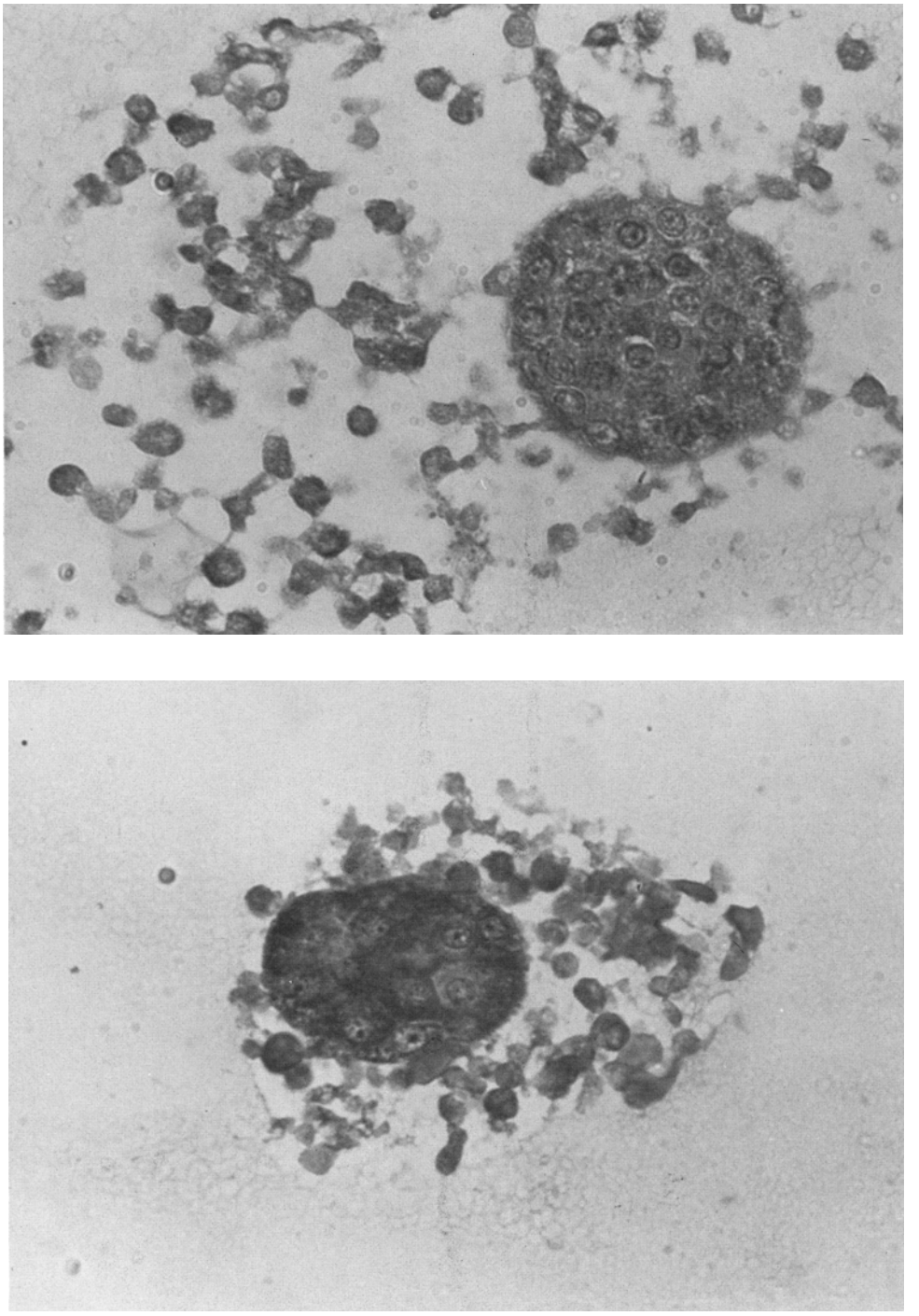

Fig. 2. Light micrographs of encapsulated pancreatic islets: Top: islets in alginate–polylysine–polyethyleneimine membranes after 90 d in culture; Bottom: islets in alginate–polylysine–alginate membranes after 135 d. Aldehyde thionin stain. Magnification 30×.

TABLE 1
Duration of Normoglycemia and Weight Increase in Diabetic Rats Following Multiple Transplants of Encapsulated Islets

	Duration of normoglycemia, d					
	Transplant No.					
Animal	1	2	3	4	5	Wt increase, g
A	16	16	18	17	18	55
B	15	16	17	20	13	60

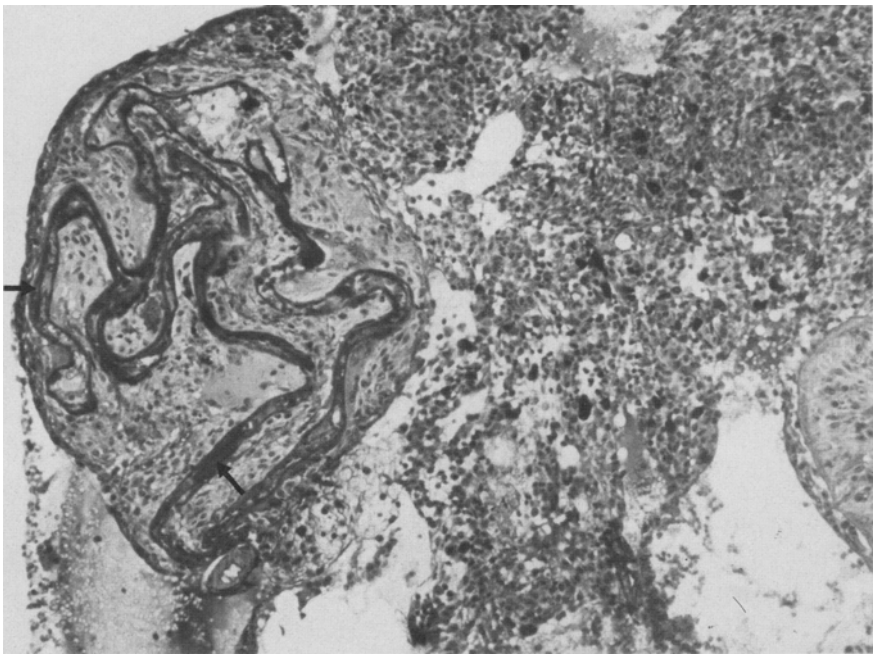

Fig. 3. Light micrograph of foreign body granuloma in a diabetic rat that had received 5 ip transplants of 3×10^3 microencapsulated islets. The animal was sacrificed 90 d after the initial transplant and the liver processed for histochemical staining. A capsule membrane (arrow) can be seen completely surrounded by giant cells and fibrous tissue. Magnification 7.5×.

trix could be stored for substantially longer periods than those kept in suspension.

The allograft experiments with islets encapsulated in alginate–polysine–polyethyleneimine membranes demonstrated that in addition to a single intraperitoneal transplant of 3×10^3 islets restoring normoglycemia for 2–3 wk as reported by Lim and Sun (*7*), repeated transplants maintained the animals in a near-normoglycemic state during the 90-d experimental period. Each time the animals became

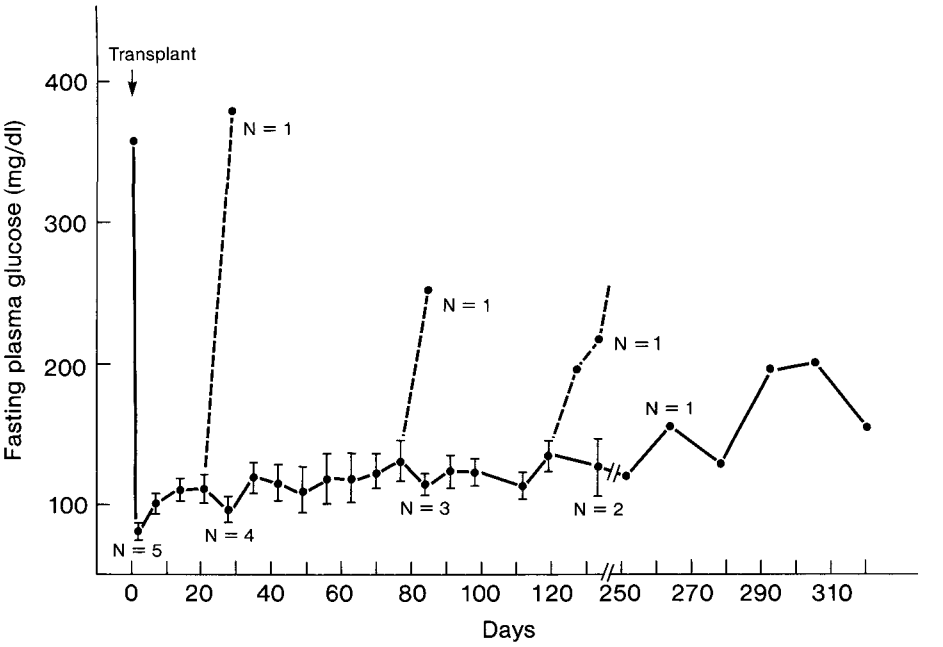

Fig. 4. Fasting blood glucose levels in rats transplanted with encapsulated islets. Male Wistar rats rendered diabetic by iv injection of streptozotocin (65 mg/kg) were transplanted with approximately 4.5×10^3 islets encapsulated in alginate–polylysine–alginate membranes. Samples for glucose assay were collected by bleeding through the orbital sinus. The points are mean values and the bars represent the SEM.

hyperglycemic an additional transplant restored normal fasting glucose concentrations for a period lasting at least as long as the previous transplant. This suggests that a simple immunologic rejection of the transplanted tissue was not occurring. Histology studies revealed the presence of foreign body granulosa in the tissues of the host animals. Capsule membranes were engulfed by giant cells and fibrous tissue, indicating that the capsules were not biocompatible. The composition of the capsule membrane was subsequently modified to improve its biocompatibility. The thickness of the polylysine layer was increased and the outer polyethyleneimine layer replaced by inert alginate. A single allotransplant of islets encapsulated in this modified membrane restored normoglycemia in diabetic recipients for up to 10 months.

The modifications to the composition of the capsule membrane increased the biocompatibility of the capsules very significantly. With the alginate–polylysine–alginate membrane, capsules were recovered from the peritoneal cavity 5 months post-transplantation. In comparison, with the original alginate–polylysine–polyethyleneimine capsules, no free capsules were found; a rapid foreign body reaction was induced within 20 d. The SEM studies showed that the alginate–polylysine–alginate cap-

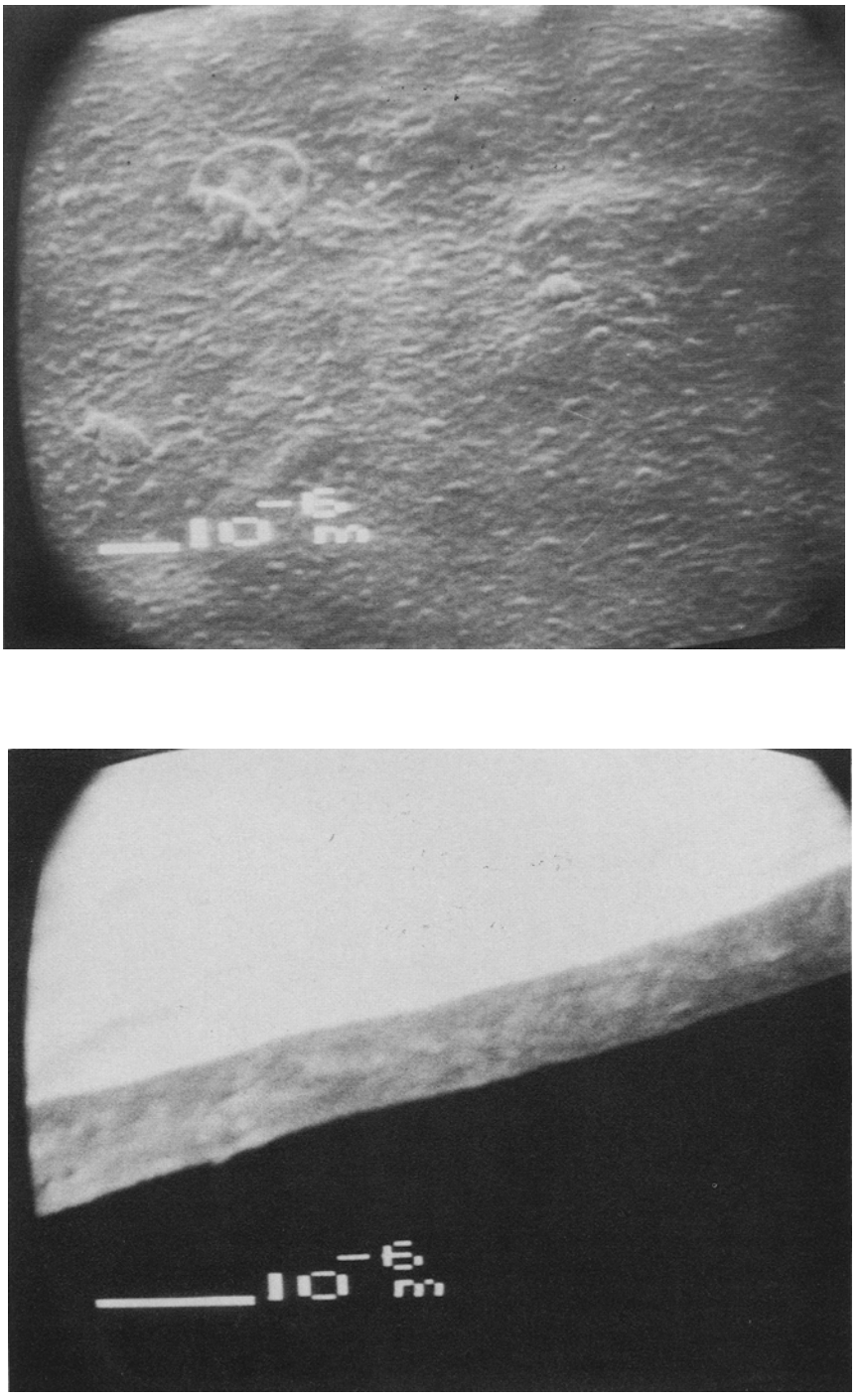

Fig. 5. SEM of alginate–polylysine–alginate capsules: (a) external surface of capsule; (b) cross-section of the membrane wall (dark diagonal line, 0.5 μm thick, running from lower left to upper right).

sules have a relatively smooth surface. The wet wall thickness is approximately 14 times greater than the dry-wall thickness indicating that the capsule wall is a hydrogel containing approximately 90% water. Hydrogels are receiving considerable attention as candidates for good biocompatible materials (*17–19*). The low interfacial tension between the swollen gel surface and the aqueous biological environment minimizes protein interaction. This interaction (*19*) may serve as a trigger mechanism for rejection mechanisms. The soft rubbery consistency of most hydrogels may also contribute to their biocompatibility by reducing frictional irritation to surrounding tissues (*20*).

CONCLUSION

Islets encapsulated in a biocompatible and durable hydrogel membrane composed of polylysine sandwiched between two alginate layers can be transplanted into the animal body and survive for at least several months. Such capsules have a great clinical potential for the treatment of diabetes and other hormone or enzyme replacement therapies.

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