Å) might exhibit a shifted amide I frequency. In particular, an increase in hydrogen bond distance of 0.05 Å could produce a rise in the frequency of the amide I band by as much as 10 cm^{-1} (*16*). Two examples of distorted α-helical polypeptides with amide I frequencies near 1665 cm^{-1} are poly-β-benzyl-L-aspartate, which forms a left-handed helix (*17*), and poly-β-benzyl-DL-glutamate, which forms an $\alpha_{L,D}$-helix (*18*). In the case of bacteriorhodopsin, the existence of distorted α-helical conformation is also supported by x-ray scattering on dried purple membrane (*19*), which reveals an anomalous pitch $P = 5.05$ Å. A second possibility that cannot be excluded is that factors other than α-helix structure, such as the two-dimensional crystalline arrangement of bacteriorhodopsin in the purple membrane, give rise somehow to the anomalous amide I frequency.

Other measurements have revealed features of bacteriorhodopsin that may lead to distortion in the α-helices. First, partial amino acid sequencing of bacteriorhodopsin (90 residues) (*20*) shows that at least 50 percent of the sequence that is expected to be α-helical based on the electron diffraction model (*5*) is not α-helical (*20*). Second, electron diffraction shows the existence of supercoils in the α-helices of bacteriorhodopsin (*19, 21*). It is not known whether the degree of supercoiling is sufficient to account for the observed shift in the amide I frequency. Infrared spectroscopy of highly supercoiled proteins such as fd phage coat protein might help shed light on this question.

In summary, infrared absorption measurements of purple membrane in D_2O suspensions and partially dried in sucrose, considered together with x-ray data, indicate that there is distortion in the structure of bacteriorhodopsin α-helices. It will be important to know whether this feature is common to other membrane proteins, or is related in some specific way to the proton transport function of bacteriorhodopsin.

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Prolongation of Islet Allograft Survival Following in vitro Culture (24°C) and a Single Injection of ALS

Abstract. *Isolated rat islets remain morphologically and functionally intact during a 7-day period of in vitro culture at 24°C. In vitro culture of islets at 24°C for 7 days prior to transplantation, in conjunction with a single injection of antiserum to lymphocytes into the diabetic recipient, results in islet allograft survival of 100 days when the islets are transplanted across a major histocompatibility barrier.*

Lafferty *et al.* (*1*) obtained prolonged survival of allografts of the thyroid gland by in vitro culture of the donor thyroid in the presence of 95 percent O_2 and 5 percent CO_2 for 26 days prior to transplantation. They suggested that organ culture of the thyroid removed passenger lymphoid cells and that these lymphoid elements played a major role in the sensitization of the host to foreign antigens of the thyroid cells. We attempted to utilize this in vitro approach for prolongation of islet allograft survival and found that 95 percent O_2 produced disintegration of isolated islets after 4 to 5 days of in vitro culture. Because of this limitation, we determined the effect of prior treatment of donor rats with total body irradiation and intravenous silica in conjunction with either in vitro culture of the islets in the presence of rabbit antiserum to rat lymphocytes (ALS) for 1 to 2 days or a single injection of ALS into the recipients at the time of transplantation (*2*). Prolongation of islet allograft survival was obtained across a minor histocompatibility barrier (Fischer to Lewis) by pretreatment of the donors and in vitro culture of the islets with ALS and across a major histocompatibility barrier (ACI to Lewis) by pretreatment of the donors in conjunction with a single injection of ALS into the recipients.

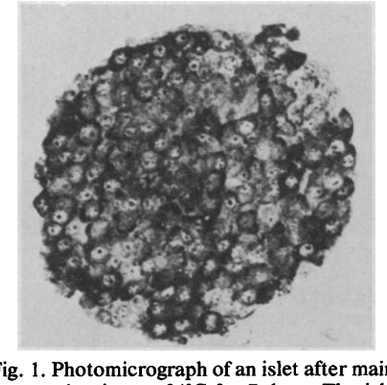

Fig. 1. Photomicrograph of an islet after maintenance in vitro at 24°C for 7 days. The islet cells appear normal and the beta cells have a normal degree of beta granulation. Small groups of alpha cells are present in the upper right and lower left portion of the photograph. Aldehyde fuchsin stain; magnification, × 300.

Opelz and Terasaki (*3*) reported that in vitro maintenance of lymphocytes at a low temperature (22°C) for more than 4 days resulted in loss of the ability of the lymphocytes to stimulate allogeneic lymphocytes in mixed lymphocyte cultures. Since we were attempting either to diminish or to alter the lymphoid elements in the islets prior to transplantation, we determined whether isolated islets would survive for 7 days when they were incubated in vitro at room temperature (24°C). Surprisingly, the islets not only survived but remained morphologically and functionally intact at the end of the 7-day incubation period. Figure 1 illustrates a normal degree of beta granulation and the presence of alpha cells at the periphery of an islet after 7 days of culture at 24°C. Insulin secretion during in vitro culture (24°C) was 16 μU per islet per hour in the presence of glucose (1.5 mg/ml) and increased to 60 μU per islet

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per hour when the temperature was raised to 37°C. The daily rate of secretion ranged from 60 to 90 μU per islet per hour during the subsequent 7-day period of culture at 37°C. Studies were then undertaken to determine the effect of in vitro culture at 24°C on the survival of islets transplanted across a major histocompatibility barrier. Islets were isolated from ACI rats (AgB^4), maintained in vitro for 7 days (24°C), and transplanted into diabetic Lewis rats (AgB^1); a single injection of ALS was administered to the recipients at the time of transplantation.

Male Lewis rats (250 to 300 g) were used as recipients and made diabetic by the intravenous injection of streptozotocin (65 mg per kilogram of body weight). These rats were housed in metabolic cages, and 24-hour urine volumes, urine glucose per 24 hours, and weights of the rats were obtained daily before and after islet transplantation. The diabetic animals were monitored for 4 to 5 weeks prior to transplantation and the mean 24-hour urine glucose was determined for the 7 days immediately before transplantation of the islets. The pre- and posttransplant levels of glucose for each rat were used to determine the time of rejection of the islets. Rejection was considered to have occurred when the posttransplant urine glucose exceeded the mean minus 1 standard deviation of the pretransplant level. Islets were isolated by the collagenase technique (*4*) and separated on a Ficoll gradient (*5*). Islet tissue was removed from the gradient and, with the aid of a dissecting microscope, only islets free of attached vascular and ductal tissue were selected and removed with a Pasteur pipette. The isolated islets were maintained in vitro in tissue culture medium CMRL-1066 containing fetal calf serum (10 percent), penicillin (100 U/ml), streptomycin sulfate (100 μg/ml), and D-glucose (1.5 mg/ml). The islets were incubated in untreated, plastic culture dishes to prevent attachment of the islets to the dish and were maintained in an atmosphere of air and 5 percent CO_2 at 24°C for 7 days. The portal vein technique (*6*) was used for islet transplantation. Either cultured or freshly isolated islets (1200 to 1500 islets) were injected into the portal vein of the diabetic recipients. One animal received only 830 islets. Rabbit antiserum to rat lymphocytes (1 ml) was injected intraperitoneally into the recipients immediately following transplantation of the islets.

Freshly isolated, ACI islets transplanted into diabetic Lewis rats were rejected rapidly with a mean survival time of 5.2 days and a mean weight loss of 2.8 g in the recipients (Fig. 2, A and B). The survival time of the allografts was increased slightly by a single injection of ALS at the time of transplanting freshly isolated islets, resulting in a mean survival time of 8.6 days and a mean weight gain in the recipients of 5.8 g. When the islets were maintained in culture for 7 days (24°C) and a single injection of ALS was administered to the recipients, none of the recipients had rejected the islet allografts at 100 days after transplantation (Fig. 2A). Two of the animals were aglycosuric at 100 days and their mean pretransplant levels of urine glucose were 12.0 and 9.9 g/24 hours, respectively. The urine glucose at 100 days in the other three rats of this group was 5.5, 6.4, and 6.6 g/24 hours, respectively, as compared to mean pretransplant levels of 9.8, 10.8, and 10.5 g/24 hours. One of these rats (5.5 g of urine glucose per 24 hours at 100 days) received only 830 islets instead of 1200 to 1250 islets. The diabetic recipients in this group gained weight at a very rapid rate for 30 days after transplantation and continued to gain weight at a slower rate during the subsequent 70 days (see Fig. 2B). The mean total weight gain for this group was 100.2 g for the 100-day period.

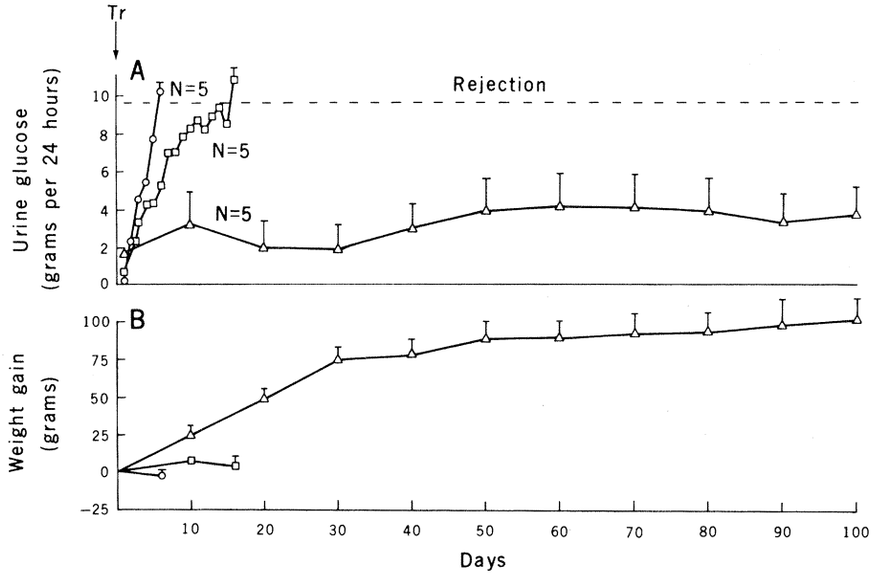

Fig. 2. (A) Urine glucose (in grams per 24 hours) following transplantation of islets from donor rats (ACI) into diabetic recipients (Lewis). Group 1 (○) received 1200 to 1250 freshly isolated islets; group 2 (□), freshly isolated islets and a single injection of ALS (1 ml) at the time of transplantation; and group 3 (△), islets that had been maintained in culture (24°C) for 7 days and a single injection of ALS at the time of transplantation. The line representing rejection (- - -) is the mean minus 1 standard deviation of the 24-hour urine glucose levels for all three groups for the 7-day interval prior to transplantation. The vertical lines indicate the standard error of the mean (S.E.M.). (B) Weight gain (mean ± S.E.M.) from the time of transplantation to rejection or continued survival of the allografts for each of the three groups.

These initial findings indicate that a marked prolongation of islet allograft survival across a major histocompatibility barrier can be obtained by the use of in vitro culture (24°C) for 7 days in conjunction with a single injection of ALS into the recipients. The immune barrier is a major deterrent for the utilization of islet transplantation as a therapeutic approach to human diabetes. Further investigations with the simple model described in this report should provide additional basic information on the mechanism of induction of immune rejection and possibly may be of assistance in the eventual application of islet transplantation to the treatment of diabetes in man.

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