biflorus combined. At all localities, the density distributions of total myrmecochore populations on mounds are statistically indistinguishable. These results suggest that the two myrmecochorous chenopods are competing for a spatially limiting microhabitat (*16*).

What is the mechanism of the competition? Depression of *S. diacantha* populations in the presence of *D. b. biflorus* is probably mediated both by competition for dispersal to ant nests and by competition for space on mounds. We cannot yet assess the relative contributions of these processes or evaluate the factors favoring one competitor over another (*17*). It seems clear, however, that competition for dispersal agents may be important in regulating the composition of plant communities in this and similar floras where plant establishment depends on highly directional transport of seeds to favorable microhabitats.

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10. Student's *t*-test: $P < .02$ to $P < .001$ ($N = 5$ sites) for *S. diacantha*; $P < .005$ and $P < .001$ ($N = 2$) for *D. b. biflorus*. A second variety of *D. biflorus* (*D. b.* var. *cephalocarpus*) was not ant-dispersed and did not grow in association with ant mounds ($N = 2$). Likewise, *S. ventricosa*, a nonmyrmecochore related to *S. diacantha*, was not associated with mounds ($N = 5$).
11. In general, soils of the Australian arid zone are old, leached, and nutrient-poor. Depending on depth, concentrations of available nitrogen average from more than 100- to more than 200-fold higher on mounds than off mounds, and available phosphorus is more than two to three times more concentrated [$P << .0005$ for each comparison (*7*)]. Soils are also less compacted on mounds ($P << .0005$) and may allow greater water penetration and storage.
12. Off the mounds, bare scalds alternate with patches of vegetation, but our sampling scheme reduced microhabitat heterogeneity among samples. We sampled contiguous quadrats (0.25 m^2) along a transect and harvested plants only from those quadrats containing individuals of both species. Plants from both mound and off-mound quadrats were oven-dried to constant weight. For each quadrat sample, we measured the above-ground biomass of *S. diacantha* and *S. ventricosa* and the combined above-ground biomass of all other plants.
13. Mounds differ in both size and nutrient concentration (*7*) as well as in proximity to a source of diaspores.
14. Between sites, mean plant densities per mound quadrat ranged from 7.50 to 23.80, with the densities of nonmyrmecochores averaging ≤ one individual per mound at each site. We adjusted plant densities at each locality to the mean density across all sites (17.15 plants/mound). By standardizing the data in this way, we assume implicitly that the effect of an individual of *S. diacantha* on an individual of *D. b. biflorus* (and vice versa) is the same at all five sites, regardless of plant density. Variability in plant density probably parallels between-site differences in the suitability of growth conditions, such as access to limiting water and nutrients as a function of rainfall or soil type.
15. Over the three sites where *D. b. biflorus* was absent, there were averages of 9.00 low density, 5.33 moderate density, and 5.67 high density populations on mounds, in comparison to means of 15.50, 2.00, and 2.50, respectively, for sites at which *D. b. biflorus* was present.
16. On mounds, individuals of *D. b. biflorus* have an average of 1.18 times greater biomass than individuals of *S. diacantha*. Adjustment of mound populations by this equivalency factor does not alter the distribution of density classes at any site.
17. Differences in the diaspores of *S. diacantha* and *D. b. biflorus* may influence their relative success in becoming established. Those of *D. b. biflorus* contain more food material (both per propagule and per gram of diaspore) than diaspores of *S. diacantha*, and *D. b. biflorus* has two seeds per propagule in contrast to one in *S. diacantha*.
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Reversal of Diabetes by Islet Transplantation: Vulnerability of the Established Allograft

Abstract. *Nonspecific stimulation of the immune system of CBA mice carrying a functional BALB/c islet allograft failed to trigger graft rejection. Only three of six animals rejected their graft when injected intravenously with 10^5, 10^6, and 10^7 peritoneal cells of BALB/c origin over a 3-month period commencing 100 days after transplantation.*

Organ culture of pancreatic islets before transplantation can facilitate allograft acceptance by normal recipient animals (*1, 2*) or by recipients conditioned with a single dose of antiserum to lymphocytes at the time of transplantation (*3*). Such allografts are functional and reverse streptozotocin- or alloxan-induced diabetes (*1–5*). These findings indicate that pancreatic islet transplantation might be used to reverse insulin-dependent diabetes in humans. However, before considering clinical application of this technology, we need to know more about the susceptibility of the established allograft to rejection.

Organ culture before transplantation reduces the immunogenicity of islet, thyroid, and parathyroid tissue (*5*) but does not destroy tissue antigens; cultured allografts are consistently rejected when the recipient is immunized with lymphoreticular cells of donor origin at the time of transplantation (*5, 6*). This reduction in tissue immunogenicity is thought to result from a loss, or inactivation, of lymphoreticular stimulator cells during culture in an oxygen-rich atmosphere (*7–9*). T cell activation by alloantigens is very efficient when the stimulator cell provides both alloantigens and a source of costimulator activity, the second signal required for T cell activation (*10*). When these stimulator cells are removed from the tissue before transplantation the tissue retains recognizable antigens, but these are much less immunogenic in the recipient (*5, 7*).

While cultured allografts can be transplanted without a need for suppression of the recipient's immune system, such grafts are constantly under the threat of rejection. Nonspecific stimulation of the recipient's immune system could raise the level of costimulator activity and trigger irreversible rejection. In the case of transplants in humans, transfusion of blood for some therapeutic reason unrelated to the tissue transplant could trigger rejection if the transfused blood carried histocompatibility antigens similar to those in the graft. In this study we investigated the extent of these threats to the continued survival of an established allograft of cultured islet tissue in the mouse.

We first investigated the effect of nonspecific stimulation of the immune system on islet allograft function. CBA mice were made diabetic by intravenous injection of streptozotocin (300 mg/kg). Approximately 2 weeks later animals with blood sugar concentrations greater than 20 mmole/liter were given cultured

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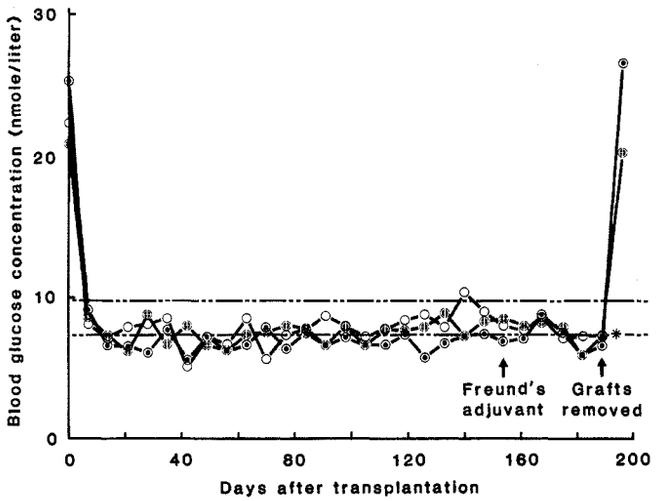

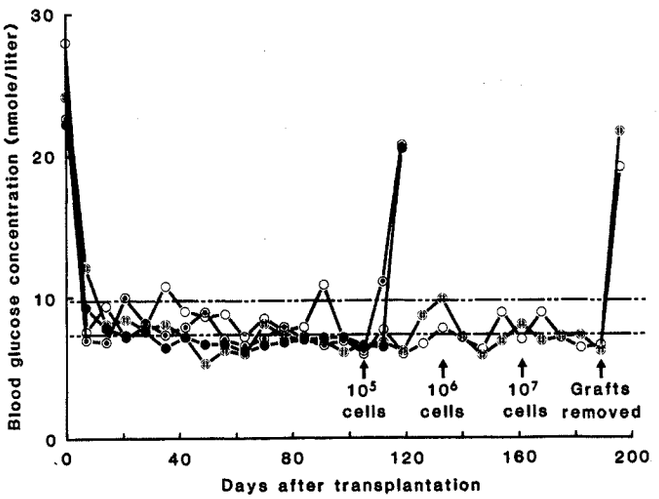

Fig. 1 (left). Blood sugar concentrations in the three nonfasting diabetic mice with islet allotransplants and receiving complete Freund's adjuvant 155 days after transplantation surgery. The antigenic substance did not effect allograft function, but the animals returned to the diabetic condition when the allograft was removed. The broken lines show the 95 percent confidence limits for blood sugar levels in normal CBA mice. The asterisk represents the death of one animal due to a technical error. Fig. 2 (right). Blood sugar concentrations in four nonfasting diabetic mice with islet allotransplants and receiving 10^5 BALB/c peritoneal cells 105 days after transplantation surgery. Two animals immediately rejected their grafts; the other two did not, even after the injection of 10^6 and 10^7 additional BALB/c peritoneal cells at the times indicated. These animals became diabetic when the transplanted islet tissue was removed.

BALB/c pancreatic islet tissue. Each recipient had seven clusters of islet tissue (equivalent to 350 islets) placed under a kidney capsule; organ culture of islet tissue and transplantation was carried out as previously described (*4*). Blood sugar concentrations in the recipients rapidly returned to normal or slightly below normal and remained so for more than 100 days (Fig. 1). In this strain combination, uncultured islet tissue is rapidly rejected following allotransplantation (*4*). At 155 days after transplantation three animals were injected intraperitoneally with complete Freund's adjuvant (*11*) emulsified with phosphate-buffered saline (*12*). Blood sugar levels in these animals were observed for an additional 35 days. None of the animals showed any evidence of a return to the diabetic state. However, when the allografts were removed by nephrectomy two animals became diabetic; the third died for technical reasons associated with the nephrectomy. Microscopic examination showed that the islet tissue in the kidneys was intact, with no evidence of rejection.

In a separate series of experiments a group of five animals carrying functional islet allografts showed the same response following treatment with complete Freund's adjuvant. Thus nonspecific stimulation of the recipient's immune system does not trigger rejection of an established cultured allograft.

The effect of antigen-specific stimulation was examined in a group of four CBA mice whose diabetes had also been reversed by the allotransplantation of cultured BALB/c islet tissue (Fig. 2). These animals were challenged by the intravenous injection of 10^5 peritoneal cells from the BALB/c donor strain 105 days after transplantation. Two animals promptly became diabetic, and their transplants were seen to have been rejected when examined histologically. However, the remaining two animals showed no evidence of rejection when challenged with 10^6 and 10^7 additional donor leukocytes at monthly intervals. Blood sugar control in these animals was dependent on the grafted tissue, and when this was removed by nephrectomy the animals became diabetic. The islet tissue carried under the kidney capsule of these two animals showed no histological evidence of rejection. Similar results were seen in a separate experiment in which one of two animals receiving allografts rejected its graft when challenged in the same way with donor peritoneal cells. These experiments show that although antigen-specific stimulation can trigger graft rejection, a proportion of the animals fail to reject their grafts when challenged with donor peritoneal cells.

The latter finding was unexpected. Lacy *et al.* (*13*) showed that rats with long-term islet allografts could be rendered diabetic by challenge with leukocytes of donor origin, and we had expected a similar result in the mouse. It should be mentioned, however, that some of the rats studied by Lacy *et al.* were rather resistant to the induction of rejection. We do not know whether the failure of some specifically challenged animals to reject their graft is due to specific tolerance or allograft enhancement.

In conclusion, it appears that nonspecific stimulation of the recipient's immune system is unlikely to trigger rejection of an established islet allograft. Specific stimulation of the recipient's immune system can cause rejection. However, the finding that requires further investigation is the inability of a proportion of the animals to reject their graft following challenge with donor peritoneal cells.

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