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Review Articles

Pancreas and Islet Transplantation

I. Experimental Studies

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Contents

I. Introduction

Total endocrine replacement therapy for insulin dependent diabetes mellitus is possible by transplantation of either immediately vascularized pancreatic allografts or by transplantation of the islets of Langerhans as free grafts. The rationale for these treatments rests on the hypothesis that the microangiopathic and other lesions associated with diabetes mellitus are secondary to disordered metabolism. Presumably, perfect control of carbohydrate metabolism would prevent the development or halt the progression of the lesions affecting the eye, kidney, nervous and other systems. Although the point is controversial, clinical and experimental observations (not reviewed here) support this hypothesis [1–6a].

Nondiabetic individuals maintain plasma glucose concentrations within a narrow range. In diabetic individuals, exogenous insulin, administered by standard techniques, cannot reliably prevent wide excursions of plasma glucose levels [7]. Systems designed to administer insulin contin-

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Table 1. Techniques for whole or segmental pancreas transplants

	References
1. Whole pancreas	
A. Pancreaticoduodenal	
With cutaneous-duodenostomy	[9, 14, 15]
With Roux-en-Y duodenojejunostomy	[9, 12, 16–19]
B. With cuff papilla of Vater and anastomosis of duct to loop of jejunum	[23, 24, 24a]
C. En block with other organs	[21, 26]
2. Segmental pancreas graft – Body and tail	
A. Duct ligated	[25,32,33,38,40,42]
B. Pancreatic ductoureterostomy	[11, 19, 27, 28]
C. Roux-en-Y pancreaticojejunostomy	[12, 19, 27, 30, 31]
D. Duct injected with synthetic polymers	[43–49]
E. Intraperitoneal with duct left open	[36, 42, 44, 50–52]

uously may be able to do so [8], but are unlikely to be as efficient as functioning B cells.

Clinical application of pancreas transplantation has been difficult. First attempts showed unequivocally that immediately vascularized pancreatic grafts were able to normalise plasma glucose levels and obviate the need for exogenous insulin, but technical complications were frequent and the early attempts were associated with a high morbidity and mortality [9–12]. For that reason, a surge of interest developed in experimental transplantation of free grafts of islet tissue.

Islet grafts can reverse diabetes in a variety of experimental models [13]. Unfortunately free grafts of allogeneic islets appear to be extremely vulnerable to rejection effector mechanisms. In addition, the yield of islets from one donor pancreas is small. The clinical attempts at islet allotransplantation have been performed with little morbidity, but almost without exception they have not been successful. Islet transplantation still has great potential, but pancreas transplantation is making a comeback. New techniques for handling of exocrine secretions have made the procedure safer and a few patients currently have functioning grafts.

The experience with pancreas and islet transplantation in animals is extensive. There are over 1,000 articles in print which will be summarized in detail to provide a background for a description of the clinical experience. In addition, these experiments define the problems and form the basis for future application of pancreas and islet transplantation to the treatment of human diabetes.

II. Experimental Transplantation of Intact Immediately Vascularised Pancreatic Grafts in Animals

Allograft rejection is ultimately the limiting factor in the application of pancreas transplantation to the treatment of diabetes, but the technical aspects of pancreas transplantation have to be solved first. Provision for drainage of exocrine enzymes and prevention of vascular thrombosis are the major technical considerations.

Pancreatic grafts can be either (1) whole organ (usually in association with the duodenum), or (2) segmental (tail, or body and tail of the pancreas). The technical variations of pancreas transplantation can also be divided into (1) those that establish exocrine drainage into the bowel or another viscus; and (2) those that do not (a. duct ligated; b. ductal system obliterated; c. duct left open). The techniques for whole or segmental pancreas transplantation are summarized in Table 1.

The difficulties with pancreas transplantation are apparent from the variety of techniques employed. There are further difficulties with interpretation of the results of experimental pancreas transplantation because of limitations imposed by the models used (whether they are autografts or allografts, immunosuppressed or nonimmunosuppressed, diabetic or not diabetic).

Most experiments with pancreas transplantation have been done in large outbred animals, usually dogs. If the method of transplantation is to be evaluated without the influence of immunological factors, an autograft model must be used, imposing further technical difficulties, such as preserving blood supply to other vital organs during pancreatectomy. Allograft models are technically easier to use, since the donor can be sacrificed, but allograft rejection makes assessment of the technique employed more difficult. Immunosuppression is unquestionably able to delay graft rejection in a large animal model, but has not always been employed. Thus allograft rejection may result in graft failure before technical problems become apparent; conversely, technical problems may result in graft failure even though a satisfactory immunosuppressive regimen is employed. Attempts at pancreas transplantation have been made since before the beginning of this century. The historical aspects of pancreas transplantation have been reviewed elsewhere [9] and only modern experiments are reviewed here.

A. Transplantation of the Pancreas with Exocrine Drainage Established

1. Pancreaticoduodenal Grafts. The initial attempts to transplant the canine pancreas with maintenance of exocrine secretion utilized the duodenum as a conduit to the skin [14, 15]. A modification of this technique was used for four of the first five clinical transplants that were reported by Lillehei et al. [9]. Largiader et al., in 1967, were the first to transplant successfully an entire pancreaticoduodenal allograft and establish exocrine drainage to the bowel of recipient dogs [16]. They placed the graft intra-abdominally and anastomosed the donor duodenum to a Roux-en-Y loop of recipient jejunum, with vascular anastomosis to the host aorta and inferior vena cava. Uchida modified this technique for use in an autograft model [17].

The technique of pancreaticoduodenal grafting in large animals has been used by several investigators [18–22], but technical problems have consistently frustrated these efforts. Vascular torsion and thrombosis are common. The duodenum is prone to necrosis or ulceration. Anastomotic

leaks are particularly serious, because of activation of exocrine enzymes.

Aquino et al. [23], in both autograft and allograft experiments, obviated some of these problems by transplanting the entire pancreas without the duodenum except for the papilla of Vater. They were successful in some dogs, but the procedure was technically demanding and the failure rate was high. Bewick [24], however, has been able to perform a similar operation with only a 7.7% morbidity and mortality in allograft recipients. One clinical case has been done by this technique [24a].

Heterotophic pancreaticoduodenal transplantation can ameliorate diabetes in rats [25], but the operative mortality is high, particularly if combined with en bloc transplantation of other organs [26]. Nevertheless, the rat is a useful model because inbred strains can be used and the results of experiments can be interpreted with the immunological factors controlled.

2. Segmental Pancreatic Grafts. Segmental pancreas transplantation is simpler than pancreaticoduodenal transplantation and avoids some of the problems related to the grafted duodenum. Several ingenious techniques have been developed that allow for exocrine drainage to be established without transplantation of the duodenum.

Gliedman et al. [11] transplanted the tail of the pancreas in dogs and anastomosed the pancreatic duct to the recipient ureter. The pancreas exocrine enzymes are inactive and injury to the urinary tract does not occur. Although this technique has been applied clinically, other investigators have been unable to achieve satisfactory duct to ureter anastomose in dogs [19, 27, 28].

Exocrine drainage of segmental pancreatic grafts has also been established by anastomosing the pancreas to the recipient jejunum [19, 21, 29]. The same problems with anastomotic leaks, enzyme activation, and local necrosis can occur. Dickerman et al. [27] minimized these complications by using a staged procedure in which a Roux-en-Y jejunal loop was first placed in the retroperitoneal iliac fossa. In a second stage, the pancreas graft was anastomosed to the Roux-en-Y loop. This approach has also been used clinically [12, 30, 31].

B. Transplantation of the Pancreas without Provision for Exocrine Drainage

1. Duct Ligated, Segmental Pancreatic Grafts. The simplest approach to transplantation of immediately vascularized pancreatic grafts is to ligate the pancreatic duct [32, 33]. Theoretically, exocrine tissue atrophy will occur and endocrine tissue will remain intact. There is, however, a relatively high incidence of pancreatitis and vascular thrombosis after duct ligation [29]. The incidence of vascular thrombosis can be reduced by doing end to end anastomoses or by actually interposing the donor splenic artery (which supples the pancreatic tail) into the iliac artery of the dog [34].

Acute and chronic inflammatory reactions in the pancreas after duct ligation can involve the islets of Langerhans and result in impaired function [29, 35, 36]. Various attempts to decrease pancreatic exocrine function following duct ligation have been made. These include direct irradiation of the pancreas [37], administration of steroids [38] or of drugs that inhibit pancreatic exocrine enzyme synthesis or secretion [38, 39]. These manoeuvres may not be necessary. Verschoor et al. [40] found that one year after duct ligation in dogs, fasting plasma glucose levels were unchanged. Although glucose tolerance test Kg values were 75% and insulin levels were 60% of normal, no further deterioration occurred over a 3 year period.

In rats, duct ligated pancreatic isografts undergo acute inflammatory reactions followed by progressive fibrosis [25, 41]. Orloff et al. [25] could not detect any differences in the islet function of ligated and nonligated pancreatic grafts tested up to two years after transplantation, although Fairbrother et al. [41] detected deterioration in function in the long term by glucose tolerance testing and serum insulin assay.

Duct ligation does not completely prevent fluid leakage from the pancreas, and this can give rise to local problems [38]. However, Kyriakides et al. [42] found that duct-ligated pancreatic allografts transplanted to the peritoneal cavity of totally pancreatectomized pigs did not result in local complications. These observations suggested that if contamination is avoided, by not opening the donor or recipient bowel, the peritoneal cavity can tolerate leakage of fluid from the pancreatic graft.

2. Duct Obliterated Pancreatic Grafts. Various liquid synthetic polymers have been injected into the pancreatic ducts of dogs to obliterate totally the ductal system [43–46a]. These polymers harden almost immediately after injection and form a solid cast within the major duct and its radicals. The objective of this approach is to eliminate secretions and suppress exocrine function.

Dubernard et al. [43] injected neoprene into the pancreatic tail, leaving it in situ on a vascular pedicle. The dogs had transient hyperamylasaemia, but remained normoglycaemic and normoinsulinaemic over several months of observation. Histological examinations of neoprene duct-obliterated dog pancreases showed variable results. Some pancreases had severe fibrosis that involved the islets, while other pancreases were atrophic with normal appearing islets [47].

Kyriakides et al. [44] transplanted neoprene injected pancreas to the peritoneal cavity of totally pancreatectomized dogs. The complication rate was low in recipients of autografts, but the infection rate was high in immunosuppressed recipients of neoprene injected allografts. Serial observations by Baumgartner et al. [28, 44a] of dogs with neoprene-injected pancreatic autografts showed that most dogs remain normoglycaemic and that glucose tolerance was only slightly impaired. Histological examination at one year, however, showed persistent inflammation and distortion of islets [28].

Acrylate glue [45] and polyisoprene [46] obliterated pancreatic grafts have been transplanted in dogs with a high success rate, and with fewer complications than with duct ligation or other techniques. An absorbable biological glue, prolamine, has also been used to occlude the pancreatic duct in dogs and in patients with chronic pancreatitis [46a].

Pancreatic allografts injected with these agents have been transplanted to diabetic patients [48–49b]. The long term effect of the severe pancreatic fibrosis induced by polymer injection, however, remains to be determined.

3. Intraperitoneal Transplantation of Segmental Pancreatic Grafts without Duct Ligation. As reported above pancreatic enzymes may not necessarily have a deleterious effect on the peritoneum, at least in pigs [42]. Subsequent experiments in dogs [44, 50] and rats [51] confirmed that the peritoneal cavity could absorb pancreatic secretions and that segmental pancreas transplantation could be performed with a low complication rate. The serum amylase concentration is initially increased after transplantation of a graft with an open duct, but the duct gradually closes and levels return to normal within a week [52]. Early [37, 44] and late [28] examination of pancreases transplanted with the duct left open have shown less inflammation and fibrosis than of grafts where the duct was ligated immediately.

C. Metabolic Efficiency of Whole or Segmental Pancreatic Transplantation

Successful transplantation of immediately vascularized pancreatic grafts to animals made diabetic either by drugs or by pancreatectomy uniformly restores plasma glucose to normal [9, 17, 18, 23–25, 27–28, 33–36, 44–46, 52–58] if more than 20% of the normal pancreatic mass is transplanted [54].

Several investigators have noted a tendency for hyperinsulinaemia and hypoglycaemia to occur during the first few hours after transplantation [9, 55], but this problem may be counteracted by IV administration of glucose or systemic administration of corticosteroids [55]. Blood glucose and insulin levels during glucose or tolbutamide tolerance tests have been variously reportedly as low, normal or elevated [9, 17, 18, 24, 28, 34, 36, 40, 53–58]. The factors responsible for the different results are difficult to sort out because of the variations in the models studied. However, several investigators have reported survival of recipients of autografts for months or years with normal blood glucose levels and without deterioration of graft function [9, 23, 25, 28].

Almost all pancreas transplants have been performed with venous drainage of the pancreatic graft into the iliac vein or vena cava of the recipient, providing an explanation for any elevations in systemic insulin levels. The liver – the major site of insulin degradation – is initially bypassed. In the few experiments where drainage was into the portal system, an advantage in terms of glucose metabolism could not be established [18, 53, 56, 57], although serum insulin levels were generally lower. Baumgartner et al. [56] found that only reduction in islet mass was an important factor in alteration of glucose tolerance after pancreatectomy and segmental pancreas transplantation. An advantage for either systemic or portal drainage or an effect of denervation could not be demonstrated.

Bewick et al. [55, 57] have shown that the elevation in plasma insulin after heterotopic transplantation of the entire pancreas is due to a combination of systemic venous drainage and pancreatic denervation. In physiological preparations, transposition of the portal venous drainage into the systemic circulation alone or denervation of the pancreas alone had little effect on systemic insulin or glucose levels – only the combination of the two duplicated the findings after heterotopic pancreas transplantation. In either case, plasma glucose levels were normal, and the liver was able to compensate for the route by which it was exposed to insulin.

Whether the minor abnormalities in metabolism that are seen in animals after pancreas transplantation are important in relation to the prevention of the long-term complications of diabetes has not been determined.

D. Immunological Aspects of Pancreatic Allotransplantation

Pancreatic allografts are subject to rejection just like any other organ [18, 20, 26, 27, 37, 40, 44, 58–63]. The functional criteria used to define rejection differ for various organs and may make it appear that the pancreas is more or less susceptible to rejection than another organ [9], but in experiments with en bloc allotransplantation of the pancreas, duodenum, liver, spleen and kidney in rats, histological evidence of rejection was seen in all organs either simultaneously or within a few days of each other [26].

Pancreas allografts will survive indefinitely in rats in whom tolerance is induced by classic techniques in the neonatal period [64]. In certain rat strain combinations, spleen allografts are not rejected and will protect a simultaneously placed pancreas allograft from rejection [65]. Techniques to induce tolerance in rodents have so far not been clinically applicable for any organ.

Until the advent of immunosuppressive treatment, the survival of canine pancreatic allografts was limited to only a few days or weeks. Most investigators have administered various combinations of azathioprine, corticosteroids and antilymphocyte serum (ALS) to the recipient animals [23, 27, 39, 44, 53, 59, 60]. The results are fairly uniform. In animals that do not succumb to technical complications the mean graft functional survival or recipient survival times in immunosuppressed mongrel dogs has ranged from three to five weeks. Verschoor et al. [40] reported that the median survival time of pancreatic grafts in nonimmunosuppressed partially matched beagles was 9 days, while in dogs immunosuppressed with azathioprine it was 27 days and with ALS it was 51 days.

Kyriakides et al. [58] found that the duration of pancreas graft survival in beagles correlated most strongly with the recipients' reactivity in mixed lymphocyte culture (MLC) to the donor. In MLC reactive recipients immunosuppressed with azathioprine and prednisone, graft survival ranged from 25 to 260 days. MLC nonreactive nonimmunosuppressed beagles had graft survival range from 9 to 55 days. An interesting aspect of these experiments was the indefinite survival of pancreatic allografts for DLA identical donors in beagles previously treated by whole body irradiation and autologous bone marrow transplantation.

Recently, cyclosporin A has been used to prolong the functional survival of pancreatic allografts in rats [61, 62]

and dogs [46]. McMaster et al. [46] were able to achieve a median graft survival of 55 days in dogs given cyclosporin A. Another approach, total lymphoid irradiation [66] has also been used to prevent rejection of pancreas allografts in rats [67].

The diagnosis of rejection is usually made when hyperglycaemia recurs. Serum amylase may rise when rejection occurs [9], but it is not a reliable sign [27]. Bewick et al. [60] found that the insulin reserve of pancreatic allografts in dogs fell 2–3 days before overt rejection. Paradoxically, a temporary rise in serum insulin levels preceded a rise in fasting blood glucose levels. However, insulin levels then declined as hyperglycaemia occurred. In contrast, Kyriakides et al. [58] found that changes in glucose clearance during glucose tolerance tests or in serum insulin response did not precede rejection, and that the occurrence of hyperglycaemia was rapid and unpredictable. They were, however, able to demonstrate changes in cell mediated lymphocytotoxicity against the donors before overt rejection.

Histological examination of rejecting pancreatic allografts in animals shows oedema, interstitial haemorrhage and round cell infiltration [9, 18, 24, 63]. Usually the exocrine and endocrine pancreases are equally involved, but some investigators have observed that the histological lesions are less severe in the islets [24, 30]. However, from a functional standpoint, the islets are rejected at the same time as the exocrine pancreas.

An important question is whether there is a difference in susceptibility to rejection between intact, immediately vascularized pancreatic allografts and free grafts of allogeneic islets. In nonimmunosuppressed animals, the interval between transplantation and recurrence of hyperglycaemia has been longer with pancreas than with islet allografts [64, 68, 69]. However, the quantity of free islets engrafted was probably less than that contained in the pancreas grafts. The question will remain open until rigorous quantitative experiments are done. Rats with long term enhanced renal allografts will accept immediately vascularized pancreas grafts but not islet grafts from the same donor strain without rejection [70]. For practical purposes it is easier to prolong the survival of pancreas than of islet allografts [64].

E. Preservation of the Intact Pancreas

For cadaveric organ transplantation to be logistically feasible on a large scale, preservation is essential. Idezuki et al. [71] in 1968 showed that canine pancreas grafts preserved for 22 hours would function after transplantation. Since that time, the two basic techniques for kidney preservation, cold storage in an intracellular salt solution or hypothermic pulsatile perfusion with plasma-like solutions, have been used.

Westbroek et al. [72] found that no modifications in technique were necessary for 24 h preservation of the canine pancreas by either the pulsatile perfusion or cold storage technique. With pulsatile perfusion they preserved two grafts simultaneously; they also found that 30 min of warm ischaemia was tolerated.

Other investigators have found that cold storage in Collins or similar solutions can preserve canine grafts for 24 h, but that the best results are obtained if the solution is hyperosmolar [73, 74] and for longer periods colloid must be added to the solution [75]. With these modifications preserved grafts function as well as fresh grafts.

Preservation of pancreas grafts by pulsatile perfusion is difficult because the pancreas is a low flow organ; commercially available machines are designed for high flow rates and results in considerable oedema of the gland [73]. Baumgartner et al. [76] found that if the system is modified to deliver perfusate at a low flow rate and if the mean pressure is kept at $\leq$ 12 mm mercury, grafts can be successfully preserved for 24 h.

Only short term storage has been used for human pancreatic grafts [30]. If the methods used in dogs can be used for human pancreas grafts, it should facilitate histocompatibility matching and preparation of the recipent for transplantation.

III. Experimental Transplantation of Free Grafts of Islet Tissue in Animals

The modern era of islet transplantation began a little over a decade ago. Earlier attempts at transplantation of free pancreatic fragments [77] did not reverse diabetes.

Amelioriation of diabetes is now possible by transplantation of a) islets specifically isolated from the adult pancreas; b) dispersed adult pancreatic tissue, either prepared from donors previously depleted of exocrine enzymes or transplanted to sites tolerating the introduction of exocrine enzymes; and c) intact or dispersed exocrine-poor, islet-rich fetal or neonatal pancreas.

The bulk of the experimental work has been done in rodents. Inbred strains allow the effect of transplantation to be evaluated independently of immunological factors. In addition, one of the major problems of islet transplantations, that is obtaining sufficient islets for an effective transplant, is easily by-passed by using multiple isologous donors for a single recipient. On the other hand, the problems for clinical application have to be solved in large animal models, and experiments in these models deserve to be emphasised.

Most investigations have been performed in rodents with diabetes induced by either alloxan or streptozotocin [13], but pancreatectomy [78, 79] and viral induced [80, 81] or naturally occurring diabetes [81, 82] have also been used. In large animals, diabetes has been induced either by total pancreatectomy [83, 84], an absolutely reliable model for both autograft and allograft experiments, but complicated by exocrine deficiency; or by streptozotocin and alloxan with or without partial pancreatectomy [85, 86], a less reliable model of diabetes and one that makes autotransplant experiments difficult. This is an important drawback because the lack of inbred strains dictates the use of allogeneic donors in situations where the immunological and metabolic consequences of the transplant are not easily distinguished.

A. Source and Preparation of Islet Tissue

1. Isolation of Islets from Adult Pancreases. The basic technique used for isolation of islets from adult pancreases is a composite of innovations introduced by various investigators, and is most successful in rats. The exocrine tissue is disrupted by retrograde perfusion of the pancreatic duct [87]. The pancreas is then minced and the exocrine and endocrine tissue are dissociated by collagenase digestion [88]. The islets can be hand-picked [87] or they can be separated from physically more dense pancreatic components by centrifugation on a discontinuous Ficoll density gradient [89], either dialysed [90] or adjusted to isotonicity with Hypaque [91]. Some contamination with lymphatic or other tissue is inevitable, but a clean preparation can be obtained by hand-picking Ficoll separated rat islets suspended in phenol red solution under a dissecting microscope with a reflected green light [92].

Between 150 to 400 islets can be isolated from an adult rat pancreas by the collagenase technique, an approximately 5 to 10% yield [93]. The islets are functional [87], but there is no quantitative data on the percentage of islets that are viable at the end of the isolation procedure. In general, several donors are required to obtain sufficient islets for successful transplantation to a single recipient [13].

Various modifications in the basic technique of islet isolation have been tried in attempts to improve yield and viability. Scharp et al. [85] used a filtration chamber to remove islets from the digestion mixture as they were liberated. A larger number of small islets is obtained, but the final islet mass isolated is only slightly increased [94]. Trypsin, hyaluronidase, and other proteolytic enzmyes have been added to the digestion mixture [94, 95]. Conversely, Vrbova et al. [96] found that they could double the yield of islets by pretreating rat donors with pilocarpine to reduce exocrine enzyme content.

The collagenase digestion/Ficoll gradient separation technique has been used to isolate viable islets from mouse [97], dog [86], pig [98], monkey [85], and human pancreases [99]. In general, application to large animals has been difficult [100]. The pancreas is more fibrous, idenfication of islets by gross morphology is unreliable, and the yield is low, although special instruments can help with mechanical mincing [101, 102].

New approaches to islet purification are being tried, but they have not yet been tested by transplantation. Downing et al. [103] found that disruption of the acinar tissue by venous, rather than ductal, distention resulted in a higher yield of islets from the canine pancreas, while Merrell et al. [104] reported isolated single cell suspension of the pancreas will selectively reaggregate into pseudoislets in gryrorotational culture.

2. Dispersion of Adult Pancreatic Tissue without Specific Islet Isolation. Although purified islets may be desirable for immunological reasons [92], it is not necessary to isolate adult islets for transplantation. Kramp et al. [105] successfully transplanted minced pancreatic fragments obtained from adult donor mice 6 to 8 weeks after exocrine atrophy was induced by pancreatic duct ligation.

Payne et al. [106] took a different approach, and induced exocrine atrophy in adult rats by chronic oral administration of DL-ethionine, a methionine analogue that is selectively toxic to the exocrine pancreas. Total pancreatic tissue amylase content declined, while tissue insulin content and islet architecture were preserved. Tissue was prepared for transplantation by retrograde injection of the pancreatic duct followed by collagenase digestion. Tissue prepared from one donor could ameliorate diabetes in up to four recipients after injection into the portal vein.

Manoeuvres to reduce exocrine tissue prior to donor pancreatectomy and tissue dispersal are not necessary in all species. Mirkovitch et al. [83] showed that totally pancreatectomized dogs became normoglycaemic after autotransplantation to the spleen or portal vein of pancreatic tissue dispersed by mincing and collagenase digestion alone. Kretschmer et al. [84] used a mechanical tissue chopper and defined the conditions that gave the optimal balance between the degree of tissue dispersal, depletion of exocrine enzyme content and islet recovery after collagenase digestion. Mehigan et al. [107] found that prior duct ligation gave a less satisfactory preparation. Elimination of the steps required for purification shortened the islet preparation time, increased the recovery, and reduced the need for multiple donors. This technique of tissue preparation has been extended to baboons [108] and humans [109].

3. Preparation of Dispersed Neonatal Pancreas. The fact that islet isolation is not necessary for successful transplantation was first shown in experiments by Leonard et al. [110], using neonatal rat donors. Neonatal pancreases have low exocrine enyzme contents and a large proportion of islet tissue relative to other pancreatic components [111–113]. Although only 4% by weight of an adult pancreas, neonatal rat pancreas possesses 11% of the adult islet mass.

These properties permit neonatal rat [110, 113–116] or mouse [112] pancreas to be minced, dispersed by collagenase digestion and transplanted without specific islet isolation. Matas et al. [113] found that $> 70\%$ of islet tissue is lost during the usual dispersal process. A shorter digestion period was less destructive [117], but minimally digested tissue from a few donors was not as effective as transplantation of more extensively digested tissue from multiple donors, even though the insulin content of the transplanted tissue was similar [113, 117]. Again, a balance exists between the final islet mass obtained and the optimal dispersion needed for the tissue to take as a free graft.

Intact neonatal pancreases have been transplanted amelioration of diabetes by free grafts in mice [117a]. There are no reports of successfully transplants of intact neonatal pancreases in rats or other species.

4. Preparation of Fetal Pancreas. Fetal pancreas has the same favourable ratio of high islet volume and low exocrine enzyme content [118], even though the total B cell mass of one pancreas is small. Fetal rat or mouse pancreas can be transplanted intact as a free, whole organ graft, the simplest of techniques [119–121a]. The optimal time for removal of fetal rat pancreases and for transplantation to under the kidney capsule of diabetic recipients is between 17 and 18

days of gestation. In general, at least four fetal pancreases must be transplanted if diabetes in the recipient is to be completely ameliorated. Mullen et al. [122] found that one fetal pancreas was sufficient if placed under the kidney capsule of nondiabetic hosts and allowed to grow for three weeks before transplantation en bloc with the kidney as a vascularized graft.

Hegre et al. [123] have dispersed multiple fetal rat pancreases by the same collagenase digestion technique they used for neonatal pancreas, and found that less fetal than neonatal tissue was required for successful transplantation. Fetal pancreatic fragments have also been transplanted after mechanical disaggregation alone [124, 125].

There is a long latent period between transplantation of fetal pancreas and reversal of diabetes [118–122]. This delay is a consequence of the need for growth and maturation of the endocrine component of fetal pancreas analogues [118, 123].

Purified islets can be obtained by tissue culture of collagenase [126] or mechanically [126a] dispersed fetal pancreases. Isolated fetal islets have not been tested for their ability to reverse diabetes after transplantation, but their short-term survival in nondiabetic hosts has been demonstrated [126a].

B. The Results of Transplantation in Rodents

1. Isolated Adult Islets. Younoszai et al. [127], in 1970, first reported the transplantation of isolated adult islets. They used outbred rats, diabetes was only temporarily ameliorated, and rejection almost certainly occurred.

Ballinger and Lacy [128], in 1972 were the first to demonstrate a sustained effect. They transplanted 400 to 600 isologous islets to the peritoneal cavity of rats and partially ameliorated diabetes. In 1973 Reckard et al. [129] in rats and Panijayanond et al. [97] in mice, were able to normalise completely plasma glucose levels by intraperitoneal (IP) transplantation of a larger number of islets, as were others shortly thereafter [79].

A major contribution was the demonstration by Kemp et al. [130] that the same number of islets that were only partially effective when transplanted IP completely cured diabetes when embolized to the liver via the portal vein. Either more islets survived the trauma of transplantation because a blood supply was immediately available, or there was a physiological advantage to this site because of the normal secretion of insulin into the portal system.

Since this discovery many groups have successfully applied intraportal injections of islets for long-term reversal of experimental diabetes in rats [79, 95, 131–146] and even mice [147]. The number of islets required for successful transplantation depends on several factors, including the integrity of the islets isolated by various investigators or the severity of the pre-existing diabetic state [137, 148]. As few as 240 can [95] or as many as 2100 islets may be required [140] to restore normoglycaemia in rats. The latent period between transplantation and amelioration of diabetes is shortened [95, 144] and glucose tolerance improved [144] as larger numbers of islets are injected. In general, if more than 1000 islets are injected, normoglycaemia usually occurs within a day or two of transplantation. Islets from young adult donors seem to function better than islets from older donors [141].

In any case, if adequate numbers of isologous islets are transplanted, the metabolic abnormalities in diabetic rats are reversed. Plasma glucose levels return to normal [79, 95, 130–141, 149–153]; circulating insulin levels become nearly normal or elevated [79, 131, 135, 142, 144, 145, 149]; glucose tolerance test curves are improved [134, 144, 145, 153] or normalised [79, 135, 149, 153]; glycosuria is abolished [95, 130, 131, 137, 153]; weight gain is restored [145, 148, 149]; polyuria, polydipsia, and polyphagia are alleviated [130, 131, 149, 150, 153]; and other metabolic abnormalities, such as elevated pancreatic glucagon [150], plasma lipid [131, 142] and articular cartilage enzyme [151], or decreased liver guanylate cyclase [152] levels, disappear. Subtle perturbations in glucose tolerance [153] may be attributed to the ectopic location of the islets, lack of innervation, disruption of the entero-insular axis or to less than normal islet mass.

Transplanted islets lodge within the hepatic portal venules immediately after intraportal transplantation, but are eventually found in interstitial tissue in direct apposition to hepatocytes [143, 150, 154]. Neovascularization occurs rapidly [154] and both insulin and glucagon containing cells can be demonstrated in the liver by immunofluorescent techniques [79].

Quantitative studies suggest that at least one half of the islets that are embolized into the portal veins survive in the liver [150]. Between 50 and 90% of the insulin content of the originally transplanted tissue has been recovered in the liver of rats completely ameliorated of diabetes [137, 145, 155]. Ziegler et al. [156] caused reversion to the diabetic state by partial hepatectomy in rats bearing islets selectively infused into the right branch of the portal vein, suggesting that the majority, if not all of the islets, were confined to the liver.

The effect of embolisation of islets to the portal vein on liver function in rats is minimal [132, 157]. Oakes et al. [157] found that serum liver enzyme levels increased acutely, but the levels returned to baseline by three days and remained normal in long-term followup after intraportal islet transplantation.

Isolated islets have been transplanted to other sites with variable results. Intrasplenic injection is nearly as efficient as intraportal injection in rats [143, 149, 158, 159] and mice [160], although some islets may escape into the liver [149]. Embolisation of islets to the lung via a systemic vein [133] and direct injection into the liver [161] has also ameliorated diabetes. Diabetes has rarely been reversed after SC [78, 130, 162], IM [130, 148], intratesticular [163], intrapancreatic [164], or intrasalivary gland [164] implantation of isolated islets, although histological survival has been observed in some instances.

2. Dispersed Adult Pancreas without Islet Purification. Kramp et al. [105, 162] were the first to produce a long-term cure of experimental diabetes by transplantation of adult pancreatic fragments without specific islet isolation. Fragments from three to four duct-ligated hemi-pancreases

were transplanted subcutaneously to syngeneic mildly diabetic mice. Most recipients became normoglycaemic over 3 to 14 weeks, although glucose tolerance test results were abnormal. Measurement of tissue insulin content showed that approximately 15% of the transplanted islets survived. The subcutaneous space is an inefficient site for transplantation, but these experiments were important because they showed that islets from adult pancreases did not have to be isolated for successful transplantation.

Payne et al. [106] obtained better results by intraportal transplantation of unpurified islet tissue prepared by collagenase digestion of pancreases harvested from donors in whom exocrine atrophy had been induced by prior administration of DL-ethionine (DLE). Diabetes was ameliorted in almost all of the rats who received tissue prepared from a single or a half and in two-thirds of the rats who received tissue prepared from one-third or one-fourth of a pancreas.

Pancreatic islet tissue from DLE treated donors (one donor per recipient) has also been transplanted to the spleen of diabetic rats [165]. Normoglycaemia occurred less rapidly after intrasplenic than after intraportal transplantation, but the results of glucose tolerance tests were similar and normal when tested at 8 weeks. Splenectomy resulted in recurrence of diabetes.

Further experiments [68] showed that more than 40% of the original islet mass was recovered after adequate dispersal of DLE treated donor pancreas by collagenase digestion. Several weeks after intraportal transplantation, between 50 and 70% of the original insulin content of the transplanted tissue could be detected in the liver of recipient rats. This technique is efficient for laboratory investigations, since intraportal injection of islet tissue prepared from ≤ 2 DLE treated donors can rapidly restore normoglycaemia in diabetic rats [61, 67].

3. Neonatal Pancreas. Shortly after the report by Ballinger and Lacy [128] on IP transplantation of isolated adult islets, Leonard et al. [110] showed that diabetes in rats could also be ameliorated by IP transplantation of multiple neonatal pancreases dispersed by collagenase digestion without specific islet isolation. Subsequent studies by several investigators have shown that the diabetic state in rats and mice can be reversed by either IP [112, 114–116], intraportal [113, 142, 166], intrasplenic [112], or IV [142, 166] administration of dispersed neonatal pancreas.

Again, a relationship exists between the severity of diabetes and the amount of dispersed neonatal pancreatic tissue required to reverse the diabetic state [113, 116, 123]. The latent period between transplantation and reversal of diabetes also depends upon the quantity of tissue transplanted, the site of transplantation, and the severity of diabetes [113, 123, 166]. The period can range from a few days to weeks, but if a sufficient quantity of dispersed neonatal pancreas is transplanted, permanent normoglycaemia occurs [110–117, 142, 166–168], glycosuria is abolished [110, 116, 123, 168], weight gain is restored [110, 113, 116, 166], and other metabolic abnormalities disappear [142, 167, 168].

Glucose tolerance test curves after amelioration of diabetes by intraportal or intravenous injection of neonatal islet tissue resembles those of normal rats [166]. The results after IP transplantation have been variable, usually [110, 114–116, 123] but not always [116] normal. Plasma insulin levels have been low [114, 116], normal [110, 123, 115, 116, 166], or elevated [142, 169], in the fasting or non-fasting state or during glucose tolerance testing.

Immunohistochemical studies after IP [123], intraportal [170] or IV [166] transplantation of dispersed neonatal pancreas have shown insulin-, glucagon-, and somatostatin- and pancreatic polypeptide-containing cells in the implants or targed organs. Islets of normal architecture as well as of one cell type can be identified. Mitotic figures are sometimes present [111, 123]. Acinar elements largely disappear [123, 170]. Serial studies of the liver after intraportal transplantation have shown that islet cells become endothelialised and gradually moved into the hepatic lobules from the portal spaces [170].

The quantitative aspects of transplantation of dispersed neonatal pancreases to diabetic rats have been studied in detail by Matas et al. [113, 117, 166]. The minimum number of donors required for successful transplantation was one by the intraportal, two by the IV and more than four by the IP route, but the interval between transplantation and occurrence of normoglycaemia was several weeks. As more donors were used, normoglycaemia occurred progressively more rapidly.

Once normoglycaemia was achieved, however, there were no observable metabolic differences in the rats receiving larger or smaller amounts of tissue. The B cell mass of one neonatal pancreas after collagenase digestion is 6% that of the normal adult B cell mass [113]. These findings suggest that proliferation of neonatal islets is required for amelioration of diabetes when small quantities of tissue are transplanted.

Even when adequate quantities of dispersed neonatal pancreas are transplanted, the interval between transplantation and occurrence of normoglycaemia is longer than when equivalent quantities of isolated adult islets are transplanted. Vialettes et al. [155] gave adult islets or dispersed neonatal pancreas with the same tissue insulin content into the portal vein of diabetic rats. The decline of plasma glucose was more rapid with adult islets. The B cell mass that ultimately survived in the liver, however, was higher from neonatal than from adult donors (70 versus 50% according to tissue insulin content).

Neonatal pancreas does not have to be dispersed for successful transplantation as a free graft. DaFoe et al. [117a] reversed diabetes in mice by implantation of four to nine neonatal pancreases under the renal capsule.

Thus, dispersed pancreas can be transplanted without donor pretreatment or specific islet isolation. Only a few neonatal pancreases are needed if transplantation is to a vascularised bed. For rapid amelioration of diabetes, however, a very large number of neonatal donors are required, and adult donors may be a better source of islets.

4. Fetal Pancreas. Fetal islets have the capicity to grow and differentiate after transplantation [118, 123], but multiple donors are usually required to treat a single diabetic animal. In addition, the latent period between transplantation and reversal of hyperglycaemia is usually several weeks.

Most investigators have transplanted fetal islets as intact free pancreas grafts, but there are a few reports of the use of dissociated fetal pancreas. Usadel et al. [125] observed histological differentiation of islets after subcutaneous transplantation of mechanically dispersed fetal pancreas (14 to 16 days gestation) in nondiabetic rats. Feldman et al. [124] found that intraportal injection of eight chopped 18 day fetal pancreases ameliorated diabetes after several weeks in approximately half of the recipients.

Hegre et al. [123] used collagenase dispersed tissue and found that 3 mg of 20 day fetal islet tissue reversed diabetes with a mean latent period of 2.5 weeks, while it required 20 mg of 9 day old neonatal islet tissue. On a weight basis, fetal pancreas was more effective. In separate experiments, Hegre et al. [121] found that the mean latent period between transplantation and reversal of diabetes was 15 weeks after IP transplantation of collagenase dispersed fetal pancreas obtained from 28 donors at 17.5 days gestation.

Transplantation of intact fetal pancreases avoids the problem of enzymatic destruction of islets and requires fewer donors [121]. Brown et al. [119] found that transplantation of at least four 17 day fetal pancreases to under the kidney capsule consistently reversed diabetes within 2 to 3 weeks. Circulating insulin levels were elevated and glucose tolerance tests were nearly normal. Except for ducts, exocrine tissue did not survive but alpha and beta cells were present in the grafts. Brown et al. [119] showed that modulating the severity of the diabetic state by temporary administration of insulin enhanced engraftment.

Spence et al. [171] also found insulin administration to be useful. In addition, they found the splenic pulp to be nearly as good a site as the renal capsule for implantation, while IM, SC and intratesticular implants did not reverse the diabetes in rats.

Mullen et al. [122] reduced the number of fetal donors required by two complicated manoeuvres. First, one fetal rat pancreas was sufficient to treat diabetes if it was placed under the kidney capsule of syngeneic normal hosts and allowed to mature for three weeks before en bloc transplantation of the kidney and intact pancreas to the recipient. Second, three fetal pancreases were sufficient if a renal vein portal caval shunt was performed after grafting to under the renal capsule. Detailed studies by Brown et al. [172] support the concept that there is a physiological benefit from diversion of secreted insulin into the hepatic portal circulation. These results are complemented by those of Spence et al. [171] who found that two fetal pancreases transplanted to the spleen could reverse diabetes in syngeneic rats. Mandel et al. [121a] observed gradual reduction of hyperglycaemia after transplantation of one cultured fetal pancreas to the spleen of diabetic mice, but the course of diabetes in non-transplanted mice was not reported.

McEvoy et al. [120, 173, 174] and Hegre et al. [121, 123] have also performed detailed quantitative studies of transplantation of intact fetal rat pancreases. They found the mean interval to reversal of diabetes was 11 weeks after transplantation of 8 and 19 weeks after transplantation of four pancreases to under the renal capsule. The B cell mass of the implants increased 8 fold after transplantation, and insulin treatment for one week resulted in a further two- to threefold increase. Insulin treatment had a variable effect on the outcome after transplantation. These paradoxical observations suggest that control of extreme hyperglycaemia may be beneficial during the period needed for neovascularisation, growth and differentiation, but that an endocrine deficiency state is also important for establishment of graft function. Further investigations are needed to define the role of insulin treatment after islet transplantation.

C. *The Results of Transplantation in Large Animals*

1. Isolated Islets. There are only a few reports on transplantation of isolated adult islets in large animals. The problems of islet yield are formidable. If multiple donors are used, allogeneic islets may be rejected before function of a technically successful transplant is established. If rejection is avoided by using an autotransplant model, the number of islets may be too few to have a significant effect on the diabetic state.

A minimal effect on diabetes has been observed after transplantation of isolated islets to the peritoneal cavity of totally pancreatectomized pigs [98] or to the portal vein of monkeys made diabetic either by partial pancreatectomy and streptozotocin [85] or total pancreatectomy [176]. Scharp et al. [85] found that serum insulin and Kg values increased after intraportal transplantation in monkeys with mild diabetes induced by partial pancreatectomy and streptozotocin, but they did not report the long term fate or the actual course of diabetes in this model. Jonasson et al. [175] found granulated B cells in the liver parenchyma after intraportal transplantation to totally pancreatectomized monkeys, but there were an insufficient number of islets to restore normoglycaemia.

Lorenz et al. [176] reversed a relatively mild diabetes induced by 80% pancreatectomy and streptozotocin injection in partially inbred Alastain dogs by intraportal transplantation of minimally histoincompatible allogeneic islets. Glucose tolerance test results were improved and serum insulin responses were intermediate between normal and diabetic dogs. Islets were demonstrated histologically in the liver, but unless special measures were taken to prevent rejection the dogs ultimately reverted to the diabetic state.

The experiments of Lorenz et al. [176] are the only ones showing a long lasting effect of isolated islets in a large animal. There are no reports of transplantation of isolated islets to dogs with diabetes induced by total pancreatectomy, a more severe and reliable model of diabetes.

2. Dispersed Adult Pancreas without Islet Purification. The problem of islet yield was partially solved by Mirkovitch et al. [83]. They reversed diabetes in totally pancreatectomized dogs by intrasplenic autotransplantation of dispersed pancreatic tissue prepared from the left limb by collagenase digestion alone, with no attempt at islet purification. Glucose tolerance tests curves were similar but peripheral vein insulin levels were half those of normal dogs. In transplanted dogs mean plasma insulin concentration in the

splenic vein was seven times higher than in the peripheral circulation and splenectomy lead to severe hyperglycaemia and death. Histological examination showed both endocrine and exocrine pancreatic tissue with no apparent detrimental effect of the latter on the spleen, a rich vascular organ.

Kretschmer et al. [84] dispersed the entire pancreas and found that dogs transplanted with tissue digested with collagenase for 20 min had the best outcome. However, metabolic studies in recipient dogs were not completely normal. The animals had fasting normoglycaemia, but Kg values during glucose tolerance tests were only half those of normal controls and peripheral vein insulin levels were very low. The results of splenectomy and histological examination were similar to those of Mirkovitch et al. [83].

Several investigators have since used the model of intrasplenic transplantation of dispersed pancreatic tissue in dogs to study a variety of problems, including preservation [179, 180], systemic versus portal venous drainage [181], the effect of endogenous pancreatic tryptic activity on the islet preparation [178] and the metabolic responses after transplantation [102, 177]. Mehigan et al. [107] found that the success rate was lower with transplantation of tissue prepared from dogs with chronic pancreatitis induced by duct ligation even though the insulin reserve of the pancreas before dispersion was nearly normal.

Mirkovitch et al. [182] found that if completion of the pancreatectomy was delayed to more than 3 weeks after intrasplenic transplantation of dispersed tissue prepared from the left limb the animals became diabetic. This outcome suggests that an endocrine deficiency state is important for engraftment. The results are interesting because of their similarity to the paradoxical results noted by McEvoy et al. [120, 172a] on the effect of insulin treatment after islet transplantation in rats.

Diabetes has also been treated by infusion of collagenase dispersed pancreatic tissue into the portal vein of pancreatectomized dogs [177, 183]. Serum liver enzyme levels are transiently elevated. Dogs are particularly susceptible to the development of portal hypertension, and Kretschmer et al. [183] found transplantation by this route not to be as effective as transplantation to the spleen. Mehigan et al. [184] described disseminated intravascular coagulation and portal hypertension after intraportal transplantation. This syndrome was secondary to tissue thromboplastin in the preparation and could be prevented by prophylatic administration of heparin and aprotinin.

Diabetes has also been obviated in three of four baboons after total pancreatectomy by infusion of collagenase dispersed autologous pancreatic tissue into the portal vein [108]. After transplantation insulin levels were higher in the hepatic than in the portal vein, conclusive proof of intrahepatic insulin secretion.

In summary, experiments in large animals show that pancreatic tissue dispersed by mincing and collagenase digestion alone can reverse severe diabetes after transplantation to the spleen or portal vein. Elimination of the steps for purification allows sufficient islet tissue to be obtained from one donor for an effective transplant.

D. Islet Transplantation on Natural Models of Diabetes

Most islet transplant experiments have been in animals with diabetes induced by either B cell toxins or by total pancreatectomy. The syndrome produced resembles insulin dependent diabetes mellitus, but it is an artifical counterpart. In addition, pancreatectomy causes metabolic and nutritional changes that complicate the animal studies. For these reasons animals with spontaneous diabetes are useful for evaluation, although not all of the models are akin to human diabetes.

Two of the models, the C57BL/6J (ob/ob) and the C57BL/KsJ (db/db) mice, are characterized by hyperinsulinaemia, hyperphagia, hyperglycaemia, islet hyperplasia early in life, and, particularly in db/db mice, insulin resistance [185]. Strautz [186] reported that islet transplantation partially reversed the syndrome in ob/ob mice and suggested that the islets supplied a missing satiety factor. Barker et al. [82] obtained slightly different results. They found that transplantation of histocompatible normal islets resulted in reduction of plasma glucose for only 2–3 weeks; hyperglycaemia and progressive weight gain then recurred, suggesting that the transplanted islets either became involved in the pathological process or eventually failed to function for non-immunological reasons.

Barker et al. [82] did not even observe a temporary effect on plasma glucose levels in db/db mice after transplantation of histocompatible normal islet, even though the transplants were done at an age when plasma insulin concentrations were falling from endogenous islet exhaustion. Conversely Barker et al. [82] found that transplantation of islets derived from db/db mice completely reversed the diabetic state in congeneic mice with streptozotocin induced diabetes. This observation seems to provide definitive proof that whatever the defect in db/db mice is, it does not reside in their islets.

New Zealand obese (NZO) mice have only mild elevations of basal blood glucose levels; but they are hyperinsulinaemic and glucose tolerance test results are abnormal. Gates et al. [187] reported that transplantation of islets enclosed within millipore filtre chambers corrected the abnormalities, although Swenne et al. [188] could not reproduce these results. Barker et al. [82] found that after transplantation of rat islets to immunosuppressed NZO mice glucose tolerance test results returned to normal. Normal islets may produce a factor that decreases insulin resistance and alleviates the syndrome [189].

Although the results of the experiments in the obese hyperinsulinaemic mice models indicate that transplantation may not be appropriate treatment for all forms of diabetes, the syndromes in these mice bear little resemblence to insulin dependent diabetes in humans. There are other animal models of naturally occurring diabetes that are more appropriate for comparison to the human situation.

Viruses have been implicated in the aetiology of some cases of human diabetes [190]. Howard et al. [80] reversed the diabetic state that follows infection with encephalomyocarditis virus (EMCV) in SWR/J mice by intrasplenic transplantation of islet tissue from uninfected isologous donors. Following transplantation, re-exposure to the virus

was not followed by recurrence of diabetes. This finding is not surprising, since the mice would be expected to be resistant to secondary infection; however, SWR/J mice that previously had been cured of streptozotocin induced diabetes by islet transplantation to the spleen also did not become diabetic after primary exposure to the virus, while normal littermates infected at the same time became diabetic. This finding is surprising, suggesting that either intrasplenic islets are not susceptible to infection or that streptozotocin induces changes in the mice that makes them resistant to the virus.

Naji et al. [81] reversed EMCV induced severe diabetes in DBA mice by transplantation of syngeneic fetal pancreas to under the kidney capsule. Removal of the graft 30 days later was followed by recurrence of the diabetes. DaFoe et al. [117a, 191] performed islet transplantation in Fl hybrids of mice strains either susceptible or resistant to viral induced diabetes on mice with susceptibility or resistance dependent upon their sex. The hybrids had intermediate suspectibility. Transplantation of islets from one or the other parental strain to hybrids or from donors of the opposite sex ameliorated streptozotocin induced diabetes. Subsequent infections with virus resulted in diabetes with the same frequency as normal hybrids or as in the recipient's sex. Together, these experiments show that islet transplantation can reverse virus induced diabetes [80, 81, 117a, 191], that latent virus or recurrent virus does not damage the transplanted islets [80], and that host factors are more important than intrinsic pancreatic factors in determining susceptibility to virus induced diabetes [117a, 191].

There is one other animal model with spontaneous diabetes that has been used for islet transplantation, the "BB" Wistar rat [81]. A variable percentage of rats in this outbred line develop insulitis and rapidly become hypoinsulinaemic, severely hyperglycaemic and ketosis-prone. Autoimmunity is probably involved in the pathogenesis, since administration of ALS early after the onset will restore normoglycaemia [192]. Naji et al. [81] restored normoglycaemia by intraportal transplantation of allogeneic islets from normal histocompatible donors to immunosuppressed "BB" recipients. If the recipients are not immunosuppressed, diabetes will recur even before rejection of the allogeneic islets [192].

Although not a natural model, isologous islets can reverse diabetes in mice with drug-induced, cell mediated insulitis in the absence of immunosuppression [160]. Autoimmune phenomena associated with diabetes will not necessarily persist to adversely effect transplanted islets.

Thus, islet transplantation can reverse diabetes in natural models characterized by B cell destruction and insulin deficiency. These results strongly argue that transplantation in humans with insulin-dependent diabetes will be similarly effective if technical and immunological problems can be overcome.

E. Immunological Aspects of Islet Allo- and Xenotransplantation

Multiple factors make it difficult to interpret and compare the results of the islet allograft experiments reported by various investigators. These factors include: 1) heterogeneous combinations of donor-recipient pairs; 2) variability in the donor source, the site of transplantation and the number of islets transplanted or engrafted; 3) variations in the potency of biological immunosuppressants, such as ALS and enhancing antibodies; 4) the effect that some immunosuppressive agents, such as corticosteroids, may have on the severity of diabetes; and 5) the lack of uniformity in the definition of rejection. At leat two consecutive days of normoglycaemia should be a minimum criterion for successful islet engraftment [193]. Since a fall in blood glucose concentration on the first day of transplantation may be due to release of insulin from damaged islets, experiments reporting allograft survival data of 1–2 days duration should be viewed with extreme caution. Rejection may actually have occurred later, although before the islets were physiologically effective, since in many isograft models there is a latent period of days to weeks before sustained normoglycaemia occurs. Ideally, in allograft experiments a large number of islets should be transplanted to an appropriate site so that normoglycaemia is definitely established before rejection occurs. Many but not all islet allograft experiments have met this criteria.

1. Islet Antigenicity and Susceptibility to Rejection. Islets are definitely immunogenic [131, 194], although there is some doubt as to whether mouse islets express all H-2 antigens [195, 196]. Zeigler et al. [131] found that Fisher rats injected with hand-picked Lewis islets rejected subsequent Lewis skin grafts in an accelerated fashion. Lacy et al. [197] and Bowen et al. [198] have provided evidence that rat islets putatively depleted of passenger leucocytes are not or are weakly immunogenetic, but B cells are definitely susceptible to immune destruction once the afferent arc of the immune response is activated [197].

A hamster insulinoma was apparently not rejected and ameliorated streptotozocin induced diabetes after SC transplantation to nonimmunosuppressed outbred Syrian hamsters [199], but a rat insulinoma has been shown to express histocompatibility antigens and to be rejected after transplantation to allogeneic recipients [200]. Fetal [198, 201–204] and neonatal [110, 205] islets are definitely antigenic and reject just as rapidly as adult islets, although well established fetal allografts may be less susceptible to immune destruction [172]. In nonimmunosuppressed recipients, fetal pancreatic islet allografts are almost always rejected before they mature sufficiently to reverse diabetes [203].

The vulnerability of islets to immune destruction by both humoral and cellular mechanisms has been demonstrated in a series of experiments by Naji et al. [206, 207]. They transplanted the islets and skin across a major histocompatibility barrier to diabetic rats who had been made tolerant to donor strain antigens at birth by IV injection of bone marrow from donor recipient strain hybrids. The rats stayed normoglycaemic with no further treatment, confirming the tolerant state. However, when tolerance was broken by injection of lymphocytes obtained from normal or sensitized recipient strain rats, hyperglycaemia occurred within 3 to 8 days and the skin grafts were also rejected.

Table 2. Functional survival (mean days) of intraportal islet allografts relative to other organ allografts in nonimmunosuppressed rats

Donor:	AsxAg	BN	DA	DA	Fisher	Lewis	Lewis	Fisher
Recipient:	As	Lewis	Lewis	ACI	Lewis	W=Lewis	Fisher	Lewis
Barrier:	Major	Major	Major	Minor	Minor	Minor	Minor	Minor
Tissue	Days	Days	Days	Days	Days	Days	Days	Days
Islets[a]	3.2	3.5	3.3	22.0	2.1	>77[b]	4.4	5.2
Pancreas[a]	–	7.6	8.3	–	–	–	16.5	12.1
Skin	8.2	–	–	11.4	13.2	11.5	–	–
Heart	7.9	7.5	–	–	–	–	>40	–
Kidney	8.8	–	–	–	–	–	–	–
References	[139]	[64]	[218]	[154]	[215]	[214]	[64]	[68]

[a] Endpoint of rejection different for each tissue except pancreas and islets. Differences in rejection time between pancreas and islets may relate to quantitative, nonimmunological factors since number of islets transplanted or engrafted may have been less than number of islets in vascularized pancreas graft

[b] 4/12 recipients did not reject islets; the other 8 never became normoglycaemic

Similarly, daily injection of antiserum directed against donor antigens resulted in hyperglycaemia within 1 to 7 days, although skin grafts were rejected much more slowly. Damage to islets occurred when immunological tolerance was not broken, since it was possible to restore normoglycaemia by retransplantation after the effect of antiserum had dissipated. Frangipane et al. [208, 209] obtained different results in one and similar results in another model. Tolerant mice previously cured of diabetes by islets transplanted across an extremely weak histocompatibility barrier did not revert to the diabetic state after administration of normal recipient strain lymphocytes or donor specific antiserum [208]. However, immunosuppressed mice ameliorated of diabetes by transplantation of rat islets became hyperglycaemic within 24 h of administration of donor specific alloantiserum [209]. These experiments show the rapidity by which islets can be damaged by humoral mechanisms.

If immunosuppression is not used, fresh islet allografts can be rejected very rapidly [129, 139], particularly apparent if transplantation is across a strong histocompatibility barrier and comparison is made to other organ allografts using different endpoints to define rejection [129, 139]. The rejection time, however, depends on several factors, including the number of islet transplanted, the site of transplantation and the histocompatibility barrier.

In most experiments, the day of rejection has been defined in terms of graft function. The physiological manifestations of rejection depend in part on the functional reserve of the transplanted tissue. If the number of islets engrafted is just sufficient to ameliorate diabetes, the destruction of only a few islets will result in the return to the hyperglycaemic state. Finch and Morris [210] were able to improve the functional survival of islet allografts in rats by increasing the number of islets transplanted from 600–800 to 1500–3000.

In regard to the site of transplantation, Barker et al. [211] and Ziegler et al. [156] found that the median functional survival of the same number of islets transplanted across a weak histocompatibility barrier was threefold longer after intraportal than after IP administration. Although Barker et al. [211] broached the possibility that the liver is an immunologically privileged site, the differences may just as well have been a reflection of the number of islets that initially survived transplantation to the respective sites, since transplantation to a vascularized bed is more efficient in the isologous situation as well [130]. This contention is supported by the observations of Slijepcevic et al. [133]. They showed that allogeneic islets embolized to the lung by IV injection, also functioned longer than islets transplanted to the peritoneal cavity, and the results were comparable to those obtained by intraportal injection.

The functional survival of allogeneic islets transplanted across major histocompatibility barriers [143, 156, 159] to the spleen or portal vein of diabetic rats has been compared. Reckard et al. [158] found the mean functional survival time was 4.4 days after intrasplenic and 4.8 days after intraportal transplantation. Finch et al. [159] reported rejection times of 5.4 and 4.8 days for intrasplenic versus intraportal islets, and immunosuppression with ALS equally prolonged grafts transplanted to either site. Allogeneic islets have also been transplanted to the spleen [69, 179, 212] or portal vein [213] of totally pancreatectomized dogs. Comparative experiments by the same investigators have not been done, but in nonimmunosuppressed dogs, normoglycaemia is not achieved or rejection occurs in a few days at either site [212, 213]. In immunosuppressed dogs rejection of intrasplenic islets has either been delayed [69] or prevented in one-third of the recipients [179]. The results of intrasplenic transplantation in the various models is interesting because the spleen is an immunological organ and rejection might be expected to occur earlier. This does not, however, appear to be the case.

The results of the experiments comparing the rejection time of islets and other organs have been variable when transplantation has been across a minor histocompatibility barrier (Table 2). Functional survival may be shorter for islets [64, 129, 215], but in at least three experiments intraportal [131, 214] or IP [208] islet allografts were rejected less rapidly [131, 208] or less frequently [214] than skin allografts from the same donor strain in rats [131, 214] and mice [208].

When transplantation has been across a major barrier, islets always seem to be rejected earlier than skin [129, 139], heart [64, 129, 139] or kidney allografts [139] (Table 2). In addition, immunosuppressive regimens that prolong the survival of skin, kidney, or heart allografts have also been ineffective or minimally effective in promoting the survival of islet allografts [70, 129, 139, 216]. These observations led to the hypothesis that islets transplanted as a free graft are more susceptible to immune destruction than islets transplanted as part of an immediately vascularized intact pancreatic graft [216, 217]. Comparative experiments in rats support, but do not necessary prove, this hypothesis [64, 68, 70].

In nonimmunosuppressed diabetic rat recipients of either islet or pancreas allografts, hyperglycaemia recurred more rapidly after islet transplantation (Table 2). Again, it is possible the difference was due to quantitative nonimmunological factors related to the final islet mass engrafted. Sutherland et al. [68] tried to compensate for this problem by transplanting the same islet mass in both types of grafts. While the insulin content of the islet and pancreas grafts were equivalent in isologous controls, the final islet mass engrafted in the liver (according to tissue insulin content) was only 50 to 70% of the original islet mass transplanted, so the question could not be answered satisfactorily. Quantitative factors are less likely to be responsible for the observations by Reckard et al. [70], that pancreas, but not islet, allografts survival times were prolonged following transplantation to adult recipients bearing immunologically enhanced heart or kidney allografts.

The rapid rejection of allogeneic islets appears to be atypical when defined in terms of graft function. It may be that in the dissociated state islets are more vulnerable and that functional deterioration occurs more rapidly. Histological studies after islet transplantation to various sites have been performed by several investigators [110, 111, 143, 198, 202–204, 219–221]. Leonard et al. [110, 111] found degranulated β cells surrounded by lymphocytes in peritoneal implants at the time recipients of neonatal islets became hyperglycaemic. Kretschmer et al. [219] observed progressive cellular infiltration and degeneration of adult allogeneic islet tissue in the spleen of totally pancreatectomized dogs. Franklin et al. [143] and Slater et al. [220] found that by 2 days intraportal islets were surrounded and by 4 days infiltrated by mononuclear cells. B cells were intact, but functional deterioration was evident by 4 days. By 7 days there was focal disintegration of islets and B cells were degranulated. By 2 weeks islets were hard to recognize, although there were focal collections of mononuclear cells. The findings were similar in the spleen [143]. Both organs remained morphologically normal [143, 220].

The histological appearance of fetal islet allografts in diabetic [201–204] and nondiabetic [221] recipients has also been described. There is variability depending on the histocompatibility barrier, but cellular infiltration is seen as early as 3 days [203] and β cell deterioration and loss of insulin progresses rapidly [202].

In summary, no fundamental differences have been detected in the immune processes leading to rejection of islets and other tissue. Islets are, however, exquisitively sensitive to rejection effect or mechanisms. For example, protocols that enhance other organ allografts may actually curtail the survival of islet allografts [66, 70]. These results emphasize the problems that must be overcome for diabetes to be successfully treated by transplantation of free grafts of allogeneic islets.

Several approaches have been used in attempts to prevent rejection of islet allografts, some of them remarkably effective. These include: 1) immunosuppression by drugs or antilymphocyte preparation; 2) induction of specific tolerance or enhancement; 3) minimization of histocompatibility differences; 4) reduction of islet immunogeneicity by tissue culture or donor pretreatment; and 5) use of immunoisolation devices or mechanical barriers to prevent contact of islets with host lymphocytes antibodies.

2. Prevention of Rejection by Generalized Immunosuppression. A variety of drugs and immunosuppressive protocols have been tested in the various islet transplant models (Table 3). Experiments testing a single agent are instructive, but their failure to prolong islet allograft survival significantly does not necessarily mean they are ineffective, since combination of drugs are usually used clinically.

The drugs currently used for clinical immunosuppression have had variable effects. Marquet and Heystek [134] extended the functional survival of islet allografts in 2 of 5 rats by administration of azathioprine, while Bell et al. [222] found azathioprine to be ineffective. Finch and Morris [210] prevented rejection indefinitely in 2 of 5 rats receiving greater than 1500 islets by daily administration of cyclophosphamide; only a minimal effect was observed in rats receiving 600 to 800 islets. Vialettes et al. [205] found that a single dose and Bell at al. [222] that 5 doses of cyclophosphamide slightly prolonged [205] or did not prolong [222] the survival of neonatal [205] or adult [222] rat islets transplanted across weak [205] or strong [222] histocompatibility barriers.

Corticosteroids are the backbone of most clinical immunosuppressive regimes, but are potentially diabetogeneic. Steffes et al. [223] found that prednisone did not have an adverse effect on the function of islet isografts in rats, but Nelken et al. [138] and Bell et al. [222] could not prevent and Schulak et al. [224] could not reverse islet allograft rejection in rats with steroids alone [138, 224] or in combination with azathioprine [222]. Furthermore, Kretschmer et al. [219] found that pancreatectomised dogs treated with azathioprine and prednisone after islet autotransplantation took longer to achieve normoglycaemia and had lower Kg values than nonimmunosuppressed recipients. Nevertheless, one-third of canine allograft recipients treated by this regimen became normoglycaemic; in the others it was difficult to distinguish the contribution of rejection or the diabetogeneic action of prednisone to graft failure [179]. Zammit et al. [69] reported a slight prolongation of intrasplenic islet allograft survival in dogs treated with azathioprine and prednisone, but Kolb et al. [213] observed almost no effect with a similar regimen after intraportal islet transplantation in dogs.

ALS or one of its derivatives have been the most effective agents in delaying rejection of islet allografts (Table 3).

Table 3. Summary of experiments testing various immunosuppressive protocols in islet allograft models

Species[a] and histo-compatibility barrier	Transplant[b] site and donor source	Islet graft[c] Functional survival days		Treatment schedule[e]	Reference
		No Treatment	Treatment		
Rats S	PV – A	8.8	31.6	Azathioprine 4 mg/kg × 21 d	[134]
Rats S	PV – A	⩽ 5	⩽ 2	Azathioprine 4 mg/kg × 4 d	[222]
Rats S	PV – A 800	4.2	6.8	Cyclophosphamide 10 mg/kg/d	[210]
Rats S	PV – A 1500	4.2	28.3	Cyclophosphamide 10 mg/kg/d	[210]
Rats W	PV – N	2.4	8.2	Cyclophosphamide 25 mg/kg × 1	[205]
Rats S	PV – A	⩽ 5	⩽ 2	Cyclophosphamide 10 mg/kg × 5 d	[222]
Rats S	PV – A	2	2	Prednisone 2 mg/kg/d	[138]
Rats S	PV – A	< 1	⩽ 5	Aza 4 mg/kg + pred 4 mg/kg × 8 d	[222]
Dogs	SP – A	–	33.1 (8/22)	Aza 3 mg/kg + pred 0.5 mg/kg/d	[218]
Dogs	SP – A	8.5	14.8	Aza 2.5 mg/kg + pred 0.5 mg/kg/d	[69]
Dogs	PV – A	2.4	2.3	Aza 3 mg/kg + pred 2 mg/kg/d	[213]
Mice W	IP – A	⩽ 7	26.5	ALS × 7 d, pre-tx	[208]
Rats W	IP – A	8.1	30.5	ALS × 5 d, pre-tx	[129]
Rats W	IP – A	8.0	18.0	ALS × 5 d, pre-tx	[154]
Rats W	PV – A	2	13	ALS × 3 doses	[138]
Rats W	PV – A	2	37.7 (13–68)	ALS × 3 + B. pertussis + donor liver extract	[138]
Rats W	PV – A	2.5	209.0	ALS × 3 doses	[225]
Rats W	PV – A	7.8	> 128.6 (41–209+)	ALS × 1 dose	[194][d]
Rats W	PV – A	8–18	> 215	ALS chronic	[135]
Rats W	PV – A	1–3	> 150	ALS chronic	[215]
Mice S	IP – A	< 12	⩽ 12	ALS × 2 doses	[226]
Mice S	IP – A	⩽ 12	⩽ 63	ALS × 4 + donor bone marrow	[226]
Rats S	IP – A	< 3	1.8	ALS × 5d pre-tx	[129]
Rats S	PV – A	8.8	59.4 (10–118)	ALS × 2 doses	[134]
Rats S	PV – A	1.2	11.8	ALS × 3 doses	[115]
Rats S	PV – A	5.2	24 (7–91)	ALS × 1 dose	[194][d]
Rats S	PV – A	5.2	> 59.0 (10–189+)	ALS × 1 + donor pre-tx with silica and irradiation	[194][d]
Rats S	PV – A	5.8	8.6	ALS × 1 dose	[228][d]
Rats S	PV – A	5.8	> 100	ALS × 1 + islet culture 24 °C	[228][d]
Rats S	PV – A	7.2	> 60.7 (14–100+)	ALS × 1 dose	[197][d]
Rats S	PV – A	7.2	> 91.2 (12–100+)	ALS × 1 + islet culture 24 °C	[197][d]
Rats S	PV – A	7.2	> 44.8 (7–100+)	ALS × 1 + islet culture 37 °C	[197][d]
Rats S	PV – A	4.3	52.0 (7–120)	ALS × 2 pre-tx	[229]
Rats S	PV – A	4.3	> 95 (18–180+)	ALS × 2 + Pd-109-H pre-tx	[229]
Rats S	PV – A	4.3	31.0 (8–90)	ALS × 1 + islet culture	[229]
Rats S	SP – A	5.4	32.0 (28–38)	ALS × 4 doses	[159]
Rats S	PV – A	4.6	> 52.2 (22–100+)	ALS × 4 doses	[159]
Rats S	PV – A	3.4–4.2	26.8–>51.2	ALS × 4 doses	[227]
Rats S	PV – A	3.4–4.2	26.4–29.1	ALS × 3–4 + enhancing sera	[227]
Rats W	PV – N	1–6	7–35	ALS × 7 + donor sol. antigen	[205]
Rats S	KC – F	14	50–70	ALS + Procarbazine + donor liver extract	[230]
Dogs W	PV – A	⩽ 70	> 365	ALS × 7 + Aza 1–2 mg/kg × 49 d	[176]
Rats W	PV – A	5.2	15.3	TLI 200 rads × 5 pre-tx	[67]
Mice W	SP – N	9	> 63 (7/11)	EHNA 10 mg/kg/d × 27	[230]
Rats S	PV – A	2–5	> 100 (4/5)	Silica 50 mg/100 g 6d pre-tx	[232]
Rats S	PV – A	2–5	0–5 (1 > 100)	Carageenan 5 mg/kg/ 0–5d pre-tx	[232]
Rats S	PV – A	3.3	10.3 (2–22)	Cyclosporin A 40 mg/kg/14 d	[218]
Rats S	PV – A	2.3	12.5 (8–12)	Cyclosporin A 20 mg/kg × 7 d	[222]
Rats W	PV – A	4.0	> 41.0 (13–56+)	Cyclosporin A 20 mg/kg/d	[61]
Rats S	PV – A	3.4	> 24.9 (6–60+)	Cyclosporin A 10 mg × 14d	[232]

[a] W = weak or minor and S = strong or major histocompatibility barrier
[b] A = adult; N = neonatal; F = fetal islet tissue; PV = portal vein; SP = spleen; IP = intraperitoneal; KC = kidney capsule
[c] Mean or range given
[d] Rejection defined by recurrence of glycosuria to –1 SD pretransplant level by Lacy et al. [194, 228]. In all other experiments rejection defined by recurrence of hyperglycaemia
[e] AZA, azathioprine; ALS, antilymphocyte serum; EHNA, erythro-9-(2-OH-3-nonyl)adenosine; TLI, total lymphoid irradiation; tx, transplant

If islets are transplanted across a minor histocompatibility barrier in rats [129, 138, 154, 194, 225] or mice [208] only a few doses of ALS can prolong their survival. Lacy et al. [194] found that even a single dose of ALS greatly prolonged the survival of hand-picked Fischer islets in Lewis rats. Gray and Watkins [135] prevented rejection for over a year in Lewis recipients of Fischer islets by chronic administration of ALS, while Beyer et al. [215] were able to prolong the functional survival of islets indefinitely in some rats of this strain combination by administration of ALS whenever plasma glucose rose to greater than 200 mg/dl.

A beneficial effect of ALS could not be demonstrated [129] or was small [226] in early experiments with IP transplantation of strongly histoincompatible islets in rats [129] and mice [226]. In more recent experiments, however, ALS has uniformly prolonged the survival of intraportal islet allografts transplanted across a major histocompatibility barrier in rats [134, 194, 197, 225, 227–229].

The effect of ALS has been enhanced by combining the treatment with other modalities. Lacy et al. [194] found that pretreating donors of hand-picked rat islets with irradiation and silica (to reduce passenger leucocytes) more than doubled the survival time seen with ALS alone after transplantation across a major histocompatibility barrier and islets cultured at 24 °C for one week were not rejected in ALS treated animals [228]. Reemtsma et al. [229] nearly doubled the graft survival time over that seen with anti-lymphocyto-globulin (ALG) alone by adding selective lymphatic irradiation with intravenous palladium-109-hematoporphyrin to recipients of strongly histocompatible rat islets.

In other experiments ALS was part of a protocol intended to induce a donor specific immunological responsiveness, although none of the investigators showed that this was actually the case. Panijayanond et al. [226] extended the survival of islet allografts in mice to nine weeks by combining ALS with administration of donor strain bone marrow. Nelken et al. [138] prolonged the survival of allogeneic rat islets transplanted across a major barrier more than threefold over that seen with ALS alone by injection of B. pertussis culture fluid and donor strain liver extract. Vialettes et al. [205] found that an ALG regimen ineffective by itself was able to prolong the survival of neonatal rat islets transplanted across a weak histocompatibility barrier when combined with administration of donor specific soluble antigen. On the other hand, Finch and Morris [227] were not able to show a synergistic effect of ALS and enhanding antiserum in diabetic rat islet allograft recipients. Mullen [230] found that a combination of donor antigen, procarbazine and ALS increased the survival of fetal pancreatic islet allografts transplanted across a major histocompatibility barrier more than fourfold and resulted in indefinite survival of fetal grafts transplanted across a minor barrier.

Administration of ALS has also been combined with prednisone [85] or azathioprine [85, 174] in large animal islet allograft models in an attempt to mimic clinical protocols. Scharp et al. [85] improved the diabetic status of monkeys after islet transplantation, but the experiments were short term. Lorenz et al. [176] found that duration of normoglycaemia was extended from 4 months in nonimmunosuppressed recipients to greater than one year in dogs treated with ALG and azathioprine after transplantation of islets from closely matched donors. Although the results of the experiments with ALS are encouraging for clinical application, for long term maintenance other drugs will be needed.

New treatments have been tested. Rynasiewicz et al. [67] found that a short course of total lymphoid irradiation in rats tripled the survival time of islets transplanted across a weak histocompatibility barrier. Lum et al. [231] found that erythro-9-(2-hydroxy-3-nonyl) adenosine, an adenosine deaminase inhibitor, could prolong the survival of mouse, but not rat, islet allografts across a weak histocompatibility barrier. In rats another inhibitor, 2-deoxycoformycin, had a slight effect when combined with adenine arabinoside [231]. Nash and Bell [232] evaluated two macrophage suppressing agents. They found that silica, but not carageenan, prolonged the survival of allogeneic rat islets in nearly half of the recipients. This treatment was more effective than other chemical immunosuppressants in their hands [232].

A drug with great potential for clinical application, cyclosporin A, has also been tested in rats. Garvey et al. [218] found that high doses of this drug were needed to prolong the survival of islets transplanted across a major histocompatibility barrier, and infections occurred. Bell et al. [222] reported that islet allografts were rejected subsequent to but not during, a 7 day course of cyclosporin A. Rynasiewicz et al. [61] found continuous administration of cyclosporin A very effective at preventing the rejection of islet allografts transplanted across a weak histocompatibility barrier, but infections occurred at high doses. Vialettes et al. [233] increased the survival of rat islet allografts transplanted across a strong histocompatibility barrier by administration of cyclosporin A for 2 weeks.

Thus, generalized immunosuppression has prolonged islet allograft survival in some, but not all animal models. In many of the experiments where an effect was not seen it is difficult to draw conclusions, because in some immunosuppression was aborted early. In others, only a small number of islets were transplanted and an effect of immunosuppression might have been seen if a larger number of islets had been transplanted.

3. Prevention of Rejection by Induction of Tolerance or Enhancement. Islet allografts will survive indefinitely in inbred rodents if immunological tolerance to the donor strain is induced by the classic technique of bone marrow injection in the neonatal period [64, 206, 208]. Induction of specific immunological unresponsiveness is more difficult in adult animals. In the experiments described in the previous section [138, 205, 226, 227, 230] injection of donor antigen and ALS according to defined protocols prolonged the survival of islet allografts, but rejection ultimately occurred.

Immunological enhancement protocols [64, 70, 139, 210, 227, 231] effective in prolonging the survival of heart or kidney allografts have had variable effect on islet allografts. Finch and Morris [210, 227] found that the survival of DA islets in Lewis rats was increased from 2–5 days in untreated rats to 5–11 days in rats treated with anti-DA serum; in Lewis recipients of semi-allogeneic (DA × Lewis) islets, normoglycaemia was maintained for 12–16 days

in three of five rats and for > 360 days in two rats. Nash et al. [139, 234] found that enhancing sera prolonged the survival of islet allografts to > 100 days in 20 to 40% of rats. Perloff et al. [64] found that administration of donor specific antigen and alloantibody actually curtailed the survival of allogeneic islets. Reckard et al. [70] found the survival of allogeneic islets was only slightly prolonged in recipients bearing enhanced hearts from the same donor strain. The difficulties with enhancement protocols are not surprising, since Naji et al. [206] showed the sensitivity of islets to destruction by antibodies.

In another approach Kramp et al. [105] made mice allogeneic radiation chimeras by injection of bone marrow after otherwise sublethal total body irradiation. In mice that did not die from graft versus host disease, diabetes induced by streptozotocin was ameliorated for at least 16 weeks in 7 of 13 recipients of allogeneic islets derived from the original bone marrow donor strain. This technique is probably not clinically applicable because of its risks.

Many of the experiments were performed under highly artificial conditions, e. g. tolerance was induced before the diabetic state, and the specific protocols are not relevant to transplantation in humans. Nevertheless, specific immunological unresponsiveness is the most important concept in transplantation; hopefully, the experiments are a prelude to development of a practial approach for its application.

4. Prevention of Rejection by Alteration of Graft Immunogenicity. Passenger leucocytes are thought to play a major role in sensitizing a host to an allograft [235]. Their elimination, either by donor pretreatment or by maintenance of tissue in vitro before transplantation, has been associated with prolonged survival of a variety of organ allografts [235].

Again, attempts to apply these techniques to delay rejection of islet allografts have given variable results. Hegre et al. [123, 201] found that fetal rat pancreatic tissue cultured in 95% oxygen for ten days was rejected < 15 days after allotransplantation. Mandel et al. [237] found that fetal mouse and Garvey et al. [203] that fetal rat pancreases cultured for 21 days in an ambient environment were rejected within 2 weeks after allotransplantation. Monkey islets cultured for 7 to 9 days by Jonasson et al. [175] and adult mouse islets cultured for 10 days by Andersson and Buschard [236] were also rejected after allotransplantation.

In contrast, Simenovic et al. [221] found that 17 days of culture in 95% oxygen reduced the immunogenicity of Balb/C fetal mouse pancreas, while 7 days of culture were sufficient to prevent histological rejection of adult islets transplanted to nondiabetic histoincompatible CBA mice. In functional studies, Kedinger et al. [238] partially reversed diabetes in 11 of 13 recipients by direct injection into the liver of adult allogeneic islets cultured in an ambient environment for 4 days. Nine of the recipients maintained fasting normoglycaemia for 90 days without immunosuppression, while recipients of noncultured allogeneic islets reverted to the diabetic state by a mean of 8.2 days after transplantation.

Lacy et al. [194] found that culture of adult rat islets in 95% oxygen for more than 5 days was associated with islet disintegration. For that reason they combined a shorter period of culture with other treatments such as ALS. In a separate set of experiments, they [228] found that hand-picked allogeneic islets cultured for 7 days at 24 °C in an ambient atmosphere did not disintegrate. According to their criteria, the cultured islets were not rejected during 100 days of observation after intraportal transplantation across a major histocompatibility barrier to diabetic rats immunosuppressed with a single injection of ALS. In rats bearing long term islet allografts, rejection could be induced by injection of peritoneal exudate cells obtained from the original donor strain [197]. These results show (1) that culture did not alter the immunological recognition of B cells and (2) support the passenger leucocyte hypothesis [197].

The combination of donor pretreatment and islet culture prior to transplantation has also been used for mouse islets in histological studies by Bowen et al. [198]. They cultured Balb C islets in 95% oxygen at 37 °C and prevented disintegration by aggregating islets into clusters of about 50 islets. Fresh islets transplanted to nondiabetic CBA recipients were infiltrated by mononuclear cells beginning at 4 days, and were completely rejected by 14 days. Donor pretreatment with cyclophosphamide reduced the intensity of the response to fresh islets, but if donor pretreatment was combined with tissue culture for 7 to 12 days, virtually no allograft reaction was seen over 3 months.

5. Prevention of Rejection by Immuno-mechanical Barriers. The results of transplantation of islet tissue enclosed in diffusion chambers have in general been poor. Theoretically a semiporous membrane will exclude cells or molecules able to mediate rejection, while allowing flux of insulin and glucose. The composition, structure and diffusion characteristics of several membranes and chambers and the problems with their use has been assessed by Theodorou et al. [239].

Swenne et al. [188] found that mouse islets survived in millipore chambers, but they could not reproduce the results of Strautz [186], and ob/ob mice receiving islets from lean littermates remained hyperglycaemic after IP implantation. Gates and Lazarus [240] reported that streptozotocin induced diabetes in rats was cured by IP implantation of neonatal rabbit pancreatic tissue enclosed in millipore chambers and that the diabetic state recurred after removal of the chamber, but Garvey et al. [241] could not reproduce these results and found no viable tissue at the end of 6 weeks. Jolly et al. [242] reported that transplantation of dog islets in millipore chambers into the peritoneal cavity of alloxan diabetic rats was followed by slow reversal of the hyperglycaemic state, but they did not remove the chambers to see if the diabetes would recur, nor did they follow a control group of untreated diabetic rats. Archer et al. [243] reported that both syngeneic and xenogeneic mouse islets enclosed in 50 K hollow fibres restored normoglycaemia for several weeks in Chinese hamsters with mild streptozotocin-induced diabetes.

In contrast to these reports, other investigators have not been able to produce a sustained reduction of hyperglycaemia in insulinopaenic diabetic animals treated by transplantation of membrane enclosed syngeneic [243a, 244,

245], allogeneic [78] or xenogeneic [246] islets. The problems with these chambers include poor neovascularization, deposition of fibrous connective tissue, and inadequate flux of insulin and glucose between the chamber and the host.

A more sophisticated approach has been to implant a chamber within the blood stream of the diabetic animal with the islets isolated on one side of a synthetic porous membrane or artifical capillary bundle [247–249]. Although plasma glucose levels have been lowered for a short time in diabetic animals, blood clotting and other technical problems have not allowed the devices to function for a long enough period to determine whether or not the theoretical objective of preventing rejection can be achieved.

6. Islet Xenografts. If rejection of tissue transplanted from animals to man can be prevented, a major problem of organ procurement would be solved. It would facilitate the use of multiple donors, should this be necessary for islets. Unfortunately, xenografts between widely disparate species are rejected with unusual vigour, often within a few minutes [249]. For this reason, islets transplanted between discordant species have been enclosed in semi permeable membrane chambers [240–243, 246], but without success.

Xenografts between closely related species, however, can function for a reasonable period. Fresh rat islets transplanted to the peritoneal cavity of nonimmunosuppressed diabetic mice have reduced hyperglycaemia [246] or restored normoglycaemia [209, 251] for up to one week. Mice treated with ALS after receiving rat islets remained normoglycaemic for 9–21 days [209] and for 2.5–8 weeks [251]. If the recipients were injected with rat anti-mouse serum, hyperglycaemia recurred within 24 to 72 h [209, 251]. Lacy et al. [252] found that highly purified rat islets were rejected between 7 to 12 days in untreated diabetic mice, but 27% were normoglycaemic at 60 days if treated with a single injection of mouse ALS. If the rat islets were cultured at 24 °C for 7 days before transplantation, 40% of recipients treated with mouse ALS and 70% of recipients treated with mouse ALS and rat ALS (to neutralize passenger leukocytes) were normoglycaemic at 60 days.

Discordant xenografts are usually rejected promptly. Eloy et al. [253] found that islet rich, 15 day (but not 18 day) chick embryo pancreas significantly improved diabetes in rats after direct injection into the hepatic parenchyma. Except for this report, discordant xenografts have been successfully transplanted only under very special circumstances, to thymic aplastic mutant nude (nu/nu) mice or nude (rnu/rnu) rats, which are genetically unable to reject allo- and xenografts [254, 255].

Reintgen et al. [199] restored normoglycaemia in nude mice with streptozotocin-induced diabetes by SC transplantation of a hamster insulinoma. Human islets can also survive after xenotransplantation to these unusual animals. Usadel et al. [256] transplanted human fetal pancreatic fragments (6 to 23 weeks gestation) SC to nondiabetic nude mice or into a plastic lined epigastric pouch in nondiabetic nude rats. Functional studies were not reported, but histological examination of the implants between 21 and 120 days after transplantation showed well formed islets with all hormone cell types. Partial amelioration of diabetes has also been observed after transplantation of human islets to nude mice with streptozotocin-induced diabetes [257]. Despite these results, application of discordant xenografts to clinical transplantation seems remote.

7. Future of Islet Allotransplantation. The major barrier to clinical application of islet transplantation is allograft rejection. Islets may have an increased susceptibility to rejection because of an inherently strong immunogenicity or because of the cellular nature of the graft. The outlook for islet allografts, however, is not entirely bleak. A variety of immunosuppressive protocols have prolonged significantly islet allograft functional survival. The importance of transplanting a sufficient islet mass is now understood. The success with transplantation of allogeneic islets in animals underscores the potential for and the need to develop specific immunosuppressive techniques that will be applicable to human transplantation.

F. Preservation of Islet Tissue

A reliable technique for short term preservation is necessary for clinical islet transplantation to be logistically practical. Long-term preservation would allow islets to be accumulated and transplanted in one procedure to a diabetic recipient selected on the basis of histocompatibility match or other factors.

Three methods of islet preservation have been investigated: 1) tissue culture; 2) cold storage; and 3) freezing. Numerous investigators have obtained in vitro evidence of islet function after storage by these methods. There are a few experiments showing that stored islets can reverse diabetes after transplantation, a more critical test of viability, and these deserve to be emphasised.

1. Preservation by Culture. Rodent islets maintained in culture for days or weeks can synthesize and secrete insulin in response to appropriate stimuli [236, 258, 259]. In cultures of fetal or neonatal pancreas, islets preferentially differentiate [126a, 175, 201, 260] and B cells can replicate [123, 126a]. High oxygen concentrations may be detrimental to isolated islets [228] unless they are protected by aggregation [198] or cultured at 24 °C [261].

Some of the experiments on transplantation of cultured islets were described in the preceding section on islet allografts [144, 228, 238]. These experiments were designed to test the ability of in vitro passage to alter islet immunogenicity, but their technical success depended upon preservation of viability. Other experiments have been designed specifically to test the feasibility of culture for islet preservation. Scharp et al. [262] found that the interval between intraportal transplantation and restoration of normoglycaemia was delayed in diabetic rats receiving isologous adult islets stored in stationary tissue culture for 3 weeks. In another experiment, intraportal transplantation of islet pellets originally derived from the nine donor rats and aggregated by gyrorotational culture for one week reversed diabetes as well as fresh islets after transplantation, even though central necrosis was present [263].

Nakagawara et al. [259] reversed mild diabetes in rats by transplantation of 300 fresh or 7 day cultured isologous islets. Andersson et al. [236] restored normoglycaemia to diabetic nude mice within 2 weeks by transplantation of 500 fresh or 10 day cultured islets.

Selawry et al. [141] found that islets isolated from old rats lost the ability to bind concanvalin A in vitro and failed to ameliorate diabetes if transplanted immediately. A 48 h period of tissue culture restored both conconavalin A binding activity and the ability to reverse diabetes after intraportal transplantation to syngeneic recipients.

Payne et al. [106] successfully transplanted unpurified collagenase dispersed pancreatic islet tissue from DL-ethionine treated donor rats after storage for 24 to 48 h. Tissue from one-third of a donor pancreas restored normoglycaemia at the same rate as fresh tissue. Culture of adult pancreatic tissue from dogs [101] and monkeys [175] for 24–48 h resulted in reduction of exocrine enzyme content, but transplants to diabetic hosts were only partially successful [13, 101, 175].

Weber et al. [115] reversed diabetes in inbred rats after transplantation of collagenase dispersed neonatal pancreatic tissue stored at 37 °C for 24 h. Hegre et al. [123, 264] maintained intact neonatal rat pancreases in organ culture for 2 to 9 days before collagenase dispersal and intraperitoneal transplantation. Tissue from 10–25 syngeneic donors restored normoglycaemia between 2 and 8 weeks. Intact fetal rat pancreases cultured for 8 days similarly reversed diabetes after transplantation to under the kidney capsule [126a]. Mandel et al. [121a] found that intact fetal mouse pancreas cultured for 21 days reduced hyperglycaemia after intrasplenic transplantation to syngeneic diabetic mice; the results were better if grafts were cultured in media with a low rather than a high glucose concentration.

At this point, culture is only a means of islet storage. No pure B cell lines have been established to provide a continuous source of insulin producing tissue for transplantation. Rodent islets can be stored for at least a week before transplantation. The relatively sophisticated equipment and fastidious conditions required for maintenance of islets in tissue culture, however, makes it difficult to store the quantities necessary for transplantation in large animal models.

2. Cold Storage of Islets. Storage in the cold is the simplest preservation method and requires the least amount of equipment. In vitro studies have defined some of the parameters that may be important in maintaining short [265] or long term viability [266].

Matas et al. [267, 268] reversed diabetes in rats by transplantation of collagenase dispersed neonatal pancreatic islet tissue stored for up to 63 h [267] in a small amount and up to 101 h [268] in a large amount of Gibco culture medium at 4 °C. In addition, they found that variable periods of ischaemia (1–7 h) could be tolerated with [268] or without [269] a 48 h period of storage at 4 °C. In complementary studies, Henriksson et al. [95] successfully transplanted adult rat islets isolated from donor pancreases after 40 min of warm ischaemia.

In contrast to the relatively poor results of transplantation in large animal models after storage of pancreatic tissue at 37 °C, cold storage has been applied successfully to islet transplantation in dogs. Sutherland et al. [179] found that 8 of 16 and Schulak et al. [180] found that 5 of 5 dogs became normoglycaemic after intrasplenic transplantation of autologous islet tissue stored at 4 °C for 24 but not 48 h. The latent period between transplantation and normoglycaemia was prolonged and glucose tolerance was impaired in some [179] but not in other [180] dogs.

Thus, both rat and dog pancreatic islets have been successfully transplanted after storage in the cold. If the reliability of this technique is improved and if the period of storage can be increased in a large animal model, this method should be suitable as a practical means for short term preservation of human islet tissue before transplantation.

3. Cryopreservation of Islet Tissue. A variety of cells or tissue fragments can be frozen and stored at −196 °C for months or years without evident loss of viability [270]. Several investigators have used in vitro tests to determine the optimal conditions for freezing and thawing of adult islets [271, 272] or fetal rat pancreas [273]. Critical factors include the cooling and warming rates, the concentration and penetration of agents added to protect against intracellular ice crystal formation, and the osmatic changes that occur during removal of the cryoprotectant [270].

Mazur et al. [273] found that 17.5 day fetal rat pancreases suspended in 2 mol/l DMSO, cooled to −8 °C, nucleated with an ice crystal, frozen at 0.3 °C/min to −78 °C, thawed at room temperature, and then slowly diluted at 0.75 mol/l sucrose solution to preclude osmotic shock, could synthesize protein and insulin in vitro. Kemp et al. [274] transplanted fetal pancreases, stored for 13 weeks at −196 °C, to under the kidney capsule of rats with renal-portal vein shunts and reversed diabetes within 30 days.

Similar experiments have been carried out using isolated adult rat islets. Banks et al. [272] found that freezing in 1 mol/l DMSO at 75 °C/min gave optimal viability by in vitro parameters. Rajotte et al. [275] found different conditions to be optimal: stepwise suspension in 2 mol/l DMSO, seeding with ice at −7.5 °C, freezing to −75 °C at 0.25 °C/min, storage at −196 °C, and warming at 7.5 °C/min. Bretzel et al. [276] have also normalized blood glucose in diabetic rats with isologous islets frozen in 1 mol/l DMSO at 2 °C/min and stored for 4 weeks at −196 °C before rapid thawing and intraportal transplantation.

Payne et al. [277] used a protocol similar to that of Rajotte et al. [275] to freeze neonatal rat pancreatic fragments to −70 °C. Fragments were thawed at room temperature, dispersed by collagenase digestion and transplanted intraportally to syngeneic diabetic rats. Only half of the rats receiving no further treatment were cured of diabetes, but 12 of 13 rats treated with insulin for 1 to 2 weeks became permanently normoglycaemic. These results suggest that freeze-thaw induced injury is compounded by the diabetic state, but that the injury is reversible if the effect of diabetes is blunted by temporary administration of insulin.

Standard cryopreservation techniques, appropriately modified, thus allow rat islets to be stored before transplan-

tation. Human fetal pancreatic fragments have been evaluated by in vitro studies [278] and have even been transplanted [279], but there are no reports of successful transplantation of frozen islets obtained from large animal pancreases.

IV. Effect of Pancreas and Islet Transplantation on Long Term Complications in Experimental Diabetes

One of the most exciting aspects of experimental pancreas and islet transplantation has been the demonstration that the renal and other lesions associated with induced diabetes in animals are secondary to the diabetic state and the finding that early, but established, lesions either regress or stabilise following correction of the metabolic abnormalities by transplantation.

The renal lesions that develop in diabetic animals are similar, in some respects, to those associated with diabetes in humans [4, 6, 280]. In rats with streptozotocin or alloxan diabetes there is a progressive increase in mesangial matrix volume [281, 282, 283] and in basement membrane thickness [282, 283, 284]. Tubular vacuolisation and hyalinisation of arterioles is present [280, 281, 283]. Immunoglobulin and other macromolecules are deposited within the mesangium [285]. Lee et al. [286] provided proof that these lesions are not a direct result of the agent inducing diabetes but are secondary to the abnormal metabolic environment to which the kidney is exposed. Kidneys transplanted from normal rats to diabetic rats develop lesions identical to those occurring in the diabetic recipient's own kidneys; conversely, lesions in diabetic rats kidneys disappeared or failed to progress after transplantation to normal rats [286]. The secondary nature of those lesions has also been inferred by their failure to develop in animals receiving pancreas [284, 287] or islet [6, 168, 281, 283, 288] transplants soon after the induction of diabetes.

The influence of islet transplantation on established renal glomerular lesions has been shown in a series of experiments in diabetic rats by Mauer et al. [167, 281, 289] and Steffes et al. [282, 290]. After islet transplantation to rats with diabetes of more than 6 months duration, light microscopic lesions present at the time of transplantation either failed to progress or their was an actual decrease in mesangial matrix material [281]. Immunoglobulin and complement progressively disappeared as the metabolic abnormalities were corrected [289], while in untreated diabetic rats, the renal lesions continued to progress. Quantitative electron microscopic morphometric studies confirmed that the increased glomerular and mesangial matrix volume in rats with diabetes of 9 months duration decreased after successful islet transplantation [282]. The increased proportion of glomerular capillary surface and glomerular basement membrane-epithelium interface juxtaposed to the mesangium also declined after transplantation. In separate experiments [290] the increase in basement membrane thickness in rats 7 months after induction of diabetes did not decline over a 6 months observation period after islet transplantation. Failure to reverse this, but not other lesions, may be due to the slow turnover of basement membrane in rats.

Bretzel et al. [291] have also performed quantitative studies in rats with diabetes of 3 to 6 months duration. Four months after islet transplantation there was a reduction of mesangial space and a re-widening of the capillary lumen. Koesters et al. [292] found that islet transplantation in diabetic rats restored phagocytic and clearance function of mesangial cells.

There are a few reports on the effect of islet transplantation on the nerve [25] and eye [288, 293, 294] lesions that develop in diabetic rats. Gray and Watkins [288] found that new vessel formation and retinal capillary dilation did not develop in rats transplanted within a month of induction of diabetes. Worthen et al. [293] obtained very similar results in rats treated with whole pancreas transplantation soon after the induction of diabetes. Actual regression of eye pathology in diabetic rats was shown by Krupin et al. [294] using ocular flurophotometry. In diabetic rats, fluorescein acclumation in the vitreous and anterior chamber was twice normal one hour after injection. Two weeks after islet transplantation the integrity of the blood occular barrier was restored and the values returned to baseline.

These reports that pancreas and islet transplantation can stabilise or induce regression of early lesions provide an impetus and a rational basis for pursuing such an approach in humans.

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