Progress report

Transplantation of insulin-secreting tissues

The past 15 years have brought the unwelcome realisation that parenteral insulin and oral hypoglycaemic agents have failed to prevent the macro- and microvascular complications associated with diabetes mellitus. The inability of current therapeutic approaches to alter significantly the morbidity and mortality caused by the vascular disease seen in diabetic patients has been measured by the University Group Diabetes Program¹.

Although discussion of the exact nature of the biochemical lesion in diabetes has led to great controversy, it is reasonably well accepted that at least one basic defect resides in the beta cell of the islets of Langerhans². Thus, all forms of diabetes are characterised by a 'relative' deficiency of circulating insulin³. Detailed studies of insulin secretion in normal and diabetic subjects indicate that the cells are sluggish in their response to glucose stimulation².

The failure of exogenous agents—for example, insulin and the oral hypoglycaemic agents—to effect a rigorous control of blood sugar from minute to minute may explain the failure of such agents to prevent the complications of diabetes. As the basic defect in diabetes probably lies in the beta cells, it has been postulated that successful transplantation of normal beta cells into the diabetic patient may prevent the development of vascular complications in the brain, eye, kidney, peripheral nervous system, and other tissues.

Since 1965, the feasibility of beta cell transplantation, as intact isolated islets of Langerhans, in fragments of pancreas or in the whole pancreas, has been actively investigated. The majority of experiments have involved animals made diabetic by the administration of specific beta cell poisons (alloxan and streptozotocin) as recipients of islet-containing tissue.

Transplantation of pancreas with vascular anastomoses between gland and recipient

ANIMAL EXPERIMENTS

Transplantation of the entire pancreas with vascular anastomoses to the recipient arterial and venous system has been accomplished by many investigators. The recipient animals were usually rendered diabetic by pancreatectomy or the administration of alloxan. Most investigators described only a modest survival rate in homotransplant recipients. Some of those animals surviving transplantation have maintained endocrine function of the graft for varying, but short, periods of time. Largiader *et al.*⁴ reported transplantation of the entire pancreas into unrelated dogs. The pancreas and duodenum were transplanted *en bloc* and the duodenum was drained into the gall-bladder. Approximately 50% of the animals died in the immediate post-operative period; the remainder survived only four to nine days. These

short-lived survivors maintained normoglycaemia until death. Circulating insulin levels were not measured. Pemberton and Manax⁵ found that one-third of diabetic dogs receiving pancreatic allografts were normoglycaemic up to 32 days. Some of these dogs demonstrated significant increases in insulin secretion rate when challenged with a glucose tolerance test, indicating the presence of functioning endocrine tissue.

Studies of whole pancreas homotransplantation in dogs have combined the problems of tissue rejection with the technical challenges of the surgical procedure. A study by Aquino *et al.*⁶ using an autotransplant model (thereby eliminating tissue incompatibility) demonstrated that surgical problems alone caused a significant percentage of failures (only two of 12 dogs survived longer than 17 days). Mortality in most of the animals was attributable to vascular thrombosis, anastomotic leaks, and intercurrent infection.

Studies of inbred strains of rats have also been designed to obviate tissue incompatibility factors. The microsurgery necessitated by the calibre of rat blood vessels was difficult and a 48 hour postoperative mortality rate of 60% was described for transplantation of the pancreas⁷. Nevertheless, some inbred rat pancreas recipients survived and were normoglycaemic. Resection of the transplants in the survivors was followed by hyperglycaemia. In a more recent study⁸, Lee *et al.* were more successful with isologous pancreatoduodenal transplants in inbred Lewis rats using a new microsurgical technique.

Most failures of whole pancreas transplantation have been due to one of three causes:

1. *Leakage of pancreatic exocrine secretion*

Attempts to solve this problem have included (a) transplantation of the duodenum with the pancreas as a drainage conduit⁴; (b) administration of ethionine in an attempt to suppress exocrine function⁷; (c) ligation of the pancreatic duct six weeks before transplantation in an effort to devitalise exocrine tissue⁷; and (d) radiation therapy⁹.

2. *Vascular thrombosis*

An interesting approach to this problem is to interpose the graft into the iliac circulation in such a way that blood flow to the leg must pass through the graft⁹.

3. *Immunological rejection*

Limited experience with the management of rejection has been reported. Radiation therapy, azothiaprime, steroids, and antilymphocyte globulin have all been used in an attempt to thwart the rejection process (see below).

CLINICAL EXPERIENCE

Whole pancreas transplantation in two patients was reported in 1966. The first patient graft survived six days, the second functioned for five months¹⁰.

Up to 1 April 1977, the American College of Surgeons, National Institutes of Health Transplant Registry in Chicago, Illinois, reported a total of 52 pancreas transplants in 50 patients. At that time there were no functioning grafts remaining. The longest survival was for 4.2 years and the transplant was carried out by Gliedman.

The complications besetting the recipients of human pancreas transplants have paralleled the experience reported in animals. Exocrine function has been handled by duct ligation or drainage *via* the duodenal conduit. Gliedman *et al.*¹¹ have advocated direct anastomosis of the pancreatic duct to a ureter, eliminating the need for cotransplantation of the duodenum (which seems to reject more readily than the pancreas itself). The exocrine function of the gland can then be assayed by urinary amylase determinations. This technique was used in the single long-term survivor mentioned above and two other patients who survived longer than any who received other types of connection. Vascular complications reminiscent of those described in animal experiments have also been reported in humans but no reliable remedy for these complications has been reported.

The immunosuppression regimes that are commonly used include azathioprine, antilymphocyte globulin, and prednisone. The use of large doses of steroids in the pancreatic graft recipient tends to obscure the relationship between graft function—for example, insulin secretion—and blood glucose levels. So-called ‘steroid diabetes’ has been reported in a number of patients.

Many of the patients receiving pancreas transplants have had histories of renal failure secondary to diabetic nephropathy. For this reason, most recipients of pancreas transplants have received coincident renal allografts. Although the clinical experience is very limited and the numbers involved are small, concern that the pancreatic graft in some way elicits a more potent rejection reaction (either to itself or to the renal allograft) had been voiced by Gliedman *et al.*¹¹. Accordingly, it has been proposed that renal and pancreatic allografting be performed asynchronously—that is, pancreas first. Using this procedure, Gliedman *et al.*¹¹ have reported improved results in terms of both number of grafts surviving and length of survival. The small number of patients in this series makes any conclusion about this proposal tentative.

A recent paper by Groth *et al.*¹² has suggested that the rising post-prandial blood sugars gave an indication of impending graft rejection that was more sensitive than fasting blood sugar or amylase. They were able to remove the grafts with the aid of angiography to evaluate rejection and salvaged all four recipients. The majority of previous patients died from complications caused by the rejection process before graft removal could be accomplished.

Transplantation of pancreas fragments without vascular anastomoses

ANIMAL EXPERIMENTS

The high percentage of whole pancreas graft failures caused by surgical complications has prompted the use of minced pancreas fragments as tissue grafts. Taking advantage of the fact that islets of Langerhans develop more rapidly than acinar tissue in most animal embryos studied, fetal or neonatal pancreas tissue has been effectively used in transplantation experiments with pancreas fragments.

Early studies demonstrated that fetal or neonatal pancreas fragments could be effectively transplanted into immunologically privileged sites such as the hamster cheek pouch or the anterior chamber of the eye. Histological examination of the transplants revealed degeneration of the acinar tissue and survival of islet tissue^{13,14}. Recent reports have described successful reversal of chemically induced diabetes in syngeneic rats by using minced

fetal or neonatal grafts of pancreas fragments into the peritoneal cavity or beneath the renal capsule^{15,16}.

Mirkovitch and Campiche¹⁷ have autotransplanted the adult dog pancreas. A portion of the pancreas was excised, minced, partially digested with collagenase, and then injected into the spleen *via* a splenic vessel. The remainder of the pancreas was then removed. The dogs demonstrated hyperglycaemia until the tenth day and thereafter had normal glucose determinations. Glucose tolerance tests and renal vein insulin determinations confirmed the functional integrity of the grafts. Splenectomy resulted in hyperglycaemia and death in all animals.

CLINICAL EXPERIENCE

A few attempts at transplantation of pancreas fragments have been described. These have usually been preparations which were intended to be isolated islets; this will be discussed below.

Transplantation of isolated islets

A major obstacle in the transplantation efforts described above is related to the anatomical reality that pancreatic islets are distributed throughout a gland, the volume of which is many times greater than that of the islets themselves. Most of the complications plaguing efforts at transplantation are direct corollaries of the fact that, in order to transplant functioning endocrine tissue, which amounts to 2% of the pancreatic tissue mass, the remaining 98%—the acinar tissue—must be either cotransplanted or eliminated. Although minced fetal pancreas tissue had been used in an attempt to increase the relative concentration of insulin-secreting tissue in the graft, the obvious objective was to transplant islets which had been isolated from all acinar tissue. In addition, as islets had appeared relatively undamaged in rejected whole organs¹⁸, it was hoped that they might be relatively protected from the ravages of tissue rejection.

The hope of isolating viable intact islets of Langerhans from the pancreas lay dormant until Moskalewski¹⁹ described collagenase digestion of the pancreas; Lacy and Kostianovsky perfected the method in 1967²⁰. This more advanced technique entailed distension of the pancreas by the injection of Hank's solution under pressure into the common bile duct. The pancreas was then excised, finely minced, and digested with collagenase. Individual islets were then readily visible in the dissecting microscope and collected with a glass loop. Islets isolated in such a fashion appeared intact histologically and responded appropriately to insulin secretagogues *in vitro*.

One further refinement of islet isolation increased the yield of islets, decreased the time required to obtain them, and has led to a burgeoning of efforts at isolated islet transplantation. This refinement consisted of using a density gradient to separate islets from acinar debris after collagenase digestion. Originally, a sucrose density gradient was proposed by Lacy and Kostianovsky in 1967²⁰. Lindall *et al.*²¹ suggested a discontinuous gradient of different concentrations of ficoll, a polymer of sucrose. This technique was explored by Scharp *et al.*⁴⁴ who found that increased yields of viable islets were obtained with the method. An additional increased yield of rat islets were reported by Scharp *et al.*³⁹ who used a digestion-filtration technique

to remove islets freed from the acinar tissue during the digestion procedure. These techniques have allowed functionally intact islets of Langerhans to be isolated from the rat pancreas in relatively large numbers.

ANIMAL EXPERIMENTS

In an early report describing the transplantation of isolated islets, Reemtsma²³ took advantage of the relative ease with which piscine islets could be obtained free of acinar tissue. Thus, fish islets were transplanted into rats which had been made diabetic by the administration of streptozotocin. Although a decrease in blood sugar in the diabetic recipient rats was described, this effect was short-lived.

The first major attempt at transplantation of isolated islets from one animal to another of the same species was reported by Ballinger and Lacy in 1972²⁴. Using inbred strains of rats, the recipients were made diabetic by administering streptozotocin. Blood glucose, urine volume, urine glucose, and weight were monitored in control and experimental animals. The transplantation of 400 to 600 isolated pancreatic islets into the peritoneal cavity or into the thigh muscle of the recipient resulted in a significant long-term reduction of hyperglycaemia, polyuria and glycosuria, and a restoration of weight gain. Although the average urine glucose, urine volume, and blood glucose levels were improved in the transplanted group, these values did not approach the normal control levels. Nonetheless, some individual animals did achieve normal control values for two months or more. Excision of islets which had been transplanted into the thigh muscle resulted in a rapid return to the diabetic state. Histological examination of the excised islets revealed intact alpha and beta cells with a marked degranulation of beta cells; this was thought to be indicative of the physiological demand for insulin placed on these cells. This report also detailed preliminary allograft experiments. Islets were transplanted across a major histocompatibility barrier and the recipients received immunosuppression. The animals subjected to this protocol evidenced some amelioration of their diabetes.

A. *Site of isolated islet implantation*

The availability of isolated islets which could be injected into an organ, a body cavity, or the blood stream led to the investigation of the question; what is the optimum site for implantation of the islets?

There are several important theoretical and practical considerations involved in the selection of an appropriate site for islet administration in diabetic animals, especially man. Ideally, the site should be surgically accessible. The organ or tissue into which the islets are placed should be expendable so that, should an untoward tissue reaction occur, the graft can be removed. Although the significance of the venous drainage of the pancreas—for example, *via* the portal vein—is not fully appreciated from an endocrinological point of view, it is likely that such a configuration is of physiological importance. The profound effects of insulin on the liver and the remarkable ability of that organ to clear the hormone imply that the metabolic relationships between normal pancreatic islets and the liver are intimate.

The importance of the site of islet transplantation was explored by Kemp *et al.*²⁵ They found that 600 to 850 islets placed subcutaneously had no

significant effect on the urine glucose, urine volume, or blood sugar of diabetic isologous rat recipients. A similar number of islets transplanted into the peritoneal cavity resulted in amelioration of the diabetic state, but none of the parameters examined reverted to normal values. When an equal number of islets were injected into the portal vein, the diabetic animals achieved normal urine volumes and blood glucose levels. Histological examination of the recipient liver revealed intact, vascularised islets, lodged in the terminal portal tracts. Alpha, beta, and delta cells were seen in the transplanted islets. Desmosomes were seen linking islets and hepatocytes²⁶. Two weeks after implantation, non-myelinated nerve fibres were observed in the pericapillary spaces of transplanted islets. Immunofluorescent staining for dopamine beta hydroxylase demonstrated the presence of nerve fibres, presumably adrenergic. As might be predicted, amelioration of chemically induced diabetes in experimental animals is dependent upon the mass of insulin-secreting tissue that is transplanted. In our laboratory, reversal of streptozotocin-induced diabetes in adult Lewis rats is consistently achieved when the isolate injected into the portal vein contains an estimated 600 islets or more. Preliminary data indicate that there is a rapid fall-off in success rate below this number of islets. However, glucose tolerance testing does not completely revert to normal until approximately 2000 islets are injected into the portal vein²⁷. Koncz *et al.*²⁸ have examined the question of islet mass and the reversal of diabetes in a different manner. Using much higher doses of streptozotocin than described by most investigators, they achieved a severe ketotic diabetic state in adult rats. Intramuscular and intraperitoneal transplants of isolated islets into these animals resulted in an improvement in the metabolic status of the recipient. They concluded that a minimum of two to three islets per gram of body weight were required to achieve a metabolic response and to maintain normal homeostasis.

Recent investigations in our laboratory examined the spleen as the target organ for isologous islets in diabetic rats²⁷. While the glucose response to an intravenous tolerance test appeared to be normal, the insulin response was not. In addition, four of seven recipients failed to return to pretransplant diabetic levels after splenectomy with islets found within the liver. Thus, although the spleen may be expendable as a transplant organ, its effectiveness may not be as great, nor does it guarantee safety from liver dispersion.

B. *Antigenicity of transplanted islets*

There have been conflicting reports on the relative ability of endocrine tissue to provoke transplantation immunorejection. Reckard *et al.*²⁹ examined the fate of isolated islets transplanted into rats which had a major histocompatibility difference from the donors. Using a recipient with diabetes induced by streptozotocin and administering 600 to 1200 islets into the peritoneal cavity, these investigators found that the islets did indeed provoke rejection. Several other interesting observations were also made:

1. Islet homografts functioned only one to three days when donors and recipients differed by a strong histocompatibility (Fischer to ACI). The period of functional survival was not lengthened by a course of immunosuppression (antilymphocyte serum).
2. Islet homografts compatible at the AgB locus (ACI to DA) resulted in normoglycaemia of the recipient for a mean of 12 days. Antilymphocyte

serum had a pronounced effect on these animals, extending the median function islet survival to 30.5 days.

3. To determine if the recipients of homologous islets had become sensitised to donor tissue, six Fischer rat recipients of 800 to 1200 AgB incompatible Lewis islets were challenged with donor skin grafts. These grafts were rejected in an accelerated manner, indicating that homologous islets did indeed stimulate the host immune system. By contrasting the period required for tissue rejection to become manifest, the authors concluded that islet tissue is at least as antigenic as skin or heart. Barker *et al.*³⁰ have demonstrated that, when donor and recipient did not differ at a major histocompatibility locus, isolated islet autotransplants into the portal vein resulted in a median survival time almost three times longer than rats receiving intraperitoneal transplants. This suggests that the liver may provide some sort of immunological protection. This phenomenon was not observed when donor and recipient differed at a major histocompatibility locus.

Finch and Morris³¹ have recently described that increasing the numbers of islets implanted increases the time to functional rejection. This is probably attributable to there being a certain critical mass of islets necessary for maintaining normoglycaemia. With additional islets transplanted, rejection of individual islets can continue longer until the number falls below that needed to maintain normoglycaemia.

C. *Effect of islet transplantation on complications of diabetes*

Some preliminary evidence concerning the effect of islet transplantation on the secondary complications of diabetes has been obtained. Sutherland *et al.*³² have described renal glomerular lesions in rats six months after induction in diabetes that were characterised by immunoglobulin and complement deposition in the glomerular mesangium. This was followed by mesangial matrix thickening. In diabetic rats which had received intraperitoneal transplantation of isologous neonatal pancreatic islet tissue, plasma glucose levels were significantly lower and several serial biopsies showed progressive decrease in mesangial immunofluorescent staining for IgG, IgM, and B₂C. Four to nine weeks after transplantation only traces of the above could be detected. Mesangial matrix thickening was arrested or actually reduced. The relationship between mesangial deposition of complement and immunoglobulin and streptozotocin-induced diabetes is not clear and the implications of these findings are difficult to interpret. There is no evidence that late complications of diabetes are reversed.

D. *In vitro storage of islets*

The ultimate clinical need for practical storage of islets after harvesting before transplantation is quite clear. Both cryopreservation and tissue culture techniques have been explored.

The ability to store certain blood elements at very low temperatures has stimulated investigation of cryopreservation of isolated islets. An impressive series of experiments detailing cryopreservation of hand isolated islets has recently been published by Ferguson *et al.*³³ They examined cold storage of islets at 4 °C; subzero cell storage at -187 °C; and subzero cell storage (-187 °C) followed by a 24-hour period of organ culture. With this last protocol, they report an 87% histological islet survival and a 75% functional

islet survival as documented by insulin release in response to a glucose challenge *in vitro*.

Islets have been successfully maintained in tissue culture by Hegre *et al.*³⁴ in 1976. They maintain their ability to synthesise pro-insulin and insulin and will release the latter in response to glucose stimulation. Acinar contamination of isolated islets seems not to survive in tissue culture. Although *in vitro* culture of the whole fetal rat pancreas yields a relative increase in islet volume in comparison with exocrine tissue, conclusions about the ability of isolated islets to replicate in cell culture has been difficult to establish. Some of the most elegant work on culturing of islet tissue has been described by Chick³⁵. Monolayer cultures of dispersed cells from the neonatal rat pancreas have been carried out. Cells cultured in such a fashion respond to glucose by releasing insulin, and they have maintained this ability for a period of one year. Furthermore, it is thought that these cells actively replicate, although this process is ultimately inhibited by fibroblast overgrowth³⁶.

Hegre *et al.*³⁴ have shown that organ culture of the neonatal rat pancreas for two to nine days did not adversely effect the ability of that tissue to reverse chemically induced diabetes when transplanted.

Matas *et al.*³⁷ have described a technique utilising short-term culture of pancreatic fragments as a method of purifying islet tissue from acinar debris. Using pancreases from adult dogs, they minced the organs and placed the tissue in culture media for 24 hours and then performed autotransplants using this 'purified' material. Some of the experimental animals survived and were not diabetic.

Ferguson *et al.*³³ have reported that mouse, rat, and guinea-pig islets suffer minimal histological damage after simple organ culture for 21 days. Despite the robust appearance of beta cells on histological examination, these organ cultured islets did not respond appropriately *in vitro*. The explanation for this phenomenon is not clear.

The theoretical possibility that islet antigenicity might be reduced during a period of organ culture has been discussed for many years. Recent impetus for this hope was described by Lafferty *et al.*³⁸. They found that the survival time of mouse thyroid, transplanted to an H-2 incompatible recipient, was lengthened by placing the thyroid in organ culture for 12 days before transplantation.

To date, allotransplant experiments after organ culture of islets and neonatal pancreas have failed to reveal any salutary effect on the rate of rejection^{34,39}.

Several laboratories are now attempting to circumvent both limbs of the immunological rejection response by constructing small implantable chambers in which the islet tissue will be housed. Pore size of the chambers would be such that nutrients, oxygen, and insulin secretagogues would gain entry but lymphocytes and complex globulin molecules would be excluded. Similarly, insulin, but not cell surface antigenic substances, would be allowed to egress.

Weaver *et al.*⁴⁰, as early as 1955 demonstrated that extravascular diffusion chambers could protect a variety of cells from destruction by immunised hosts if the pores were of the proper size. While a number of endocrine tissues have been shown to function in diffusion chambers, the effect is usually transient because of the host's fibroblastic response to the chamber. However, Jolley *et al.*⁴¹ greatly increased the length of survival by coating the membranes

with proteolytic enzymes. A more physiological approach would be to place the chamber intravascularly where the islets could more efficiently respond to glucose changes. Yet, one must then tackle the problem of coagulation as well. The feasibility of this approach has been demonstrated by Chick *et al.*³⁵, Sun *et al.*⁴² and Tze *et al.*⁴³ using islets surrounding fine capillary tubes.

CLINICAL EXPERIENCE

Islet transplantation in man poses one more major problem not encountered in rodent experiments. The pancreas in the human is compact and fibrous as compared with the readily distensible gland in the rat, making isolation of large quantities of physiologically intact islets very difficult.

Preliminary work has been reported in animal models which are thought to be more relevant to the problems posed by human islet transplantation. The isolation of islets from the monkey, which has a compact fibrous pancreas, and subsequent transplanting have been described⁴⁴. The *in vitro* assay of these isolated islets and the results of their transplantation are less impressive than the results described in the rat.

Human islets have been successfully isolated from donors who have recently died and from operative specimens. Human isolated islets have been shown to release insulin when exposed to high concentrations of glucose in a perfusion system. Isolated islets have been transplanted into the peritoneal cavity and portal vein of at least seven diabetic patients by Najarian *et al.*⁴⁵. In some patients a decrease in daily insulin dependency was noted. Rigorous assessment of successful transplantation of isolated islets in man is still awaited.

It is clear that the attempt to alter the course of diabetes mellitus by transplantation of insulin secreting tissues has progressed rapidly in the past 10 years. Many animal experiments and preliminary work in man have yielded exciting and encouraging results. The techniques recently developed for isolation of human islets will now allow direct investigation of human hormone responses to calorogenic molecules, stress, and other endocrine effector agents. The work described above has provoked as many questions as it has answered, yet the challenges of the future have been more clearly defined.

R. C. KARL, D. W. SCHARP, W. F. BALLINGER, AND P. E. LACY
Departments of Surgery and Pathology
Washington University School of Medicine
St. Louis, Missouri 63110, USA

Received for publication 21 June 1977

References

- ¹The University Group Diabetes Program (1970). A study of the effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes *Diabetes*, **19**, 747-830.
- ²Cerasi, E., and Luft, R. (eds.) (1970). *Pathogenesis of Diabetes Mellitus*. Almqvist and Wiksell: Stockholm.
- ³Felig, P. (1971). Pathophysiology of diabetes mellitus. *Medical Clinics of North America*, **55**, 821-834.
- ⁴Largiader, F., Lyons, G. W., Hidalgo, F., Dietzman, R. H., and Lillehei, R. C. (1966). Orthotopic allotransplantation of the pancreas. *American Journal of Surgery*, **113**, 70-76.
- ⁵Pemberton, L. B., and Manax, W. G. (1971). Control of alloxan diabetes in dogs by islet cell transplantation. *Surgery, Gynecology, and Obstetrics*, **132**, 75-80.

- ⁶Aquino, C., Ruiz, J. O., Schultz, L. S., and Lillehei, R. C. (1973). Pancreatic transplantation without duodenum in the dog. *American Journal of Surgery*, **125**, 240-244.
- ⁷Reemtsma, K., Giraldo, N., Depp, D. A., and Eichwald, E. J. (1968). Islet cell transplantation. *Annals of Surgery*, **168**, 436-446.
- ⁸Lee, S., Chandler, J. G., Krubel, R., Nakaji, N. T., Rosen, H., and Orloff, M. J. (1971). Relief of diabetes mellitus by heterotopic transplantation of the whole pancreas in inbred rats. *Surgical Forum*, **22**, 75-77.
- ⁹Merkel, F. K., Kelly, W. D., Goetz, F. C., and Maney, J. (1968). Irradiated heterotopic segmental canine pancreatic allografts. *Surgery*, **63**, 291-297.
- ¹⁰Kelly, W. D., Lillehei, R. C., Merkel, F. K., Idezuki, Y., and Goetz, F. C. (1967). Allo-transplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. *Surgery*, **61**, 827-837.
- ¹¹Gliedman, M. L., Gold, M., Whittaker, J., Rifkin, H., Soberman, R., Freed, S., Tellis, V., and Veith, F. J. (1973). Clinical segmental pancreatic transplantation with ureter-pancreatic duct anastomosis for exocrine drainage. *Surgery*, **74**, 171-180.
- ¹²Groth, C. G., Lundgren, G., Arner, P., Collste, H., Hårdstedt, C., Lewander, R., and Östman, J. (1976). Rejection of isolated pancreatic allografts in patients with diabetes. *Surgery, Gynecology, and Obstetrics*, **143**, 933-940.
- ¹³Gonet, A. E., and Renold, A. E. (1965). Homografting of fetal rat pancreas. *Diabetologia*, **1**, 91-96.
- ¹⁴Coupland, R. E. (1960). The survival and growth of pancreatic tissue in the anterior chamber of the eye of the albino rat. *Journal of Endocrinology*, **20**, 69-77.
- ¹⁵Brown, J., Molnar, I. G., Clark, W., and Mullen, Y. (1974). Control of experimental diabetes mellitus in rats by transplantation of fetal pancreases. *Science*, **184**, 1377-1379.
- ¹⁶Steffes, M. W., Sutherland, D. E. R., Mauer, S. M., Leonard, R. J., Najarian, J. S., and Brown, D. M. (1975). Plasma insulin and glucose levels in diabetic rats prior to and following islet transplantation. *Journal of Laboratory and Clinical Medicine*, **85**, 75-81.
- ¹⁷Mirkovitch, V., and Campiche, M. (1976). Successful intrasplenic autotransplantation of pancreatic tissue in totally pancreatectomised dogs. *Transplantation*, **21**, 265-269.
- ¹⁸Lillehei, R. C., Simmons, R. L., Najarian, J. S., Weil, R., Uchida, H., Ruiz, J. O., Kjellstrand, C. M., and Goetz, F. C. (1970). Pancreatico-duodenal allotransplantation: experimental and clinical experience. *Annals of Surgery*, **172**, 405-436.
- ¹⁹Moskalewski, S. (1965). Isolation and culture of the islets of Langerhans of the guinea pig. *General and Comparative Endocrinology*, **5**, 342-353.
- ²⁰Lacy, P. E., and Kostianovsky, M. (1967). Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes*, **16**, 35-39.
- ²¹Lindall, A., Steffes, M. W., and Sorenson, R. (1969). Immunoassayable insulin content of subcellular fractions of rat islets. *Endocrinology*, **85**, 218-223.
- ²²Reemtsma, K. (1970). Experimental islet cell grafting: a transplantation model. *Transplantation Proceedings*, **2**, 513-515.
- ²³Ballinger, W. F., and Lacy, P. E. (1972). Transplantation of intact pancreatic islets in rats. *Surgery*, **72**, 175-186.
- ²⁴Kemp, C. B., Knight, M. J., Scharp, D. W., Ballinger, W. F., and Lacy, P. E. (1973). Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. *Diabetologia*, **9**, 486-491.
- ²⁵Griffith, R. C., Scharp, D. W., Hartman, B. K., Ballinger, W. F., and Lacy, P. E. (1977). A morphologic study of intrahepatic portal-vein islet isografts. *Diabetes*, **26**, 215-221.
- ²⁶Feldman, S. D., Hirshberg, G. E., Dodi, G., Raizman, M. E., Scharp, D. W., Ballinger, W. F., and Lacy, P. E. (1977). Intrasplenic islet isografts. *Surgery*, **82**, 386-394.
- ²⁷Koncz, L., Davidoff, F., DeLellis, R. A., Selby, M., and Zimmerman, C. E. (1976). Quantitative aspects of the metabolic response to pancreatic islet transplantation in rats with severe ketotic diabetes. *Metabolism*, **25**, 147-156.
- ²⁸Reckard, C. R., Ziegler, M. M., and Barker, C. F. (1973). Physiological and immunological consequences of transplanting isolated pancreatic islets. *Surgery*, **74**, 91-99.
- ²⁹Barker, C. F., Reckard, C. R., Ziegler, M. M., and Naji, A. (1975). The liver as an immunologically privileged site for rat pancreatic islet allografts. *Diabetes*, **24**, suppl. 2, 418. (Abstract).
- ³⁰Finch, D. R. A., and Morris, P. J. (1977). The effect of increasing islet numbers on survival of pancreatic islet allografts in immunosuppressed diabetic rats. *Transplantation*, **23**, 104-106.
- ³¹Sutherland, D. E. R., Steffes, M. W., Mauer, S. M., and Najarian, J. S. (1974). Reversal of secondary lesions of diabetes in rats by pancreatic islet transplantation. *Surgical Forum*, **25**, 309-311.
- ³²Ferguson, J., Allsopp, R. H., Taylor, R. M. R., and Johnston, I. D. A. (1976). Isolation and long term preservation of pancreatic islets from mouse, rat, and guinea pig. *Diabetologia*, **12**, 115-121.
- ³³Hegre, O. D., Leonard, R. J., Schmitt, R. V., and Lazarow, A. (1976). Isotransplantation of organ-cultured neonatal pancreas: reversal of alloxan diabetes in the rat. *Diabetes*, **25**, 180-189.
- ³⁴Chick, W. L., Like, A. A., Lauris, V., Galletti, P. M., Richardson, P. D., Panol, G., Mix, T. W., and Colton, C. K. (1975). A hybrid artificial pancreas. *Transactions of the American*

- Society for Artificial Internal Organs*, XXI, 8-15.
- ³⁶Goetz, F. C. (1974). Conference on beta cell function, transplantation, and implantable glucose sensors: a summary. *Metabolism*, **23**, 875-884.
- ³⁷Matas, A. J., Sutherland, D. E. R., Steffes, M. W., and Najarian, J. S. (1976). Short-term culture of adult pancreatic fragments for purification and transplantation of islets of Langerhans. *Surgery*, **80**, 183-191.
- ³⁸Lafferty, K. J., Conley, M. A., Woolnough, J., and Walkser, K. Z. (1975). Thyroid allograft immunogenicity is reduced after a period in organ culture. *Science*, **188**, 259-261.
- ³⁹Scharp, D. W., Murphy, J. J., Newton, W. T., Ballinger, W. F., and Lacy, P. E. (1974). Transplantation of islets of Langerhans in diabetic rhesus monkeys. *Surgery*, **77**, 100-105.
- ⁴⁰Weaver, J. M., Algire, G. H., and Prehn, R. T. (1955). The growth of cells *in vivo* in diffusion chambers. II. The role of cells in the destruction of homografts in mice. *Journal of the National Cancer Institute*, **15**, 1737-1767.
- ⁴¹Jolley, W. B., Hinshaw, D. B., Call, T. W., and Alvord, L. S. (1977). Xenogeneic pancreatic islet transplantation in proteolytic enzyme-bonded diffusion chambers in diabetic rats. *Transplantation Proceedings*, **9**, 363-365.
- ⁴²Sun, A. M., and Macmorine, H. G. (1976). Cultivation of monkey and rat islets and the use of artificial capillary units to diabetic animals. (Abstract). *Diabetes*, **25**, suppl. 1, 339.
- ⁴³Tze, W. J., Wong, F. C., Chen, L. M., and O'Young, S. (1976). Implantable artificial endocrine pancreas unit used to restore normoglycemia in the diabetic rat. *Nature*, **264**, 466-476.
- ⁴⁴Scharp, D. W., Kemp, C. B., Knight, M. J., Ballinger, W. F., and Lacy, P. E. (1973). The use of ficoll in the preparation of viable islets of Langerhans from the rat pancreas. *Transplantation*, **16**, 686-688.
- ⁴⁵Najarian, J. S., Sutherland, D. E. R., Matas, A. T., Steffes, M. W., Simmons, R. L., and Goetz, F. C. (1977). Human islet transplantation: a preliminary report. *Transplantation Proceedings*, **9**, 233-236.