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CANINE PANCREATIC ALLOTRANSPLANTATION WITH DUODENUM (PANCREATICODUODENAL TRANSPLANTATION) USING CYCLOSPORIN A

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ABSTRACT

Pancreatic transplantation is still partly experimental and involves a variety of problems, such as handling of exocrine secretion, prevention of arterial thrombosis of the graft, and immunosuppression. Aiming at the best method of handling exocrine secretion and at prevention of thrombosis, pancreas transplantation with the duodenum (pancreaticoduodenal transplantation) was performed in eighteen pairs of mongrel dogs. Nine recipients (control group) were transplanted without immunosuppression, and the other nine were immunosuppressed with cyclosporin A (CsA group) after transplantation. Five dogs which died of intussusception, strangulation, hemorrhagic pancreatities etc. were not evaluated in this study. In the control group, survivals were 16, 14, 8, 7 and 6 days; in the CsA group, survivals were >395, >150, 89, 70, 25, 18, 18, and 12 days, respectively. In the CsA group there were two long-term surviving dogs without rejection; and the grafted pancreas of one of these dogs was judged to be almost normal in exocrine and endocrine glands microscopically from a specimen biopsied on the 260th postoperative day. This result is worthy of special mention in the animal experiment. CsA was more effective in prolonging survival of the dogs which received pancreaticoduodenal transplantation when compared with conventional immunosuppression. In the pancreaticoduodenal transplantation with aortic cuff, which was expected to increase the blood flow of the graft followed by a decrease in the incidence of thrombosis, there were 6 cases with thrombosis of the graft among 13 recipients. It was concluded, therefore, that CsA is effective in prolonging the survival of dogs which receive pancreaticoduodenal transplantation with physiological drainage of exocrine secretion into the duodenum, but that prevention of thrombosis by means of adequate anti-thrombosis treatment is essential.

Keywords: pancreatic transplantation, pancreaticoduodenal transplantation, cyclosporin A, rejection, thrombosis.

INTRODUCTION

Pancreatic transplantation is performed as the treatment for diabetes mellitus and for replacement after total pancreatectomy. Various techniques for pancreas transplantation have been used clinically and experimentally. In the beginning (1966–1975), 12 pancreaticoduodenal transplantations were performed by Lillehei and associates with conventional immunosuppression. One patient survived 12 months but 8 patients died of sepsis or due to other reasons in the early postoperative period.[1,2] Three patients survived after removal of the pancreaticoduodenal graft. As a result of their findings of the duodenum's increased sensitivity to rejection, Lillehei and

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associates modified their technique to transplantation of the pancreas alone.[3] This modified technique was employed in only one patient in 1973.

Connolly and associates[4] (1969–1971) performed 3 pancreaticoduodenal transplantations, one of which succeeded, but the patient was killed in a traffic accident 10 months after transplantation; two grafts were removed after 2 months. In 1971, Gliedman and associates[5] reported 10 segmental pancreatic transplantations with pancreatic duct anastomosed with ureter; in only one case did the graft survive for as long as 4.2 years. Later, there were occasional reports concerning pancreas transplantation but the results were not satisfying.[6] During this time, while pancreas transplantations were rarely performed, islet transplantations were frequently performed. In 1978, Dubernard and associates reported a new method of segmental pancreatic transplantation by obstruction of the pancreatic duct with neoprene.[7] And in 1979, Sutherland and associates reported an open-duct technique of segmental pancreatic transplantation.[8] Thereafter, segmental pancreatic transplantation increased in frequency in certain cases. [9,10,11]

A variety of problems[12] involved in pancreas transplantation have been clarified: 1) handling of exocrine secretion; 2) long-term function; 3) thrombosis; and 4) immunosuppression. These problems were discussed at the International Workshop of Segmental Pancreatic Transplantation.[13] We may reappraise the usefulness of the pancreas transplantation with duodenum (pancreaticoduodenal transplantation) in the light of these problems because it is the best method of handling exocrine secretion and is followed by good long-term function when thrombosis and rejection are prevented.

The purpose of this study was as follows: 1) to bring about immunosuppression by administering cyclosporin A (CsA), a metabolite isolated from the culture broth of the fungal species *Tolyocladium inflatum.*, and the most promising new immunosuppressive agent of recent years; 2) to prevent thrombosis in the graft by increasing the blood flow of the graft, using the cuff of the aorta with coeliac artery and superior mesenteric artery for the arterial anastomosis; and 3) to perform pancreaticoduodenal transplantation as the method of handling exocrine secretion effectively.

MATERIALS AND METHODS

Eighteen pairs of mongrel dogs weighing 8 – 15 kg were used. Weights of these donor-recipient pair dogs were similar. The animals were fasted 24 hrs prior to surgery. All animals showed normal blood glucose level (70–110 mg/dl) before operation.

Operation Technique

Heterotopic allo-pancreaticoduodenal transplantation was performed. In the donor, after preparation of the pancreas and duodenum, the coeliac artery (CA) and superior mesenteric artery (SMA) were excised in a navicular shape with the aorta wall (aortic cuff) for arterial anastomosis with the recipient's common iliac artery (CIA). The portal vein (PV) was dissected for anastomosis with the common iliac vein (CIA) under the bifurcation of the right and left hepatic branch. The spleen was removed and the ends of the splenic artery (SA) and vein (SV) were ligated. The proper hepatic artery, distal portion of the SMA and SMV, and common bile duct were each ligated and severed, respectively. The duodenum was severed near the pyloric ring and at the ligament of Treiz. The end of the oral side of the duodenum was closed with a continuous Albert & Lembert suture (Fig. 1a). The pancreaticoduodenal graft was perfused ex vivo with Lactate Ringer solution through a cannula inserted first into the CA and, second, into the SMA on the inside orifice of the aortic cuff, cooled at 4°C, and preserved by refrigeration. Total ischemic time was 3 hr. In the recipient, the CIA and CIV were exposed. The aortic cuff and

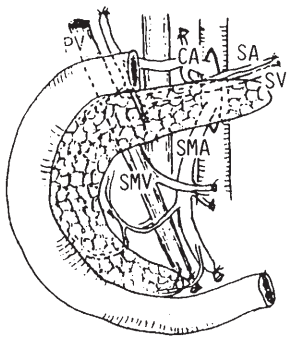


Fig. 1a

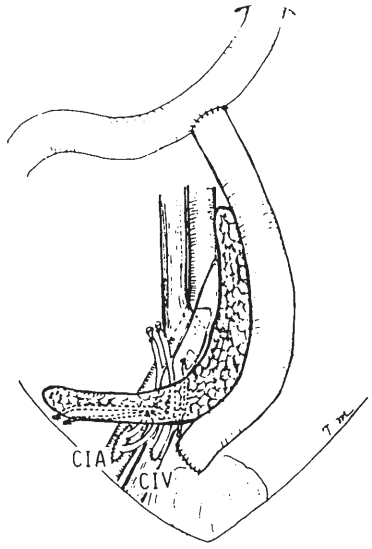


Fig. 1b

Fig. 1
a; Preparation of the pancreaticoduodenal graft (donor).
b; Heterotopic allo-pancreaticoduodenal transplantation (recipient).

portal vein of the graft were anastomosed end-to-side to the CIA and CIV. Duodenojejunostomy was performed with Albert & Lembert sutures end-to-side to the upper part of the jejunum. Total pancreatectomy was performed[14] just after transplantation. The spleen was not removed (Fig. 1b). The animals were placed on a regular diet on the third postoperative day without enzyme preparations.

Nine dogs were immunosuppressed with CsA 8 mg/kg/day intravenously until they were able to take medication orally, thereafter they received CsA 16 mg/kg/day by p.o. administration (CsA group). The other nine dogs were not administered CsA and thereby acted as the control group. Complete autopsy, including liver, kidney, spleen and graft, was performed in all animals.

Pancreas function was monitored by fasting blood glucose, and graft deterioration was defined when fasting blood glucose was above 300 mg/dl.

RESULTS

The overall graft survival and cause of death are shown in Table 1. These results excluded 5 dogs that died of intussusception (1), strangulation (1), hemorrhagic pancreatitis (1), and general malaise within 3 postoperative days of the graft working normally (2). In the control group, survivals were 16, 14, 8, 7, and 6 days. All died in a diabetic status. No. 1 and 2 dogs died due to rejection, and No. 3, 4 and 5 dogs died of generalized peritonitis due to perforation of the transplanted duodenum. In the CsA group, No. 10 and 11 dogs were surviving in normoglycemia at 395 days and 150 days. No. 12 and 14 dogs died of pulmonary thrombosis without thrombosis in the graft. No. 13 died of chronic rejection and cholecystitis and No. 15, 16 and 17 dogs died

Table 1 The overall survival and cause of death after pancreaticoduodenal allotransplantation in dogs.

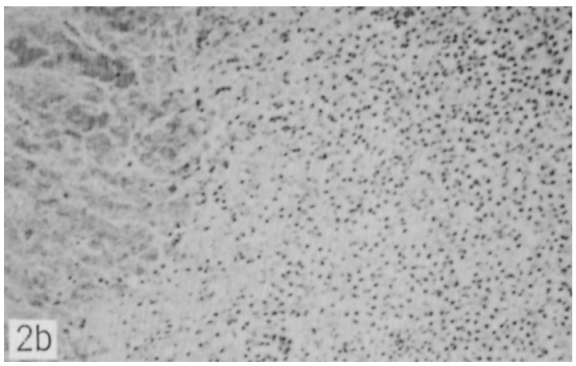
Group	No.	Body Weight	Survival (days)	Cause of death
Control Group	1	14 kg	16	acute rejection
	2	15	14	acute rejection
	3	10	8	generalized peritonitis
	4	13	7	generalized peritonitis
	5	9	6	generalized peritonitis
	6	11	2	strangulation
	7	9	0	hemorrhagic pancreatitis
	8	10	3	general malaise
	9	13	2	general malaise
CsA Group	10	10 kg	> 395	alive
	11	8	> 150	alive
	12	10	89	pulmonary thrombosis
	13	11	70	chr. rej.* & cholecystitis
	14	9	25	pulmonary thrombosis
	15	10	18	generalized peritonitis
	16	12	18	generalized peritonitis
	17	15	12	generalized peritonitis
	18	10	5	intussusception

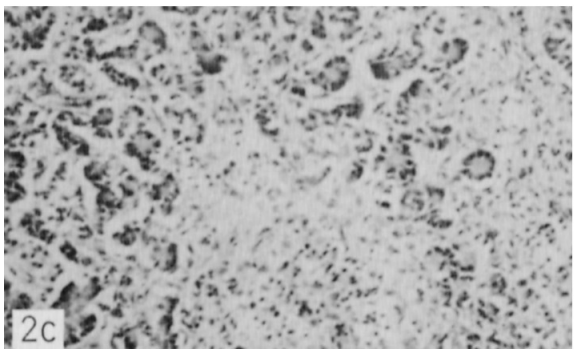
*: chronic rejection

of generalized peritonitis due to perforation of the transplanted duodenum.

Autopsy and Biopsy

In the rejected grafts (No. 1 and 2), the duodenal wall was thickened without arterial thrombosis (Fig. 2a), and remarkable infiltration by mononuclear cells and polymorphic nuclear leukocytes was noted microscopically. Mucosa was lost due to necrosis. The submucosal layer was thickened with severe cellular infiltration, but the muscular layer was not destroyed (Fig. 2b). The pancreas was destroyed by mononuclear cell infiltration, and a slight increase of fibrous tissue was noted. The exocrine lobulet structure was also destroyed and Langerhans' islets were not identified (Fig. 2c). Six dogs died of generalized peritonitis, 3 in the control group (60%) and 3 in the CsA group (37.5%). The cause of these perforations of the grafted duodenum was revealed by macroscopic and microscopic examination to be arterial thrombosis in the graft (Fig. 3a and 3b). In the perforated transplanted duodenum, there was little inflammatory reaction. The duodenal wall was thin and the mucosa and submucosal layers were fully necrotic, possibly due to arterial thrombosis which was noted in the large artery in the transplanted pancreas. Pancreas stroma were also nectrotic. Exocrine glands were loose, with lobulet structure,





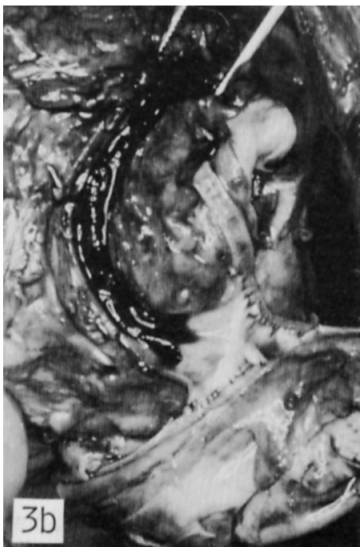
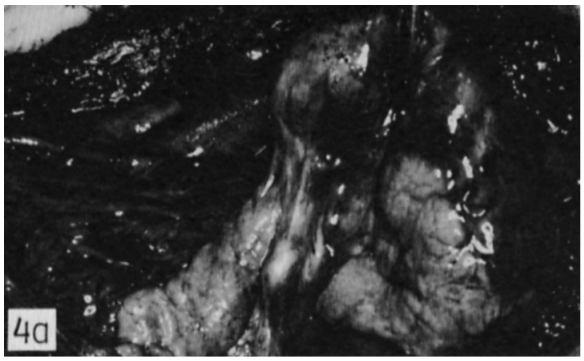
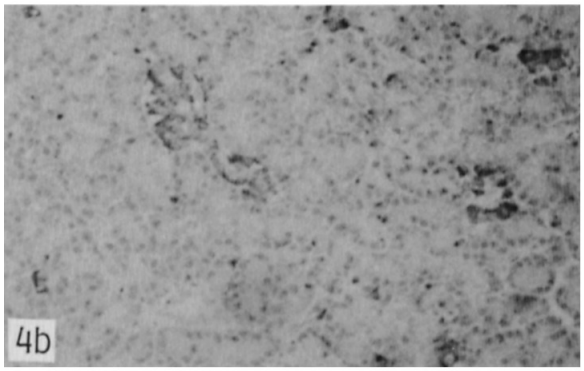


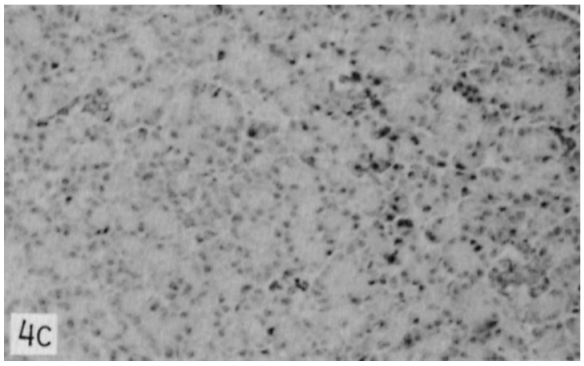
Fig. 2
a; The duodenum of the rejcted graft without CsA (Dog No. 1).
b; Photomicrograph of the duodenum of the rejected graft without CsA (×150, Dog No. 1).
c; Photomicrograph of the pancreas of the rejected graft without CsA (×150, Dog No. 1).
Fig. 3
a; Perforation of transplanted duodenum (Dog No. 4).
b; Arterial thrombosis of the graft (Dog No. 4).

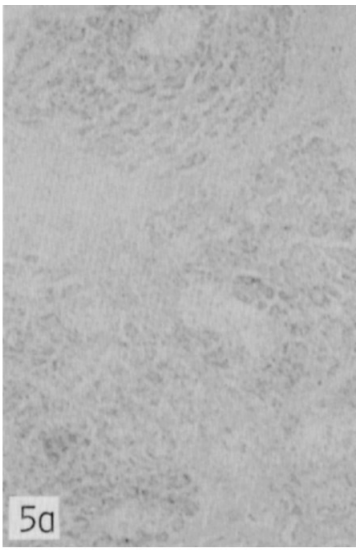
and Langerhans' islets were not identified either in Hematoxirin-Eosin stain or in Aldehyde-Fucusin stain. There was little mononuclear cell infiltration.

In the CsA group, No. 10 and 11 dogs were still alive in normoglycemia at 395 days and 150 days. The biopsied specimen (Fig. 4a) of the transplanted pancreas on the 260th postoperative day revealed almost normal structure of the exocrine and endocrine glands, and hormonal substances such as insulin, glucagon and somatostatin were well stained by immunohistochemical method (Fig. 4b and 4c).









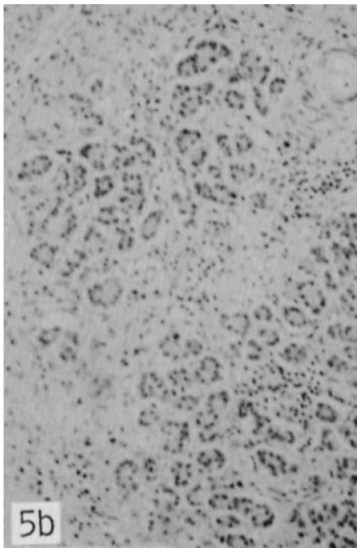


Fig. 4

a; The pancreaticoduodenal graft 260 days after transplantation (Dog No. 10).

b; Photomicrograph of insulin staining of the pancreas 260 days after transplantation by immunohistochemical method (×150, Dog No. 10).

c; Photomicrograph of glucagon staining of the pancreas 260 days after transplantation by immunohistochemical method (×150, Dog No. 10).

Fig. 5

a; Photomicrograph of the pancreas 89 days after transplantation (×60, Dog No. 12).

b; Photomicrograph of the pancreas 70 days after transplantation (×150, Dog No. 13).

No. 12 dog died of pulmonary thrombosis on the 89th postoperative day. The graft looked normal macroscopically but microscopically the duodenum was slightly thickened and was infiltrated with some mononuclear cells. In the pancreas there was much fibrosis with slight cell infiltration, and one third of the exocrine glands were replaced with interstitial fibrosis and were seen to contain many Langerhans' islets (Fig. 5a). No. 13 dog died of chronic rejection and cholecystitis 70 days postoperatively. The duodenal wall was thick, and fibrosis and mononuclear cell infiltration were noted in the mucosa and submucosal layers. The pancreas was fully fibrotic with cell infiltration. The exocrine glands were noted in small areas, like islands, in the fibrosis, and endocrine glands were not found (Fig. 5b). No. 14 dog died of pulmonary thrombosis on the 25th postoperative day in normoglycemic state, but histologically there was slight mononuclear cell infiltration in the duodenum and pancreas, indicating a mild rejection reaction.

DISCUSSION

Clinically, according to an overview report of the Pancreas Transplantation Registry,[15] 204 pancreas transplants were performed between July 1, 1977 and December 31, 1982. Of the 204 cases, 195 were segmental pancreas grafts, 8 were whole pancreas transplants (all silicone-rubber-injected), and 1 was a pancreaticoduodenal graft. The number of grafts functioning for more than 5 months was 34 (17%). One hundred seventy grafts ceased to function at less than 5 months because of technical complication, rejection, or death of the recipient. It is known that the most important technique in pancreas transplantation is the provision for handling exocrine secretions. Currently,[16] suppression of exocrine function by injection into the duct is the most popular technique[17-20] and the operation-related mortality has been relatively low,[21] but some injected grafts may have been damaged because fibrosis was induced by the injected agent and involved the islets.[22-24] It is not clear at the present time which technique is the best, and the physiological approach of pancreaticoenterostomy has been used in recent clinical trials in Minnesota,[25-27] Stockholm[28,29] and Cambridge.[30,31]

Experimentally, Ota[32] reported that the longest survivor among 20 dogs administered azathioprine died on the 63rd postoperative day. Idezuki[33] and Lillehei[34] reported that 6 of 99 dogs survived for 20 days or longer with and without immunosuppressive therapy (azathioprine); of these 6 dogs, the longest survival was 160 days with death finally occurring due to rejection and the others died within 40 days. Early animal studies using conventional immunosuppression had not achieved long-term allograft survival in pancreaticoduodenal transplantation.

Clinically, pancreaticoduodenal transplantation had not been performed. And although Langerhans' islet transplantation was tried, none of the grafted patients succeeded in long-term survival. Currently, segmental pancreatic transplantation has been performed most popularly, but the long-term success rate so far has been relatively low. Recently, we had a chance to use the new immunosuppressive agent, CsA, which was effective in prolonging survival after total small intestinal allotransplantation in the dog.[35] From the results achieved, we have re-evaluated the pancreaticoduodenal transplantation using CsA.

In our experiment, there were two long-term survivors which survived for more than 395 days and 150 days, respectively, without rejection among the 8 dogs in the CsA group, and biopsy revealed almost normal structure of the graft, especially of the endocrine glands stained by the immunohistochemical method. This result is worthy of special mention in the animal pancreas transplantation experiment as it strongly suggests that CsA is more effective in prolonging survival in pancreaticoduodenal transplantation in the dog than conventional immunosuppression.

In regard to handling exocrine secretion, experimental studies on pancreatic duct obstruction

with prolamine by Gebhardt[36] reported that exocrinal pancreatic atrophy with marked fibrosis at some time can lead to a relative reduction in the number of B cells. In our study, biopsy of long-term surviving grafts revealed no fibrosis in the exocrine gland. Taking Gebhardt's results into consideration, pancreaticoduodenal transplantation with physiological drainage of exocrine secretion into the duodenum is much better than the duct obstruction method.

In observation of thrombosis of pancreas grafts, it was seen that many grafts were lost due to thrombosis especially in the segmental pancreatic transplantation in which blood flow was very poor. Calne[37] and Dutoit[38] reported an additional distal splenic arteriovenous anastomosis to increase the total blood flow in both the artery and vein in the segmental pancreatic transplantation. McPhedran[39] performed jump graft: the splenic artery was anastomosed end-to-side at both proximal and distal ends. The purpose of this technique was to improve splenic arterial blood flow. In the pancreaticoduodenal transplantation more blood flow was expected than in the segmental pancreatic transplantation, and in our experiment, the aortic cuff technique with celiac artery and superior mesenteric artery for arterial anastomosis was expected to follow much more blood flow. But our data shows that there was 60% graft thrombosis in the control group and 37.5% in the CsA group. In addition, there were two cases of pulmonary thrombosis without graft thrombosis. In order to prevent graft thrombosis followed by perforation of the grafted duodenum, splenic arteriovenous anastomosis or some anticoagulating treatment during and two or three weeks after operation must be considered in the next step of the experiment.

From the point of view of the functional quality of the grafted pancreas, pancreaticoduodenal transplantation with CsA and with adequate anti-thrombosis treatment is one of the best techniques of pancreatic transplantation.

REFERENCES

1) Kelly, W.D., Lillehei, R.C., Merkel, F.K. *et al.*, Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. *Surgery*, **61**, 827–837, 1967.
2) Lillehei, R.C., Simmons, R.L., Najarian, J.S. *et al.*, Pancreaticoduodenal allotransplantation: Experimental and clinical experience. *Ann. Surg.*, **172**, 405–436, 1970.
3) Lillehei, R.C., Ruix, J.O., Aquino, C. *et al.*, Transplantation of the pancreas. *Acta Endocrinol*, 83 Suppl 205, 303–318, 1976.
4) Connolly, J.E., Martin, D.C., Steinberg, T. *et al.*, Clinical experience with pancreaticoduodenal transplantation. *Arch. Surg.*, **106**, 489–494, 1973.
5) Gliedman, M.L., Gold, M., Whittaker, J. *et al.*, Clinical segmental pancreatic transplantation with ureter-pancreatic duct anastomosis for exocrine drainage. *Surgery*, **74**, 171–180, 1973.
6) Largiadèr, F., Uhlschmid, G., Binswanger, U. *et al.*, Pancreas rejection in combined pancreaticoduodenal and renal allotransplantation in man. *Transplantation*, **19**, 185–187, 1975.
7) Dubernard, J.M., Traeger, J., Neyra, P. *et al.*, A new method of preparation of segmental pancreatic grafts for transplantation: Trials in dogs and in man. *Surgery*, **84**, 633–639, 1978.
8) Sutherland, D.E.R., Baumgartner, D., Najarian, J.S., Free intraperitoneal drainage of segmental pancreas grafts: Clinical and experimental observations on technical aspects. *Transpl. Proc.*, **12** Suppl 2, 26–32, 1980.
9) Sutherland, D.E.R., International human pancreas and islet transplant registry. *Transpl. Proc.*, **12** Suppl 2, 229–236, 1980.
10) Sutherland, D.E.R., Goetz, F.C., Najarian, J.S., Review of world's experience with pancreas and islet transplantation and results of intraperitoneal segmental pancreas transplantation from related and cadaver donors at Minnesota. *Transpl. Proc.*, **13**, 291–297, 1981.
11) Sutherland, D.E.R., Current status of pancreas transplantation: Registry statistics and an overview. *Transpl. Proc.*, **15**, 1303–1307, 1983.
12) Dubernard, J.M., Traeger, J., Martin, X. *et al.*, Pancreatic transplantation in man: Surgical technique and complications. *Transpl. Proc.*, **12** Suppl 2, 40–43, 1980.

13) Pfeiffer, E.F., Land, W., Landgraf, R. *et al.*, Segmental pancreatic transplantation. International Workshop Munich Spitzingsee. *Hormon. & Met. Res.*, **13** Suppl, 1–104, 1983.
14) Markowitz, J., Technic of complete pancreatectomy in the dogs. Experimental Surgery. Williams and Wilkins, Baltimore, 351–357, 1959.
15) Sutherland, D.E.R., Pancreas transplantation: Overview and current status of cases reported to the registry through 1982. *Transpl. Proc.*, **15**, 2597–2601, 1983.
16) Sutherland, D.E.R., Pancreas and islet transplant registry statistics. *Transpl. Proc.*, **16**, 593–598, 1984.
17) Dubernard, J.M., Traeger, J., Pozza, G. *et al.*, Clinical experience with 31 pancreatic allografts in man. *Transpl. Proc.*, **15**, 1318–1321, 1983.
18) Brons, G. and Calne, R.Y., Transplantation of the pancreas in man. *Hormon. & Met. Res.*, **13** Suppl, 81–83, 1983.
19) Sutherland, D.E.R., Goetz, F.C., Elick, B.A. *et al.*, Experience with 49 segmental pancreas transplants in 45 diabetic patients: Results according to technique, donor source and immunosuppression. *Hormon. & Met. Res.*, **13** Suppl, 50–52, 1983.
20) Calne, R.Y., Recent advances in clinical transplantation of the liver and pancreas. *Transpl. Proc.*, **15**, 1263–1268, 1983.
21) Toledo-Pereyra, L.H., Pancreatic transplantation. *Surg. Gynecol. Obstet.*, **157**, 49–56, 1983.
22) Gebhardt, Ch., Clinical studies on duct occlusion with prolamine. *Hormon. & Met. Res.*, **13** Suppl, 56–58, 1983.
23) Gokel, J.M., Simultaneous pancreas and kidney transplantation in man – Graft morphology. *Hormon. & Met. Res.*, **13** Suppl, 76–77, 1983.
24) Calne, R.Y., White, D.J.G., Rolles, K. *et al.*, Renal and segmental pancreatic grafting with draining of exocrine secretion and initial continuous intravenous cyclosporin A in a patient with insulin-dependent diabetes and renal failure. *Br. Med. J.*, **285**, 677–680, 1982.
25) Sutherland, D.E.R., Goetz, F.C., Elick, B.A. *et al.*, Pancreas transplantation for diabetes: Clinical experience and metabolic studies in 54 recent cases at the University of Minnesota. *Transpl. Proc.*, **15**, 1322–1325, 1983.
26) Sutherland, D.E.R., Chinn, P.L., Goetz, F.C. *et al.*, Minnesota experience with 85 pancreas transplants between 1978 and 1983. *World J. Surg.*, 8, 244–252, 1984.
27) Sutherland, D.E.R., Chinn, P.L., Elick, B.A. *et al.*, Maximization of islet mass in pancreas grafts by near total or total whole organ excision without the duodenum from cadaver donors. *Transpl. Proc.*, **16**, 115–119, 1984.
28) Groth, C.G., Lundgren, G., Gunnarsson, R. *et al.*, Segmental pancreatic transplantation with special reference to the use of ileal exocrine diversion and to the hemodynamics of the graft. *Transpl. Proc.*, **12** Suppl 2, 62–67, 1980.
29) Groth, C.G., Lundgren, G., Klintmalm, G. *et al.*, Successful outcome of segmental human pancreatic transplantation with enteric exocrine diversion after modifications in technique. *Lancet*, ii, 522–524, 1982.
30) Calne, R.Y. and White, D.J.G., The use of cyclosporin A in clinical organ grafting. *Ann. Surg.*, **196**, 330–337, 1982.
31) Calne, R.Y., Paratopic segmental pancreas grafting: A technique with portal venous drainage. *Lancet*, i, 595–597, 1984.
32) Ota, K., Mori, S., Nobori, M. *et al.*, Allotransplantation of the pancreas in dogs. *J. Surg. Res.*, 7, 207–214, 1967.
33) Idezuki, Y., Feemster, J.A., Dietzman, R.H. *et al.*, Experimental pancreaticoduodenal preservation and transplantation. *Surg. Gynecol. Obstet.*, **126**, 1002–1014, 1968.
34) Lillehei, R.C., Simmons, R.L., Najarian, J.S. *et al.*, Pancreatico-duodenal allotransplantation: Experimental and clinical experience. *Ann. Surg.*, **172**, 405–436, 1970.
35) Craddock, G.N., Nordgren, S.R., Reznick, R.K. *et al.*, Small bowel transplantation in the dog using cyclosporine. *Transplantation*, **35**, 284–288, 1983.
36) Gebhardt, Ch., Stolte, P.O., Schwille, H. *et al.*, Experimental studies on pancreatic duct occlusion with prolamine. *Hormon. & Met. Res.*, **13** Suppl, 9–11, 1983.
37) Calne, R.Y., McMaster, P., Rolles, K. *et al.*, Technical observations in segmental pancreas allografting: Observations on pancreatic blood flow. *Transpl. Proc.*, **12** Suppl 2, 51–57, 1980.
38) DuToit, D.F., Reece-Smith, H., McShane, P. *et al.*, A successful technique of segmental pancreatic autotransplantation in the dog. *Transplantation*, **31**, 395–396, 1981.

39) McPhedran, N.T., Attisha, R.A., Ross, S.A. *et al.*, Pancreatic autotransplantation. *Transpl. Proc.*, **14**, 709–713, 1982.