Long-Term Exocrine Function of the Pancreas Transplant

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The development of transplantation of the pancreas has primarily been directed towards the alleviation of diabetes mellitus by supplying an endogenous source of insulin. The major thrust of the research effort has been to evaluate the endocrine responsiveness of the transplanted gland and to demonstrate that the abnormality in glucose metabolism can be corrected. Little is known about the long-term exocrine function of the transplanted pancreas. Pancreaticoduodenal transplants utilize the duodenum as a conduit for exocrine secretion and there has been some experience with direct anastomoses of the pancreatic duct. Despite attention to the technical problems of providing for exocrine drainage, there have been few studies of the secretory capacity and responsiveness of the transplanted pancreas. Moreover, the reported studies have involved few animals and have been complicated by the problems of rejection and immunosuppression associated with allografts.

Transplantation of the pancreas, thus far, has not been used to replace pancreatic insufficiency other than the insulin defect of diabetes mellitus. The ingestion of pancreas extracts has been used to control the clinical sequelae of exocrine pancreatic insufficiency. Steatorrhea can be controlled in the majority of patients, but fat absorption almost never returns to normal. Large doses of pancreas extracts are required, and some patients cannot be managed satisfactorily. Diabetes occurring after surgical removal of the pancreas and in destructive diseases of the pancreas similarly can be treated satisfactorily in most instances by insulin therapy; however, problems associated with insulin replacement are well recognized. The widespread clinical application of transplantation of the pancreas to these deficiency states has been restricted by the problems of immunologic rejection. If these could be resolved, transplantation of the pancreas might be considered for replacement of exocrine secretion as well as for endocrine function when there has been removal or destruction of the whole pancreas.

Interaction between neural and humoral mechanisms affecting the organs of the gastrointestinal tract has received considerable attention. It has been suggested that some of the symptoms of the postvagotomy syndrome can be attributed to pancreatic exocrine dysfunction. Experimental studies of the secretory response of the pancreas after vagotomy have provided conflicting results. The transplanted pancreas provides a unique physiologic model of a denervated gland, and the response of this preparation is independent of vagal and sympathetic activity.

In the present study, the exocrine function and morphologic characteristics of pancreaticoduodenal isografts were examined in detail for up to one year after transplantation. The secretory response of the graft was compared with the secretion of the host pancreas in a comparable control model. With the use of a strain of syngeneic rats, these studies were not complicated by rejection and the need for immunosuppression.

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Material and Methods

Pancreaticoduodenal isografts were performed in seventy-eight male Lewis rats (320 to 350 gm) using our standard microvascular surgical technic. The technical details of this procedure have been described previously [*1*] and are mentioned here only as they apply to the exocrine model.

Preparation of the Transplant Model. The donor pancreaticoduodenal graft was transplanted into the host by anastomosis of the graft aortic segment to the host abdominal aorta, and the graft portal vein to the host inferior vena cava. The proximal end of the graft duodenum was ligated, and the distal end was anastomosed end to side to the third portion of the host duodenum. At one, three, six, nine, and twelve months after transplantation the animals were reoperated on, and the graft was cannulated for collection of pancreatic secretions. At the time of cannulation, the anastomosis of the graft duodenum to the host was taken down, forming a blind graft duodenal pouch. (Figure 1.) This pouch was cannulated to the exterior with a short Silastic® tube brought through a stab wound in the flank.

Preparation of the Control Model. In the control model the pancreaticoduodenal isograft was performed in a similar manner; however, it differed in that gastrointestinal continuity was established through the graft duodenum. The host duodenum was ligated and divided at the pylorus, and the host pylorus was anastomosed end to end to the proximal end of the graft duodenum. The distal end of the graft duodenum was anastomosed end to side to the host jejunum. The host bile duct was divided and reimplanted into the transplant duodenum so that the pancreatic secretion of the host pancreas would not be contaminated with bile. The host model was studied one month postoperatively. At that time, the host duodenum was ligated to form a blind pouch of duodenum and pancreas and it was cannulated to the exterior. (Figure 2.)

Exocrine Secretory Studies. Continuous twenty-four hour collections of the pancreaticoduodenal graft (fifty-nine animals) and the host pancreas (nineteen animals) were performed daily for up to two weeks after cannulation. During the period of collection, the animals were maintained in Bollman cages on standard rat chow and water ad libitum. The rats received 25 ml of normal saline subcutaneously each day to replace the fistula loss. There were 495 twenty-four hour collections made (88 in control animals, 407 in transplants). Determinations of volume, protein, trypsin, amylase, pH, and electrolytes (sodium, potassium, chloride, and bicarbonate) were made on each collection.

On the third and fifth days after cannulation, stimulated pancreatic secretion was measured. After a four hour basal collection, pancreozymin (obtained from

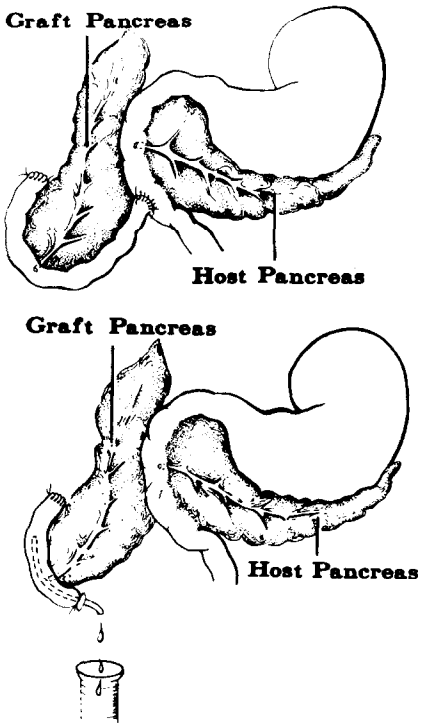

Figure 1. Pancreas transplant model. (Upper) Pancreaticoduodenal graft with the graft duodenum anastomosed to the host duodenum. (Lower) Cannulation of the graft duodenum for collection of graft pancreas secretions.

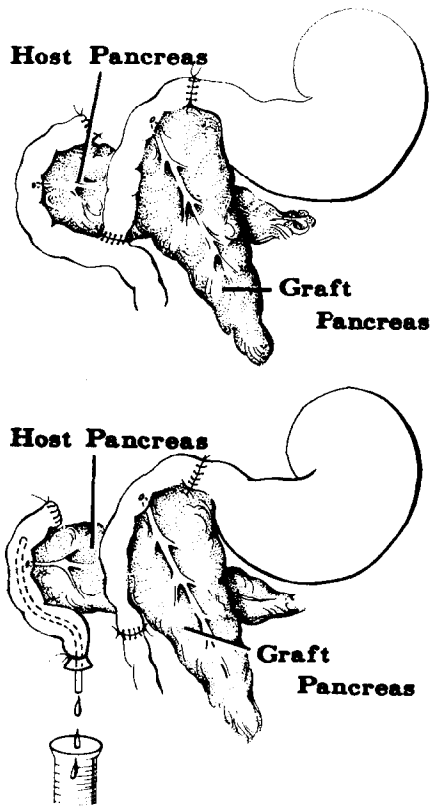

Figure 2. Host pancreas model. (Upper) Pancreaticoduodenal graft in position providing continuity of the gastrointestinal tract. (Lower) Cannulation of the host pancreas for collection of the host pancreas secretion.

TABLE I Twenty-Four Hour Exocrine Pancreas Secretion

	Host Pancreas	Graft Pancreas					
	1 month	1 month	3 months	6 months	9 months	12 months	Total
Number of observation	88	90	151	27	23	116	407
Volume (ml)*	17.0 ± 1.3	17.5 ± 0.8	16.3 ± 0.6	21.7 ± 2.1	13.3 ± 1.06	15.4 ± 0.6	16.5 ± 0.36†
Protein (mg/100 ml)	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 1.1	1.0 ± 0.1	1.9 ± 0.2	1.2 ± 0.1	1.1 ± 0.04†
Trypsin (ΔOD/min/L)	1,380 ± 120	2,852 ± 267	1,880 ± 173	1,772 ± 503	3,654 ± 429	5,900 ± 1,156	3,364 ± 363†
Amylase (Somogyi units)	89,000 ± 10,300	142,000 ± 16,842	91,900 ± 76,760	130,000 ± 16,000	195,000 ± 30,136	57,200 ± 5,600	110,000 ± 5,550†
pH	7.5 ± 0.1	8.1 ± 0.1	7.8 ± 0.1	7.8 ± 0.1	8.1 ± 0.0	7.9 ± 0.1	7.9 ± 0.04†
Sodium (mEq/L)	88.3 ± 3.7	78.9 ± 2.5	91.7 ± 2.4	61.4 ± 5.6	74.5 ± 7.5	86 ± 3.1	84 ± 1.5†
Potassium (mEq/L)	4.14 ± 0.63	3.0 ± 0.12	4.3 ± 0.14	2.4 ± 0.20	3.75 ± 0.52	3.6 ± 0.16	3.6 ± 0.08†
Chloride (mEq/L)	70 ± 3.6	75 ± 2.7	82 ± 2.5	31 ± 3.7	64 ± 6.7	68 ± 28.	72 ± 1.5†
Bicarbonate (mEq/L)	11.4 ± 1.4	10.2 ± 1.0	15.0 ± 0.8	5.9 ± 1.8	5.1 ± 1.5	9.8 ± 1.0	11.3 ± 0.51†

* Mean ± standard error of mean.

† Not statistically different from host pancreas.

Sigma Chemical Co., St. Louis, Missouri, known to be contaminated up to 10 per cent with secretin), in a dose of 10 Crick units in 0.5 ml of saline, was injected intravenously every thirty minutes for six doses. Stimulated secretion was collected for three hours. Determinations of volume, protein, trypsin, amylase, pH, and electrolytes were made on the basal and stimulated samples.

Representative animals were sacrificed at one, three, six, nine, and twelve months postoperatively for histologic examination of the graft.

Results

Over a twenty-four hour period, the host pancreas secreted a mean of 17.0 ± 1.3 (standard error of mean) ml of pancreatic juice that was alkaline in pH and contained 1.1 ± 0.1 mg per cent of protein. The mean concentrations of electrolytes were: sodium 88.3 ± 3.7 mEq/L, potassium 4.1 ± 0.6 mEq/L, chloride 70 ± 3.6 mEq/L, and bicarbonate 11.4 ± 1.4 mEq/L. The trypsin content of the juice averaged 1,380 ± 120 ΔOD (change in optical density)/min/L, and the mean amylase content was 89,000 ± 10,300 Somogyi units. (Table I.) The electrolyte composition appeared to be independent of the volume of secretion. Bicarbonate levels varied considerably but did not correlate with fluctuations in pH or chloride concentration.

The mean twenty-four hour secretory volume for the transplanted pancreas varied over a range of 13 to 22 ml, with an average secretion of 16.5 ± 0.36 ml. This was not significantly different from that of the host pancreas. The pH was alkaline, and the protein content was similar to that of the host pancreas secretion. The average concentrations of electrolytes in the graft secretion were: sodium 84 ± 1.5 mEq/L, potassium 3.6 ± 0.08 mEq/L, chloride 72 ± 1.5 mEq/L, and bicarbonate 11.3 ± 0.51 mEq/L. As was seen in the host, the greatest variation occurred in the bicarbonate concentration. These fluctuations could not be correlated with changes in volume, pH, or chloride concentrations. The concentration of enzymes in the graft pancreas secretion over a twenty-four hour period varied considerably between the animals within each study period, and the groups differed from each other; however, no distinct pattern of change could be identified. The mean twenty-four hour amylase output was 110,000 ± 5,550 Somogyi units for the graft pancreas and this was not significantly different from that of the host pancreas. The mean trypsin concentration (3,364 ± 363 ΔOD/min/L) of the graft pancreas twenty-four hour secretion was significantly higher ($p < 0.01$) than that of the host pancreas secretion. Examining the groups individually revealed higher trypsin levels at nine and twelve months after transplantation. This increase was not observed for amylase and appeared to be independent of the other constituents of the pancreatic juice.

In general, the twenty-four hour volume, pH, protein content, electrolyte composition, and concentration of enzymes in the exocrine secretion of a pancreas transplant were similar to those of the host pancreas. The pancreas grafts demonstrated normal exocrine function for up to one year after transplantation.

Seven pancreozymin stimulation studies were carried out on the host pancreas in five of the control animals. During the three hour test period the rate of secretion increased 27 per cent over basal from a mean of 0.62 to 0.79 ml/hr. There was no change in the protein content, the pH remained al-

TABLE II Pancreozymin-Stimulated Exocrine Secretion

		Host Pancreas	Graft Pancreas				
		1 month	3 months	6 months	9 months	12 months	Total
n		7	18	3	1	25	47
Volume	Basal*	.62	.51	.41	.54	.58	.55
(ml/hr)	Stimulated*	.79	.89	.74	.90	.99	.93
	% Change	27	75	80	67	71	69†
Protein	Basal	2.2	1.2	2.0	.8	1.8	1.6
(mg/100 ml)	Stimulated	2.2	1.7	2.6	1.8	2.0	1.8
	% Change	0	42	30	125	11	12
Trypsin	Basal	1,630	1,453	2,500	1,400	1,543	1,590
(ΔOD/min/L)	Stimulated	2,190	2,134	3,600	3,680	2,405	2,150
	% Change	34	47	44	162	56	35†
Amylase	Basal	174,000	216,000	240,000	138,000	224,000	221,000
(Somogyi units)	Stimulated	261,000	424,000	300,000	514,000	278,000	332,000
	% Change	50	96	25	272	24	50†
pH	Basal	7.7	8.4	7.5	8.7	7.8	8.0
	Stimulated	7.8	8.4	7.7	8.3	7.9	8.1†
Sodium	Basal	115	116	126	95	129	124
(mEq/L)	Stimulated	125	119	150	124	127	126
	% Change	9	3	19	31	2	2†
Potassium	Basal	5.3	6.0	4.4	5.8	5.2	5.4
(mEq/L)	Stimulated	5.8	6.4	4.3	6.8	5.2	5.6
	% Change	9	7	−2	17	0	4†
Chloride	Basal	127	118	96	147	107	113
(mEq/L)	Stimulated	145	117	98	125	109	111
	% Change	14	−1	2	−15	2	−2†
Bicarbonate	Basal	4.6	18.5	0.7	9.0	2.9	9.0
(mEq/L)	Stimulated	4.5	16.6	1.7	11.2	3.8	9.9
	% Change	2	−10	143	24	31	10†

* Mean value.

† Indicates that the % changes are not statistically different between graft pancreas and host pancreas.

kaline, and only small fluctuations occurred in the electrolyte composition of the juice. The mean trypsin concentration increased 34 per cent from a basal level of 1,630 to 2,190 trypsin units after stimulation. Similarly, the amylase concentration increased 50 per cent from a mean basal level of 174,000 Somogyi units to a stimulated level of 261,000 Somogyi units. (Table II.) Pancreozymin stimulation of the graft pancreas caused an increase in an enzyme-rich exocrine secretion that was comparable to the response of the host pancreas. Forty-seven stimulated secretion studies were carried out in twenty-seven animals. The volume of secretion increased a mean of 69 per cent. The variation in volume response between animals studied at three, six, nine, and twelve months was small with a range of 67 to 80 per cent. The protein concentration of the juice increased a mean of 12 per cent, and this increase was significantly different from the response of the host pancreas. Small fluctuations were seen in the electrolyte composition of the juice after stimulation; however, there was not a consistent pattern of change, and the changes were not significantly different from those seen in the control host pancreas. Trypsin concentration increased 35 per cent from a mean basal of 1,590 to 2,150 units, and this change was statistically similar to the increase observed for the host pancreas. Amylase concentration increased 50 per cent after stimulation from 221,000 to 332,000 Somogyi units, and this increase was also statistically comparable to the increase seen in the host pancreas. (Table II, Figure 3.)

The gross morphologic characteristics of the transplanted pancreas and duodenum appeared normal throughout the study period. Occasionally small organized abscesses or suture granulomas could be identified in the operated area. The transplanted gland usually became enveloped in the intra-abdominal fat. Microscopic examination of the graft and host pancreas revealed essentially normal pancreas. At one month post transplantation mild inflammatory cell infiltrates and a few dilated ducts were seen, suggestive of pancreatitis secondary to the transplant procedure. These changes resolved, and normal acini and duct struc-

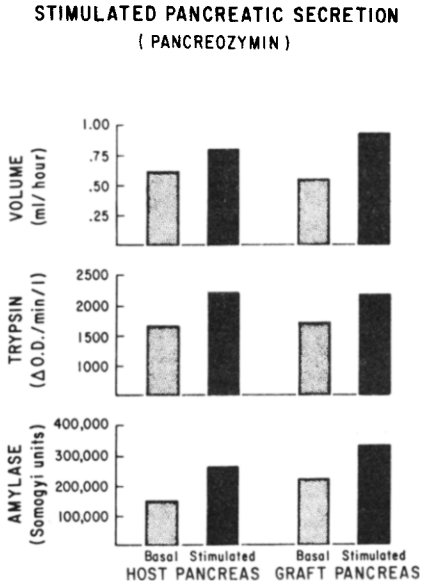

Figure 3. Stimulated pancreatic secretion. Compares the increase in the mean volume, amylase concentration, and trypsin concentration between the host pancreas and transplanted pancreas after stimulation with pancreozymin.

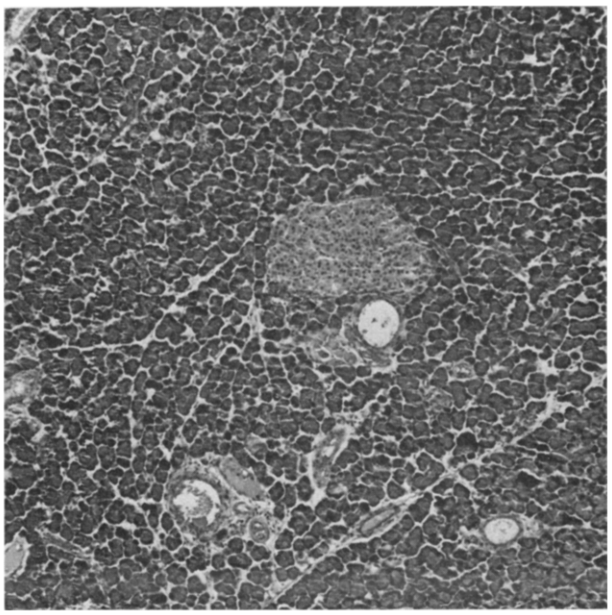

Figure 4. Graft pancreas one year after transplantation (hematoxylin and eosin stain; original magnification × 40). Demonstrates normal ducts and acinar structure without evidence of rejection, pancreatitis, or duct obstruction.

ture were found in the later study periods up to one year after transplantation. There was no evidence of rejection, duct obstruction, or pancreatitis. (Figure 4.)

Comments

The exocrine function of the pancreas has been studied in detail in large animals and in man but not in rats. The pancreaticoduodenal transplant in our host control model allowed us to measure the exocrine secretion of the pancreas in an awake, healthy animal over a prolonged period, thereby avoiding the problems of an acute fistula preparation. Moreover, the intact gastrointestinal tract permitted the normal release of endogenous hormones that affect pancreatic secretion. The host pancreas secretory studies reported herein document the normal exocrine secretion and response to pancreozymin of the rat pancreas.

The endocrine function of the transplanted pancreas has been studied in detail; however, there have been only a few reports of the exocrine function of pancreas grafts [2–6]. Previous studies have been performed on allografts in the dog and have been complicated by problems of immunosuppression and rejection. Therefore, they have been confined to studies in the immediate postoperative period. Seddon and Howard [2] demonstrated a high basal secretion and a diminished response to hormonal stimulation. Himal, Goodhead, and MacLean [4] also noted an increased secretion in the early postoperative period, but this was transient and exocrine function rapidly disappeared. Asikari and Drieling [5] noted normal twenty-four hour secretion after transplantation but a diminished secretory response to secretin, which they attributed to the development of pancreatitis, and they questioned the usefulness of pancreas transplants for exocrine replacement. Studying the autotransplanted pancreas, Ruiz and colleagues [6] observed early exocrine dysfunction manifested by diarrhea, weight loss, and abnormal results of fecal fat studies. These symptoms resolved, and biopsies at six months showed normal acinar tissue without fibrous replacement. Detailed studies of pancreatic autografts or allografts have been lacking.

Our results indicate that the exocrine function of the transplanted pancreas is maintained in the absence of rejection. The period of these studies in the rat (up to one year) represents half the expected life span of the animal. The twenty-four hour volume, electrolyte composition, and enzyme concentration of the exocrine secretion from the transplanted gland were similar to those from the host pancreas in a comparable control model. The high basal secretion noted in other studies [2,4]

may have been related to pancreatitis secondary to the operative procedure, which was not seen in these long-term studies. The histopathologic signs of acinar destruction seen in the allograft models [7] were not present in the rat isografts for up to one year post transplantation, documenting the absence of rejection in these syngeneic animals.

The clinical application of transplantation of the pancreas has been to treat severe diabetes mellitus, and it has not been used to manage other pancreatic endocrine or exocrine insufficiency states, such as occur in chronic pancreatitis and after pancreatectomy. Acinar insufficiency manifested by steatorrhea, malabsorption, and weight loss has been reported to occur in 22 [8] to 48 per cent [9] of patients after pancreaticoduodenectomy. Some authors [10] have minimized the problems of pancreatic extract therapy whereas others [11–13] have found such therapy inadequate. Marks, Bank, and Airth [13] studied pancreatic steatorrhea in eleven patients and noted marked improvement in eight with pancreatic extract replacement. However, stool fat levels returned to normal in only one of their patients, indicating abnormal fat absorption in the others despite replacement therapy. Considerable differences in the effectiveness of various commercial preparations have been demonstrated [14], and the usual recommended dosages may not be effective. Many patients require large quantities of pancreatic extract to maintain weight and prevent diarrhea, and optimal caloric absorption cannot be achieved in all patients. Six long-term survivors of pancreaticoduodenectomy were studied by Fish, Smith, and Williams [11], and they noted that half were unable to regain their weight. All had an increased number of stools (three to twelve per day), and five had abnormal fecal fat. The findings in the present study indicate that in the *absence of rejection* the long-term function of the pancreas transplant is normal, and it is capable of responding to hormonal stimulation. This would suggest that the pancreas transplant might be considered for replacement of exocrine function as well as endocrine function when the present problems of rejection can be resolved.

The pancreas is innervated by vagal fibers that synapse in the intrinsic ganglia of the pancreas with the postganglionic unmyelinated fibers that continue to the acinar and islet cells and smooth muscle cells of the ducts. Sympathetic innervation is through the splanchnic nerves and celiac ganglion. Section of the splanchnic nerves causes an increase in pancreatic secretion [15] whereas vagotomy causes a decrease [16,17]. The diarrhea and steatorrhea that occur after truncal vagotomy have been attributed to pancreatic insufficiency, and the proponents of superselective vagotomy propose that preservation of the vagal innervation of the pancreas will decrease the incidence of these complications.

The vagal control of pancreatic secretion involves a direct effect on the acinar tissue as well as an interaction with humoral stimuli. Studies of the effect of vagotomy on the pancreatic response to exogenously administered intestinal hormones have been conflicting. Recent studies [17] have indicated that the response to endogenous release of secretin and pancreozymin is decreased whereas the response to exogenously administered hormones is unchanged. The transplanted pancreas is a unique model of a denervated gland that is independent of autonomic nervous influences. Moreover, the pancreaticoduodenal transplant reflects only the effect of denervation of the pancreas in that it is still influenced by the humoral factors from the innervated host gastrointestinal tract. We observed that secretion from the transplant was more constant than that from the host pancreas. The normal response of the graft pancreas to exogenous pancreozymin, when compared with the response of the innervated host pancreas, confirms the observation of Konturek, Becker, and Thompson [17] on the effect of truncal vagotomy on the response to exogenous hormones. We did not observe a decrease in the basal twenty-four hour secretion of the denervated graft pancreas. This may indicate some compensatory effect of splanchnic sympathetic denervation. More likely, it indicates that the pure vagal influence on the acinar function is not great and that vagotomy primarily affects the humoral mechanisms regulating pancreatic secretion rather than the gland itself. This finding lends further support to the role of the vagus in the release of gastrointestinal hormones.

Summary

The long-term exocrine function of fifty-nine pancreaticoduodenal isografts was evaluated in rats for up to one year post transplantation. At one, three, six, nine, and twelve months after transplantation the grafts were cannulated and the exocrine secretion was collected. The volume, protein content, pH, amylase and trypsin concentrations, and electrolyte composition of the secretion were compared with those obtained from the host pancreas in nineteen control rats. Twenty-four

hour secretion studies demonstrated normal basal function of the pancreas transplant when compared with that of the host. Pancreozymin stimulation caused an increase in volume, trypsin concentration, and amylase concentration of the graft pancreas secretion that was similar to those seen in the host. These studies indicate that there is normal exocrine secretion of pancreas transplants in the absence of rejection and that denervation of the gland has little direct effect on over-all pancreatic function.

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