MEDICAL GRAND ROUNDS

THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER

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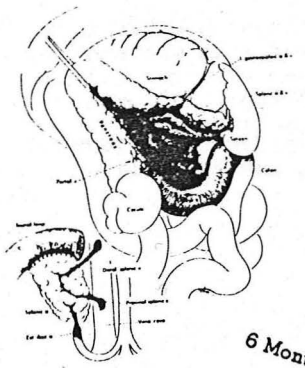
"NEWER METHODS OF BLOOD GLUCOSE REGULATION"

PHILIP RASKIN, M.D.

managing diabetes

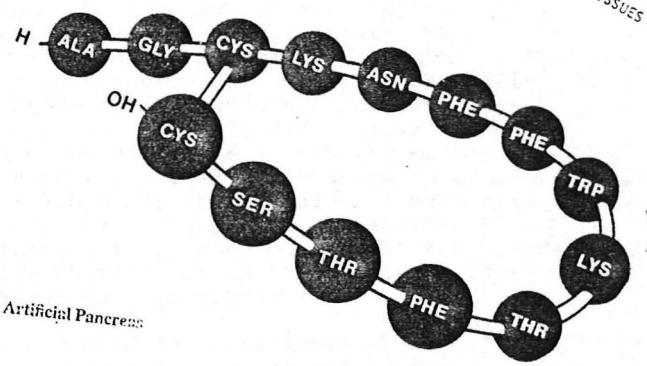
Immunosuppression

Pancreatic Transplantation

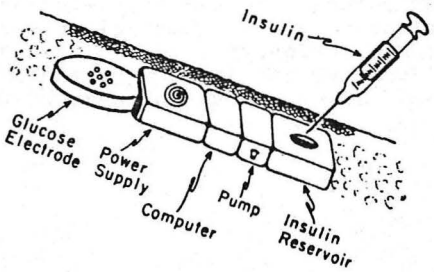


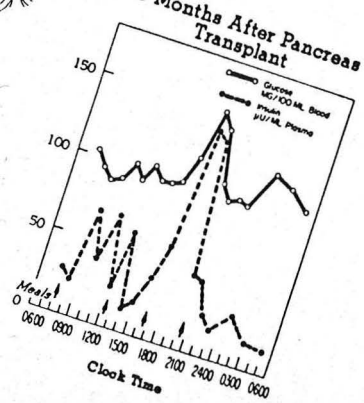
somatostatin

ACTIONS OF SOMATOSTATIN UPON ENDOCRINE TISSUES

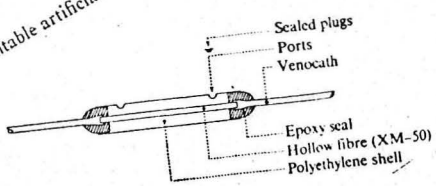


Artificial Pancreas





Implantable artificial endocrine pancreas



INTRODUCTION

Last year at these proceedings I discussed the important issue of whether or not the microvascular complications of diabetes mellitus were related in any type of cause and effect way to the metabolic abnormalities of the disease. My conclusions at that time were, that although there was an ever increasing body of data in experimental diabetes suggesting that hyperglycemia and microvascular disease are in some way related, there was little convincing clinical evidence for the view that rigid control of the metabolic abnormalities would prevent the vascular complications of the disease. However, there was also no overwhelming evidence to the contrary, i.e., that the microvascular complications of diabetes mellitus are independent of the hyperglycemia.[1,2] My reasons for this conclusion were primarily based on the fact that, with the present modalities of treatment for diabetes mellitus, i.e., diet management and intermittent subcutaneous insulin therapy, it is impossible to completely restore glucose homeostasis to normal.[3]

Today's discussion will be directed to those areas which offer promise as alternatives or adjuncts to current modalities for the treatment of the hyperglycemia of diabetes mellitus. Specific areas to be covered include: whole or partial pancreatic transplantation; isolated islet cell transplantation; artificial beta cell devices; and finally, some of our own work on the utility of the glucagon suppressing agent, somatostatin.

WHOLE PANCREAS TRANSPLANTATION

Transplantation of the pancreas was first accomplished as early as the 1920's. Viable endocrine function of whole pancreas transplants was first successfully shown by Gayet and Guillemie[4] and Houssay[5] who demonstrated that an allotransplanted pancreas with vascular anastomosis over cannulae could lower the blood glucose levels in diabetic dogs for periods of up to 12 hours. Over the years the many difficulties with this procedure are apparent from the variety of techniques that have been attempted in order to accomplish successful pancreas transplantation. In addition to the multiple problems associated with immunologic graft rejection, common to transplantation of any organ, transplantation of the pancreas has presented additional special problems. Vascular thrombosis, pancreatitis, and digestion of host tissue by exocrine pancreatic enzymes may all occur. Except for graft rejection, and associated immunological difficulties, the discussion of which is beyond the scope of this presentation, the major problems associated with attempts to transplant the pancreas are related to the exocrine secretory function of the transplanted pancreas. This has resulted in the development of several different techniques of pancreatic transplantation (total or segmental). These can be divided into those methods that establish drainage of exocrine secretions and those that do not.

The methods which provide for drainage of pancreatic exocrine secretion include pancreatic duodenal transplantation, segmental pancreatic transplantation with ureter to pancreas, direct anastamosis or anastamosis into a retroperitoneal jejunal loop. Transplantation of a pancreatic duct ligated pancreas is the only example of transplantation that does not provide for exocrine drainage.

Pancreatic Transplantation (Duct-Ligated Pancreas)

Many of the problems arising from pancreatic transplantation are those resulting from continued secretion of pancreatic exocrine enzymes. Transplantation of a pancreatic duct-ligated pancreas might provide a theoretical advantage of inducing atrophy of pancreatic exocrine tissue while presumably leaving the endocrine tissue intact. However, this does not seem to be the case in practice as transplantation of pancreatic duct-ligated pancreata results in acute and chronic inflammatory reactions involving the entire pancreas including the islets of Langerhans.[6]

Reemstsma, et al[7] described a technique for transplantation of a duct-ligated segment of pancreas (body and tail) into the groin of a dog. The results were poor with on 14 of 34 animals showing graft function. Those grafts that failed did so due to vascular thrombosis. Pemberton and Manax[8] attempted transplantation of duct-ligated segmental grafts into the neck of alloxan diabetic dogs. Failure rates were high due to vascular thrombosis. Merkel,

et al[9] reported beneficial effects of pre-treatment of the donor pancreas with 500 rads of x-ray irradiation three to seven days prior to transplantation on the subsequent development of vascular thrombosis. Following transplantation of the distal segment of the irradiated gland there was not a single case of thrombosis. Other efforts used to influence the fate of duct-ligated pancreatic transplants include the use of 5-Fluorouracil to the recipient.[10] Kyriakides, et al prevented the accumulation of amylase-rich fluid around pancreatic duct-ligated segmental pancreatic transplants placed in the neck of pigs and the administration of glucagon, which inhibited pancreatic exocrine function. Pancreatic fibrosis was inhibited by large doses of methylprednisolone to the transplant recipients.[11,12]

In general, one might state that transplantation of a portion of a duct-ligated pancreas has been less than satisfactory in most experimental animal models. At present it does not seem that ligation of the pancreatic duct prior to transplantation results in the desired effect, i.e., atrophy of pancreatic exocrine function with maintenance of adequate endocrine function.

Transplantation of the Pancreas with Provision for Pancreatic Exocrine Drainage

Pancreatic-duodenal transplantation

Lillehei and his colleagues at the University of Minnesota have described a method for transplantation of the whole pancreas including transplantation of a portion of the duodenum. This technique assures that in this way the pancreatic duct will remain intact and the portion of the duodenum that is also transplanted is anastamosed to the recipient bowel providing drainage for the exocrine secretions[13,14] (Figure 1).

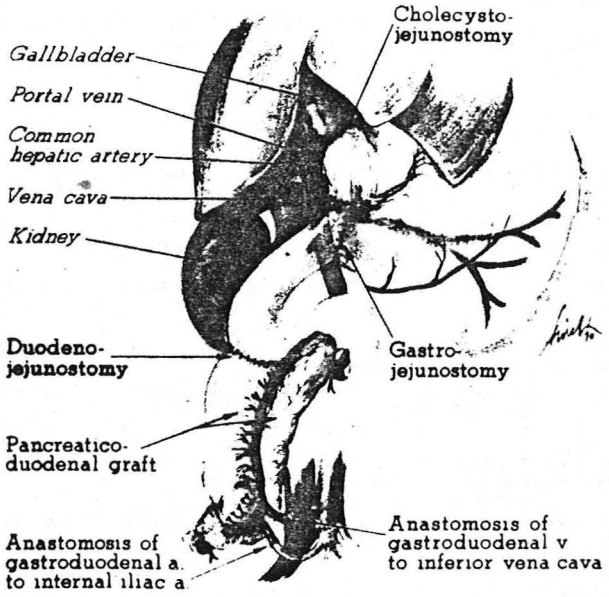


Fig.1. Technique used for pancreaticoduodenal allografts.[14]

In dogs it was possible to cause normoglycemia in pancreaticoduodenal allotransplanted pancreatectomized dogs until the death of the dogs. In most instances the dogs died because of rejection of the graft. The use of immunosuppressive drugs in such experiments has resulted in long term survival (> 1 year) in dogs receiving pancreaticoduodenal autografts.[15] In this study pancreatic grafts functioned immediately in the recipient if the surgical procedure was technically successful. When rejection of the pancreaticoduodenal graft did occur, it was often due to rejection of the attached duodenum rather than rejection of the pancreas.

Since 1966, this group has done several such transplantations in

man. Table 1 shows the experience of these 12 patients who received combined pancreaticoduodenal and renal allotransplantation.

Patient	Sex	Age	Tissue Type®	Drainage of P-D	Survival (months)	Graft Function (+ to ++++)* R	P-D	Cause of death	Comment and/or Autopsy
R.R.	F	32	–	Duodenostomy	4½	1+	3+	Sepsis	Chronic R rejection P-D normal
J.R.	M	37	–	Duodenostomy	1	0	0	Sepsis	Probable lethal ischemia of R and P-D in donor
A.Z.	F	44	–	Duodenostomy	1	– (only P-D)	4+	Sepsis	P-D normal septicemia from hemodialysis
D.O.	F	31	D	Duodenostomy	5½	1+	4+	Acute hyper-kalemia	Chronic R rejection P-D normal; ALG
G.M.	M	34	B	Roux-Y	12	3+	4+		
C.B.	M	34	B	Roux-Y	7	2-3+	4+	acute perforation Graft duodenum with sepsis	Minor bouts of R rejection; ALG
N.T.**	F	28	B	Roux-Y	1 week	0	4+	Sepsis secondary to ATN – ??	Thrombus in portal V of P-D; no histological signs of rejection in R
M.P.	F	34	C	Roux-Y	1	4+	4+	Acute necrosis R and duodenum P-D	Contaminated ALG immediately preceded death
R.K.	M	30	D	Roux-Y	5	0-2+	4+		
I.O.	M	26	C	Roux-Y	1	0-3+	4+	Sepsis	ATN-due to urinary tract infection and/or rejection; ALG

Table I. Combined Pancreatico-Duodenal (P-D) and Renal (R) Allotransplantation for Juvenile Onset Diabetes Mellitus with Terminal Renal Failure. [14]

All were juvenile onset diabetics with terminal renal failure. The source of the kidney and pancreas-duodenum organs were cadaver donors of suitable blood type. The most notable success in these experiments is shown in Figure 2.

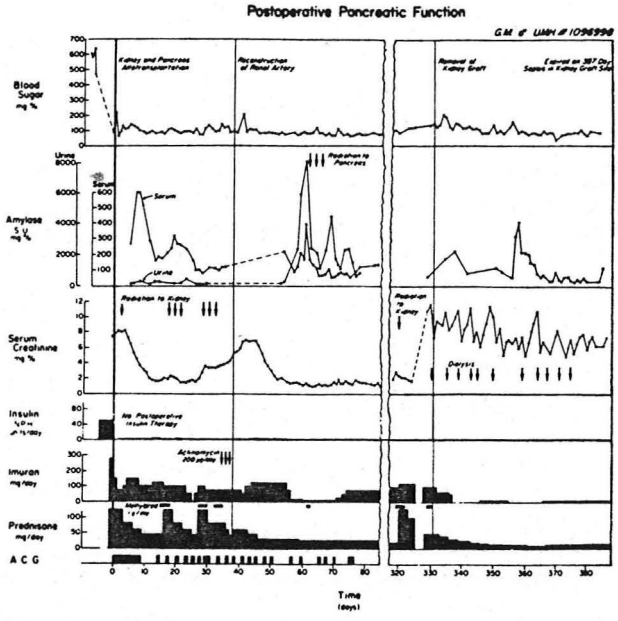


Fig.2.: Clinical course of G.M. who survived for 12 months with renal and pancreaticoduodenal allografts. Death occurred secondary to renal rejection when the patient stopped taking immunosuppressive drugs. Pancreatic allograft function remained normal until death occurred. [14]

This 34 year old man with diabetes of 25 years duration survived over 12 months with his pancreaticoduodenal allograft. Plasma glucose levels remained within the normal range without exogenous insulin and he had a normal glucose tolerance test (Figure 3), and a normal response to meals (Figure 4).

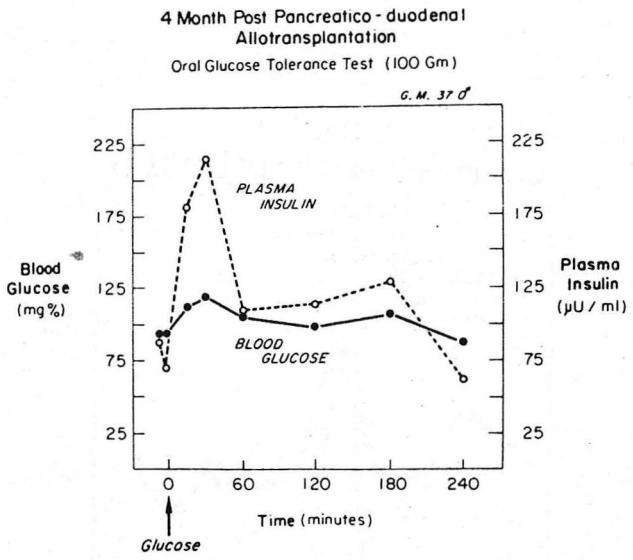


Fig. 3: Oral glucose tolerance curve in G.M. following pancreaticoduodenal allotransplantation. Note biphasic insulin secretory response.[14]

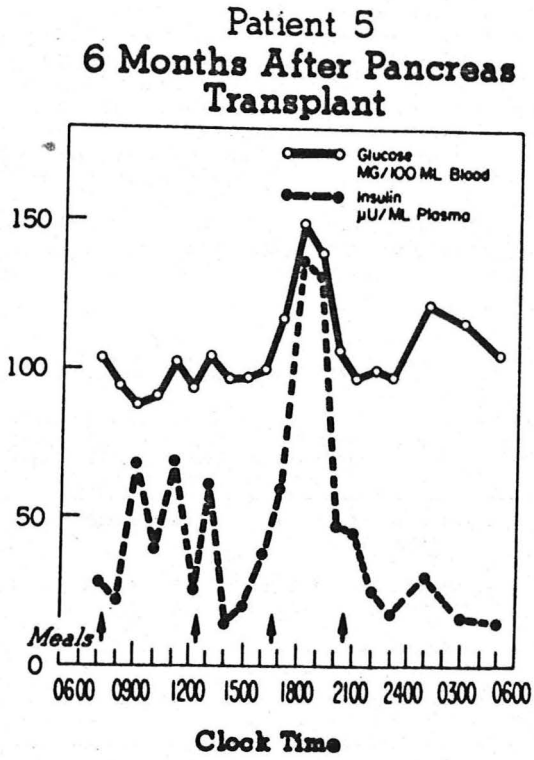


Fig. 4.: Response of glucose and insulin in G.M. to feedings. Arrows incidate meals. The 24-hour glucose profile is normal and there are insulin secretory bursts in response to meals. [14]

This patient eventually succumbed to complications associated with rejection of his transplanted kidney. In the other cases of combined pancreaticoduodenal and renal transplantation the cause of the patients' eventual demise seemed related to the renal transplantation in all but one instance. In that patient death occurred from an acute perforation of the duodenal graft. Rejection of the pancreas was not a problem, in fact, a clear cut histological picture of rejection was not seen in any of the transplanted pancreata leading these workers to suggest that the pancreas might enjoy some degree of immunologic privilege; a thought that has not held up.

Table II shows the results of pancreaticoduodenal allotransplantation in three other juvenile diabetic patients without terminal renal failure.

Patient	Sex	Age	Tissue Type	Drainage of P-D	Graft Survival (weeks)	Graft Function (+ to ++++)*		Status	Comment
C.G.*	F	19	C	Roux-Y	1	only P-D	4+	Alive after removal of P-D graft	Acute duodenal rejection. The pt. is back on insulin.
L.R.*	F	28	C	Roux-Y	12	only P-D	4+	Alive after removal of P-D graft	Arterial and venous thrombosis. The pt. is back on insulin.
R.E.**	F	24		Roux-Y	4	only Pancreas	4+	Alive after removal of pancreas graft	Clear-cut microscopic rejection of pancreatic allograft The pt. is back on insulin.

Table II: Pancreatico-duodenal Allografts in patients with Juvenile-onset Diabetes Mellitus Without Terminal Renal Failure.[14]

The first experiment failed because of an acute duodenal rejection. The second graft functioned well for 21 weeks resulting in the discontinuation of insulin therapy. The graft was removed following acute arterial and venous thrombosis. The third patient did well for 24 days when the transplanted pancreas was rejected and the graft had to be removed.

Segmental pancreatic transplantation with ureter to pancreatic duct anastamosis.

Several techniques have been developed which allow for establishment of exocrine drainage of the pancreas without transplantation of the duodenum. Gold, et al[16] transplanted the distal pancreas in dogs with anastomosis of the main pancreatic duct to the recipient's ureter to provide exocrine drainage. The canine ureter and a bladder mucosa tolerated the presence of the digestive enzymes without ill effects. Gliedman, et al[17] extended this work to man. They described pancreatic transplantation with ureter to pancreatic duct anastomosis performed in combination with synchronous (2 patients) and asynchronous (3 patients) renal transplantation. Two of these patients (asynchronous transplantation) did very well, one surviving for more than four years with a functional pancreatic transplantation with normoglycemia maintained without exogenous insulin therapy (Figures 5 and 6).[18]

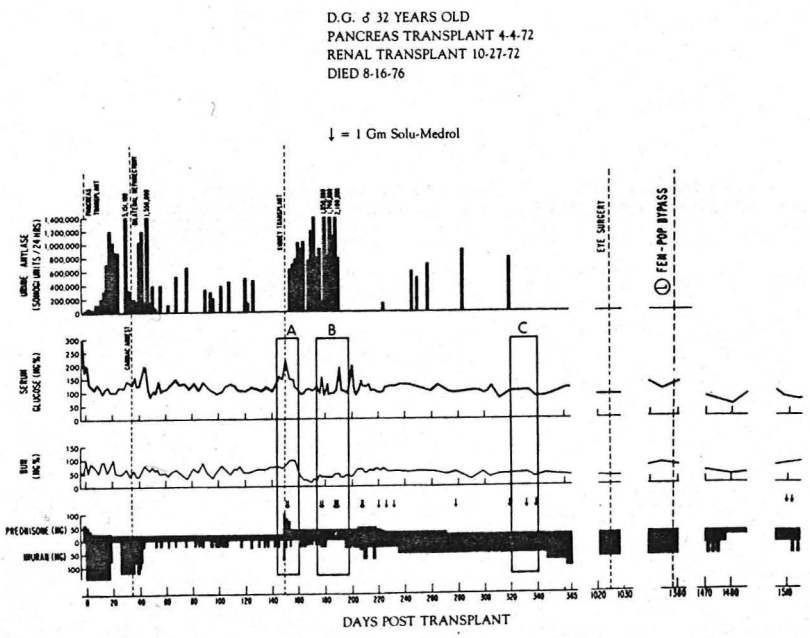


Fig. 5.: Graphic representation of the 4 year clinical course of patient D.G. following pancreatic transplantation.[18]

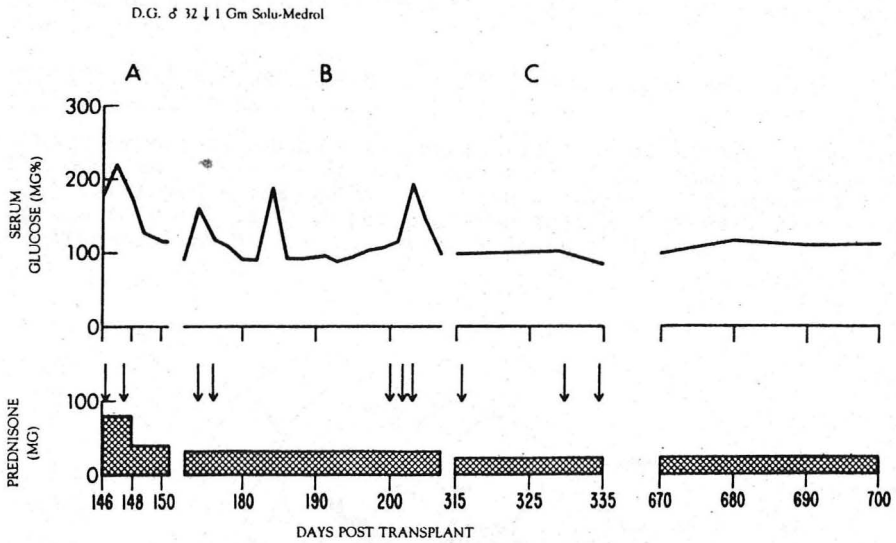


Fig. 6.: Large-scale representation of areas A, B., and C, from Fig. 5 showing the relationship between serum glucose concentration and prednisone dose.[18]

This patient eventually died on August 16, 1976 due to a combination of sepsis and terminal renal failure; however, insulin had not been administered from the time of the pancreatic transplant until his death.

Pancreatic transplantation with anastomosis into a retroperitoneal jejunal loop.

A most important advance in pancreatic transplantation technique has been made by a member of our Surgery Department. Dickerman, et al[19] developed a new operation which allows for transplantation of the body and tail of the pancreas into a Roux-en-Y retroperitoneal limb of the recipient's jejunum (Figure 7).

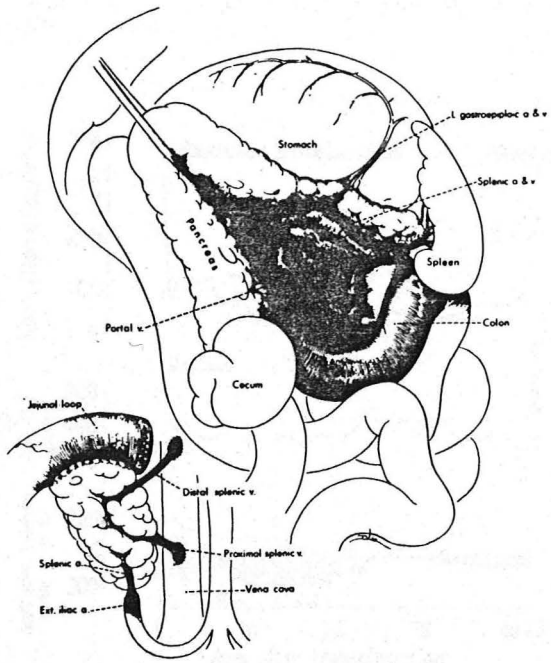


Fig. 7.: Retroperitoneal Roux-en-Y pancreatic transplantation Donor pancreatectomy is depicted at the right. The site of transection of the pancreas is indicated with a dotted line. Transplantation of the body and tail of the pancrease is depicted below. Both ends of the splenic vein are anastomosed to the vena cava to facilitate venous drainage. [19]

The use of double venous anastomosis of the spleenic vein to the inferior vena cava resulted in elimination of the problem of venous thrombosis, a major complication when other techniques have been used. Normoglycemia was maintained for up to 85 days in alloxan diabetic dogs undergoing pancreatic transplantation by this method. This procedure has several advantages over previously described methods; (1) it provides for pancreatic exocrine drainage into the recipient's gastrointestinal tract, (2) construction of a long limb of jejunum used for the pancreaticojejunostomy will prevent enteric fistula formation should rejection occur, (3) the retroperitoneal position of the graft facilitates its removal should rejection, infection, or hemorrhage occur, and (4) the donor bowel is not entered during donor pancreatectomy and contamination is reduced. The procedure has been utilized by Swedish workers[20], who treated four juvenile onset diabetics without uremia with segmental pancreatic transplantation. Three of the four grafts functioned well with resulting relative normoglycemia (Figure 8) in the absence of exogenous insulin administration.

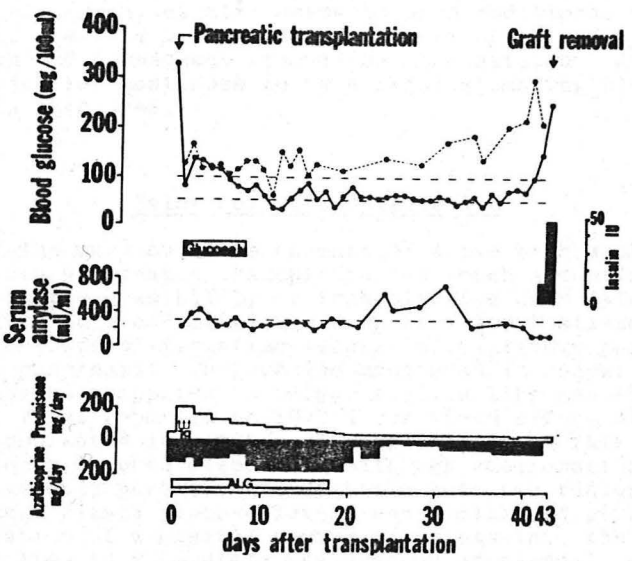


Fig. 8: Postoperative course in a patient following retroperitoneal Roux-en-Y pancreatic transplantation. The solid line_____, denotes glucose and the dotted line ....., postprandial blood glucose. [20]

The administration of an intravenous glucose load resulted in a prompt increase in plasma C-peptide levels. Unfortunately, all of these grafts had to be removed within six weeks because of irreversible rejection.

Dr. Dickerman has attempted this operation in a 21 year old juvenile onset diabetic who had a prior renal transplantation for chronic renal failure secondary to his diabetic nephropathy and who was blind from his proliferative diabetic retinopathy. Unfortunately, this experiment ended tragically when the patient suffered multiple small infarcts in his jejunal Roux-en-Y limb of bowel. However, the graft functioned well for over 72 hours with normoglycemia being present in the absence of exogenous insulin despite the administration of intravenous glucose.

To summarize, clinical pancreatic transplantation has been totally unsuccessful to date. Of the 51 transplantations attempted, none are surviving, Dr. Gliedman's patient having done the best with a four year survival post-pancreatic transplantation.[17] Certainly the techniques which have shown the most potential are those which utilized segmental pancreatic transplantation with some provision for drainage of the exocrine pancreas. The method of Dickerman, et al[19] seems to have additional benefit in that the graft is placed in a retroperitoneal and thus accessible position should subsequent removal be necessitated. Acute and chronic rejection continues to be a major stumbling block to advances in this area.

Islet Cell Transplantation

Because of the many problems enumerated above with whole or partial organ pancreatic transplantation, much attention has been paid to the possibility of transplanting only islet cell tissue. This idea became viable when Lacy and Kostianovsky [21] developed methods of isolating islets in relatively pure form from adult pancreata. The species most used is rodent but these methods have been applied to larger species like man.[22] Ballinger and Lacy[23] first reported in 1972 a sustained effect of isologous islets transplanted into the peritoneal cavity of rats with streptozotocin induced diabetes. Although amelioration of the diabetes was only partial, the diabetic rats had restored weight gain, reduced plasma glucose levels and diminished glycosuria. Transplantation of a greater number of islets into the peritoneal cavity resulted in a complete restoration of normoglycemia in diabetic rats.[24] Further improvement in technique occurred when it was shown that embolization of the islet cell tissue into the

portal vein of the recipient was much more effective in ameliorating diabetes than was giving the islets intraperitoneally.[25 26] It appears that transplantation via the portal vein provides an immediate blood supply for the transplanted islets and assures that insulin reaches the liver in high concentration, thus simulating normal physiological conditions.

The observation that the neonatal pancreas has a maximal islet volume and insulin content and minimal acinar tissue and enzymes[27 28] allowed Leonard et al[29] to study the effect of dissociated neonatal pancreas transplantation on alloxan diabetic rats (pancreata to be transplanted are minced, briefly treated with collagenase and injected without efforts to separate islets from acinar tissue). Reversal of diabetes was observed in 100% of the eight moderately diabetic and in 29% of the seven severely diabetic rats which received isotransplants. Normoglycemia persisted in several of these successfully treated animals followed for as long as 18 months after transplantation (Figure 9).

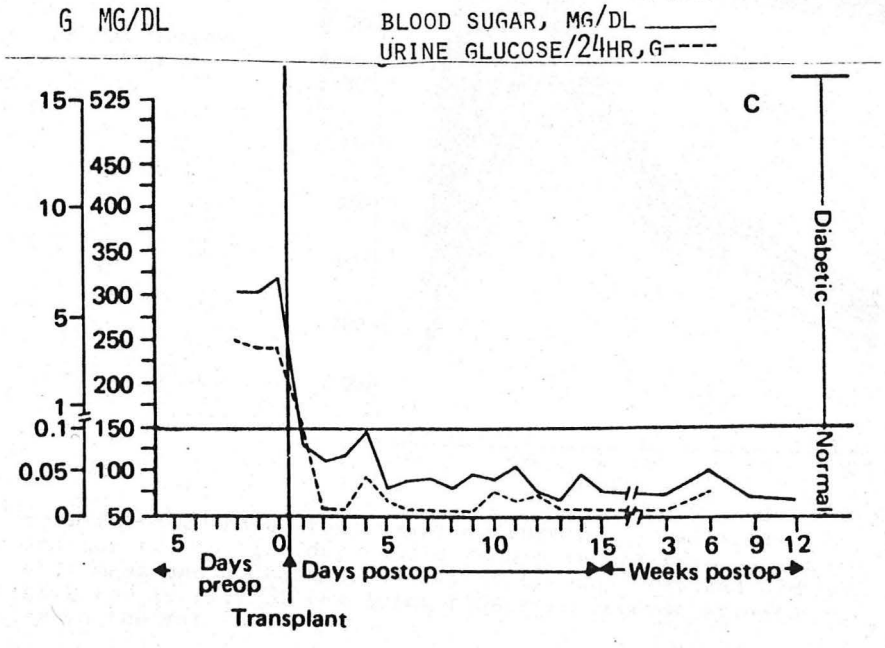


Fig. 9: Effects of dissociated neonatal pancreas transplantation in an alloxan diabetic rat. This represents a moderately diabetic recipient exhibiting an excellent response to pancreatic isotransplantation. Animals differed in the time required to reach normoglycemia.[29]

Most animals receiving dissociated neonatal pancreata showed normal glucose tolerance for five months after transplantation (Figure 10).

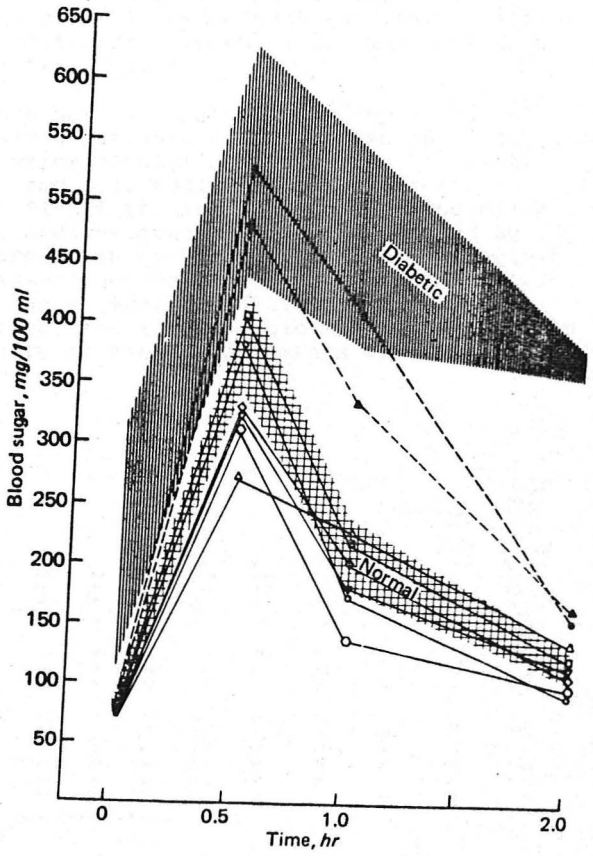


Fig. 10:
Individual glucose tolerance curves of successfully controlled diabetic animals compared to the range of glucose tolerance curves of normal and diabetic animals defined by mean values ± 1 SD. Six animals (open symbols) demonstrated normal tolerance curves 5 months following isotransplantation. Two animals (closed symbols) exhibited somewhat abnormal tolerance curves.[29]

This group further showed that organ culture of neonatal rat pancreata for two to nine days would result in an increase in islet cell mass and a decrease in acinar tissue[27,28], and did not impair the ability of the islet tissue to reverse alloxan diabetes in the rat.[30]

Other work by Mullen et al[31] showed complete reversal of streptozotocin induced diabetes in adult rats would follow transplantation of a single fetal pancreas if the organ is first grown in a normal carrier before transfer to the diabetic recipient. They also showed that careful blood sugar control with exogenous

insulin in the diabetic recipients would enhance the function and survival of the transplant.

Lundgren, et al[32] extended the observations of Leonard, et al[29] to human islets. These workers were able to maintain structure and functional integrity of isolated human islets maintained in tissue culture for one to three weeks.

Najarian and his colleagues have attempted 10 transplantations of islet cell tissue in seven patients with diabetes mellitus, all of whom had received prior renal transplantation.[33] Islet cell tissue was obtained from four adult cadaver pancreata by collagenase digestion and ficoll gradient human islet isolation techniques and six infant cadaver pancreata were dispersed by mincing and collagenase digestion without separation from acinar components. The islet tissue thus obtained was injected intraperitoneally in five instances, implanted into a muscle pocket in the groin in one instance, and infused into the portal vein in four instances. The results of these experiments are shown in Table III.

Recipient and Donor Data			Transplantation Data				Insulin Administration Data					Diabetic Control	
Recipient and Islet Trans Number	Islet Donor	No. HLA Antigens Shared Between Islet Donor and Recipient	Temporary Augmentation of Immunosuppression After Islet Transplant ALG (mg/kg)	Pred*	Per cent Adult Islet Mass Trans.	Site of Trans.	Pretrans. Daily Insulin Dose (Units)	Posttrans. Interval of Lowest Insulin Dose (Weeks)	Lowest Insulin Dose (Units)	Per cent of Pretrans. Dose (Units)	Current Insulin Dose	Average Posttrans. Plasma Glucose Level as Per cent of Avg. Pretrans. Level	Average Posttrans. 24 hr. Urine Glucose Excretion as Per cent of Avg. Pretrans Excretion
A	Adult	1	0	NC	0.4	IP		NC	NC	NC	NC	76	21
B													
1	Adult	0	0	NC	0.4	IP	96	2.4	72	75	—	78	100
2	Infant	0	0	NC	9.0	IP	80	NC	NC	NC	—		
3	Infant	1	0	*	8.5	IP	80	3.5	28	35	80		
C													
1	Adult	2	60	NC	14.5	IM	60	NC	NC	NC		75	100
2	Adult	0	180	*	0.2	IP	60	3.5	16	26	32		
D	Infant	0	270	*	5.7	PV	62	2.9	30	48	54	75	51
E	Infant	0	360	*	12.0	PV	64	2.4	30	46	62	89	124
F†	Infant	0	210	*	2.2	PV	64	2.8	46	68	—	88	55
G	Infant	0	270	*	0.4	PV	72	1.78	32	44	32	115	88

* Pred means dose was raised to 2 mg/kg and then tapered to maintenance dose over 2-4 weeks

† F died of a myocardial infarct 11 months after the islet transplant

NC, no change

IP, intraperitoneal transplant, IM, intramuscular; PV, portal vein.

Table III.: Summary of Ten Human Islet Transplants in Seven Recipients: 19-30 Months Followup.[33]

As is clear hyperglycemia and glycosuria continued unabated following islet cell transplantation although in some patients there was a temporary decrease in their daily insulin requirements. None of the patients showed an increase in circulating C-peptide levels. The reason for this failure seems straight forward enough and relates to the small amount of islet cell tissue obtained for transplantation by the isolation techniques used. In these experiemtns the percent of adult islet cell mass transplanted ranged from 0.2 to 14.5 percent. Hopefully better isolation techniques, which provide for a larger yield of islet cell tissue, will become available to permit further attempts at islet cell transplantation in man.

Islet Cell Transplantation (Artificial Capillary Units)

Islet cells are not immunologically privileged and like other tissues are rapidly rejected when transplanted across major histocompatibility barriers. Therefore, any transplantation of isolated islets would require immunosuppression of the recipient. Chick, et al[34,35] using the observation that artificial semipermeable membranes can protect grafted cells from immune rejection[36] developed what they termed a "hybrid artificial pancreas". In essence, islet cells are cultured on the outside of a hollow semipermeable tube, sealed into the ends of small glass jackets. The cultured islets can be perfused with nutrient media (Figures 11 and 12) and the semipermeable membrane protect the islets from immune response of a potential host.

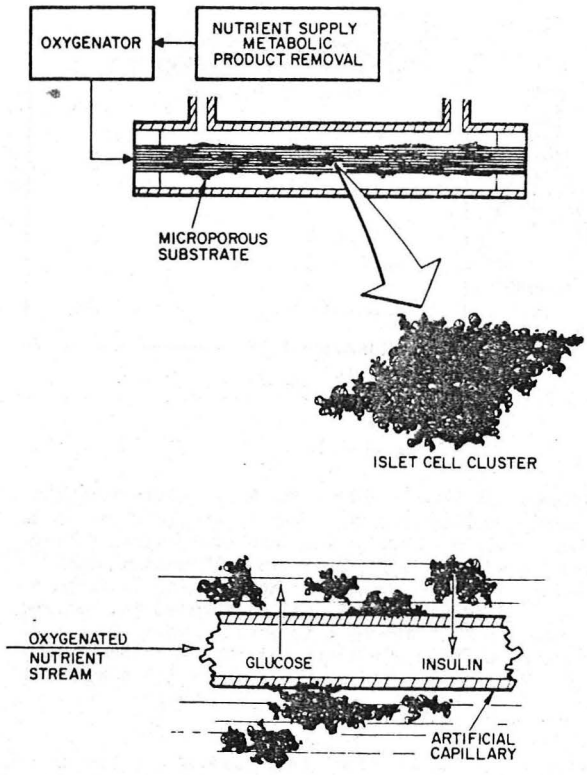


Fig 11: Beta cell culture on hollow fibers

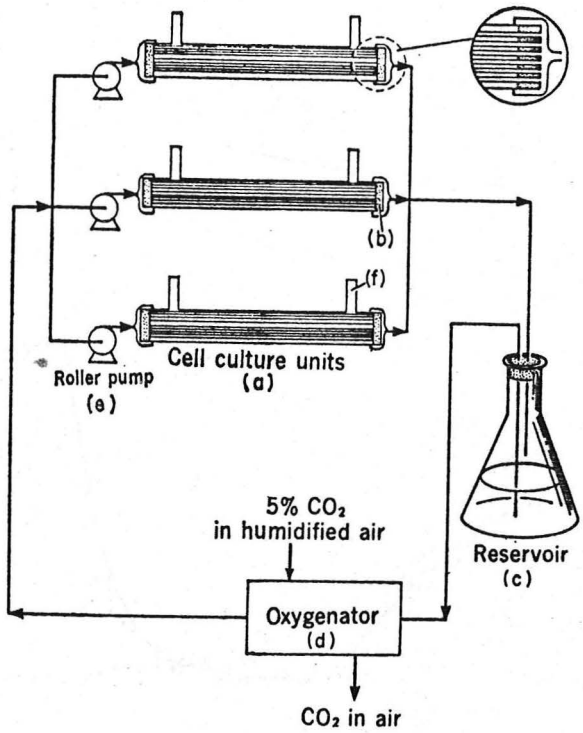


Fig. 12: Perfusion circuit. A cell culture unit (a) consisted of a bundle of one or more types of hollow fibers (capillaries) sealed into each end of an 8-mm glass shell by silicone rubber of epoxy resin (b). Units may be arranged in parallel as shown or in series. Nutrient medium oxygenated and brought to the appropriate pH by by exposure to a humidified mixture of 5 percent CO_2 and air. Cells were inoculated onto the capillary bundles through shell side pprts (f) [36]

These studies clearly showed that beta cells cultured on artificial semipermeable hollow fibers continue to synthesize, store, and release insulin. Furthermore, insulin release could be readily modulated by altering the glucose concentration in the perfusion media. This work was recently extended to alloxan diabetic rats in whom the "hybrid artificial pancreas" was implanted ex vivo as arteriovenous shunts.[37] In these studies the use of such devices clearly resulted in a return of normoglycemia (Figure 13), increased circulatory insulin levels (Figure 14), and restored intravenous glucose tolerance to normal. (Figure 15)

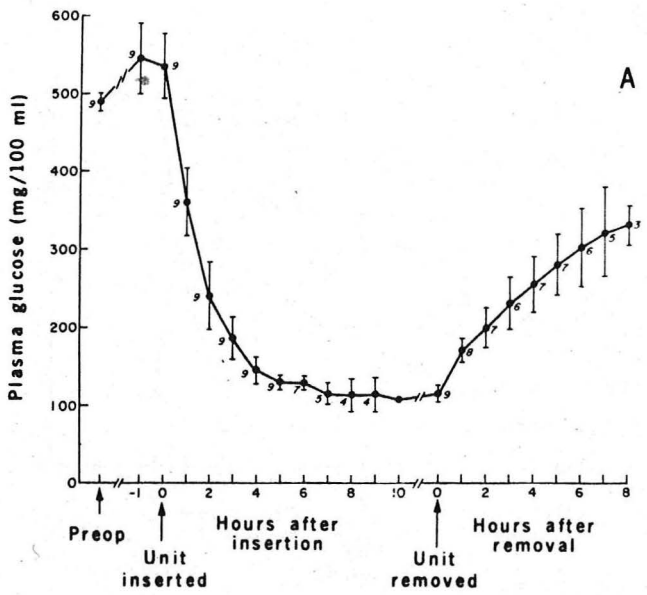


Fig. 13: Effect of the "artificial hybrid pancreas" on plasma glucose concentrations in rats with alloxan-induced diabetes.[37]

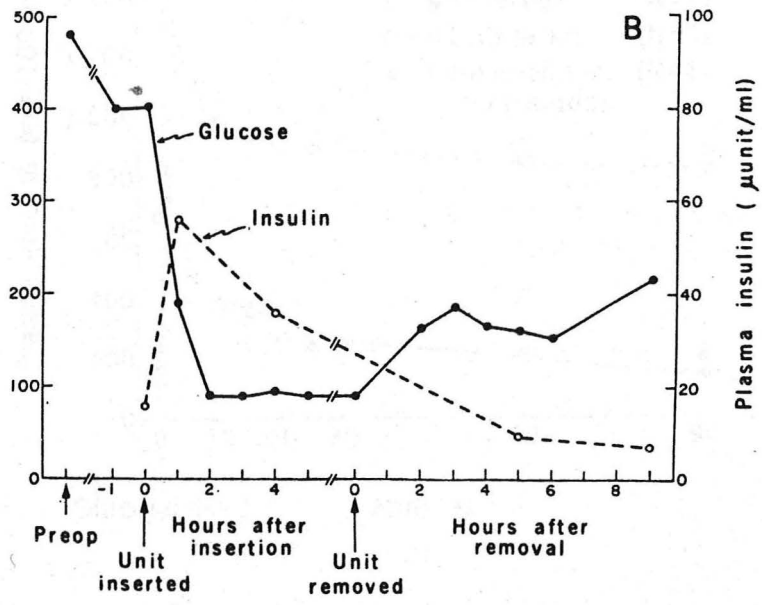


Fig. 14: Effect of the "artificial hybrid pancreas" on plasma glucose and insulin concentrations in rats with alloxan-induced diabetes[37]

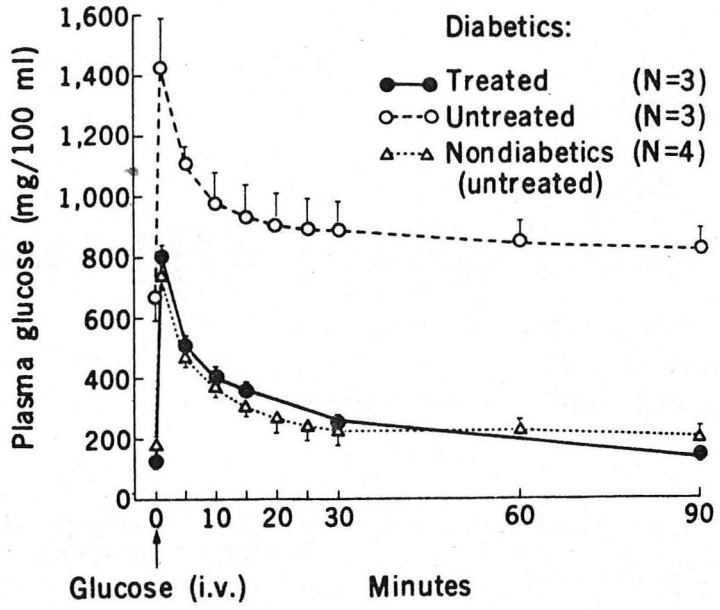


Fig. 15: Intravenous glucose tolerance tests in diabetic rats with the "artificial hybrid pancreas" inserted ex vivo in the arteriovenous shunt.[37]

Others have had equally promising results using a similar device (Figure 16).[38]

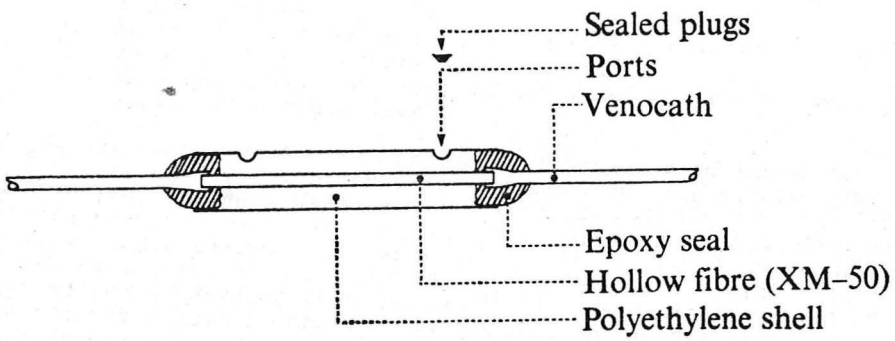


Fig. 16: Diagram to show the construction of the implantable artificial endocrine pancreas. [38]

Pancreatic islets from adult rats when cultured in artificial capillary units will remain functional for at least 97 days[39], responding to high glucose concentrations in the perfusion medium by increased insulin release throughout the duration of the culture period. Sun,et al[40] using such a device attached ex vivo to the vascular system of diabetic monkeys lowered blood glucose levels into the non-diabetic range within one hour. Furthermore, peritoneal implantation of pancreatic islets enclosed in a semipermeable membrane diffusion chamber has been shown to result in significant amelioration of the diabetic state in streptozotocin diabetic CBA mice for periods of up to two weeks.[41]

The Artificial Beta Cell

Over the past several years much attention has been devoted to the development of an artificial beta cell device, which if made small enough might be implanted into the body of a diabetic, that would constantly monitor the glucose concentration in the blood.

(or any body fluid in dynamic equilibration with blood) and control a self-contained unit to meter out insulin in proportion to need as indicated by blood glucose levels. The requirements for this artificial organ are:

1. A sensor for glucose
2. An amplifier and computer
3. A power supply
4. An insulin reservoir that could be conveniently refilled from the outside by injection.
5. An insulin emission device, activated by the sensor-amplifier-computer system.

At present such a device is available although it is large and must be attached via an intravenous connection to patients. One such device, called a Glucose Controlled Insulin Infusion System (CGIIS) has been developed by the Life Science Instrument Division of Miles Laboratories, Inc. The CGIIS permits the computation and delivery of insulin on a minute by minute basis. Several groups have helped to develop this device.[42 43] A schematic diagram of the apparatus used to monitor and regulate blood sugar is shown in Figure 17.[44]

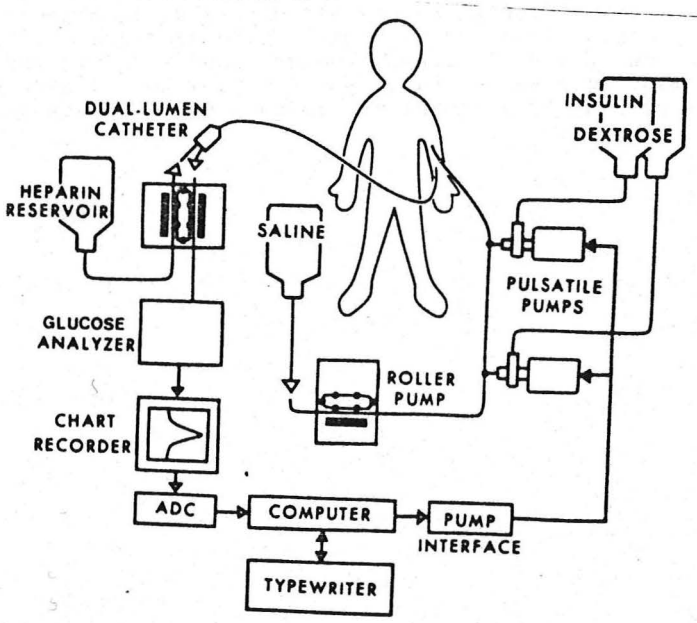


Fig.17: Schematic diagram of the Glucose Controlled Insulin Infusion System (GCIIS) used for monitoring and automatic regulation of blood sugar.[44]

Venous blood is drawn continuously from the patient into the inner lumen of a dual-lumen catheter and is fed to a Technicion® glucose analyzer modified to measure glucose in samples of whole blood withdrawn at a rate of 0.05 ml/min. The glucose concentration is computed and values averaged for one minute. The apparatus has a three and a half minute delay, i.e., that three and a half minutes elapse from the time a change in the blood glucose enters the catheter until the change is registered. The computer than reacts within one minute by altering the dextrose or insulin infusion rate. The computer controls two pumps which deliver appropriately metered amounts of dextrose and insulin which are carried to the patient by a steady infusion of normal saline.[44]

The automatic control of blood sugar in diabetic subjects is regulated by a computer which in addition to computing actual blood glucose concentration, also calculates the rate of change of the blood glucose concentration. The computer uses these two important variables to determine the required rates of dextrose and insulin infusion. The blood glucose concentration is regulated by the computer in accordance with two control algorithms which relates respective rates of dextrose or insulin infusion to the measured blood glucose level and its rate of change. Figure 18 shows a typical experiment in a diabetic subject studied over a 10 hour period on two separate days, comparing the continuous blood glucose profile sustained by subcutaneous insulin with that which was achieved when the patient was regulated by the artificial pancreas. Also shown are the minute-by-minute infusion of insulin and dextrose.

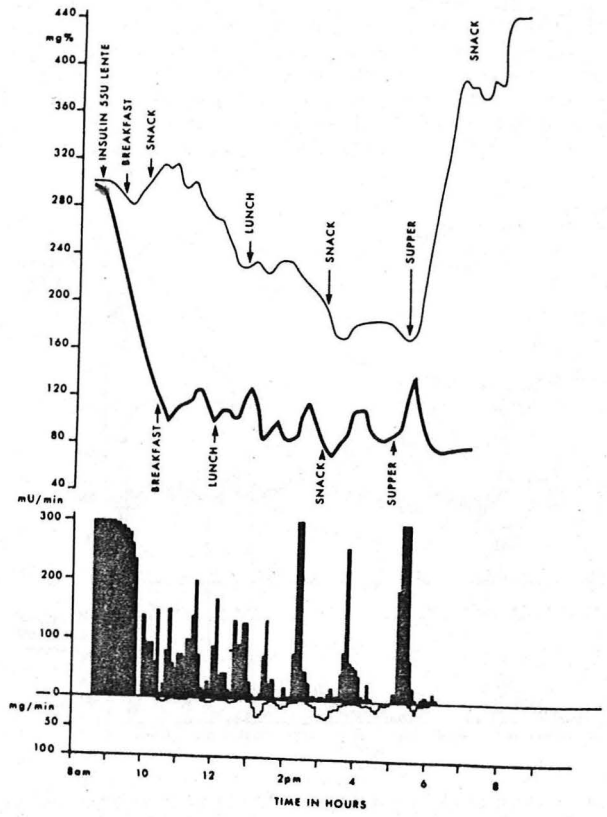


Fig. 18: Continuous records of blood sugar profiles in subject S.M. sustained by subcutaneous insulin (top curve) and regulated by artificial pancreas (center curve). Minunte-by-minute infusion patterns (lower curve) of insulin (black) and dextrose (white). Note dextrose infusion scale is inverted with respect to the insulin infusion scale. Total insulin infused is 53.1 Units.[44]

This device used by the Toronto group has been slightly modified in more recent studies, using a glucagon infusion to combat falling blood glucose levels rather than a dextrose infusion. These workers have clearly demonstrated, in short term studies, the ability of such a system to restore and maintain glucose homeostasis in human diabetics studied while consuming their usual diet (Figure 19),[45] undergoing a 50 gm oral glucose tolerance test (Figure 20),[45] or indulging in moderate physical exercise.[45 46]

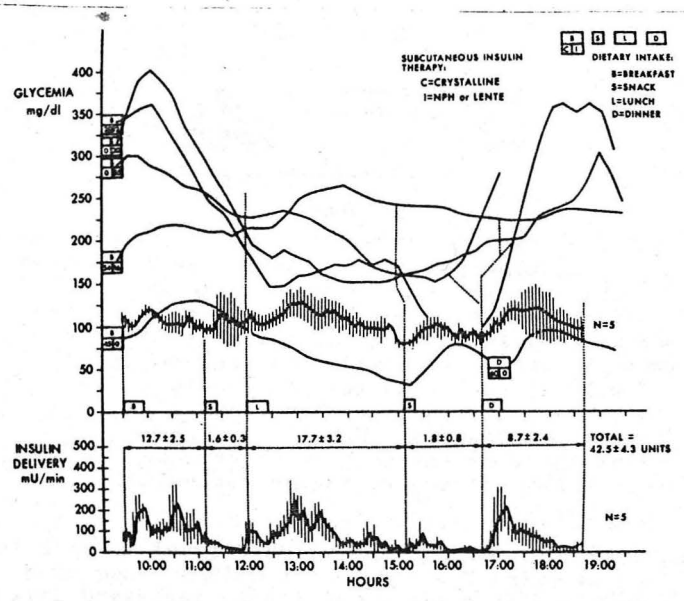


Fig.19: Upper panel shows the continuously monitored blood glucose concentrations following meals and snacks in 5 diabetics receiving their usual therapy (shown individually) and in the same subjects treated with the artificial pancreas (shown hatched as mean ± SEM). Lower panel shows insulin delivery rate by the artificial pancreas (shown hatched as mean ± SEM) corresponding to the mean glucose concentrations above.[45]

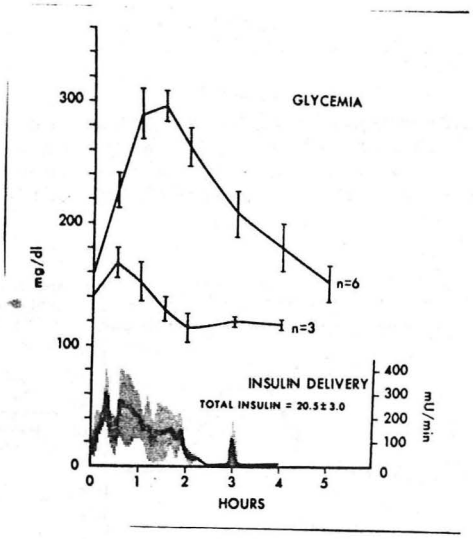


Fig. 20: Upper curve is mean glycemic response (± SEM) to repeated 50 gm oral glucose tolerance tests in 3 subjects given their usual therapy. Center curve is mean glycemic response (± SEM) to same test in same subjects given insulin by the artificial pancreas. Lower curve is mean rate (± SEM) of insulin delivery by artificial pancreas in response to glucose concentrations in center curve.[45]

The total dose of insulin delivered by the artificial pancreas during these 10 hour meal studies was 42.5 ± 4.3 units (12.7 ± 2.5 units infused with breakfast; 1.6 ± 0.3 units with the morning snack; 17.7 ± 3.2 units with lunch; 1.8 ± 0.8 units with the afternoon snack; and 8.7 ± 2.4 units infused with dinner). Recognizing that the duration of these studies with the artificial pancreas was only 10 hours and the remaining 14 hours of the day is largely "postabsorptive" and the insulin requirement for that part of the day is basal (15 mU/min.) one might calculate that the daily insulin requirement to maintain normoglycemia in these diabetics averaged approximately 50 units. This is somewhat higher than had been previously thought.

These same workers[47] compared the route of insulin administration with the artificial pancreas in pancreatectomized dogs receiving an intraportal infusion of glucose. During computer-controlled insulin administration, normal glucose tolerance could be restored by both portal and peripheral routes of insulin delivery. There

were no significant differences in (1) glycemic patterns, (2) insulin infusion patterns, (3) peripheral IRI levels and (4) total insulin requirements between the two routes (Figure 21). This work is further confirmation of the earlier work of Madison, et al.[48]

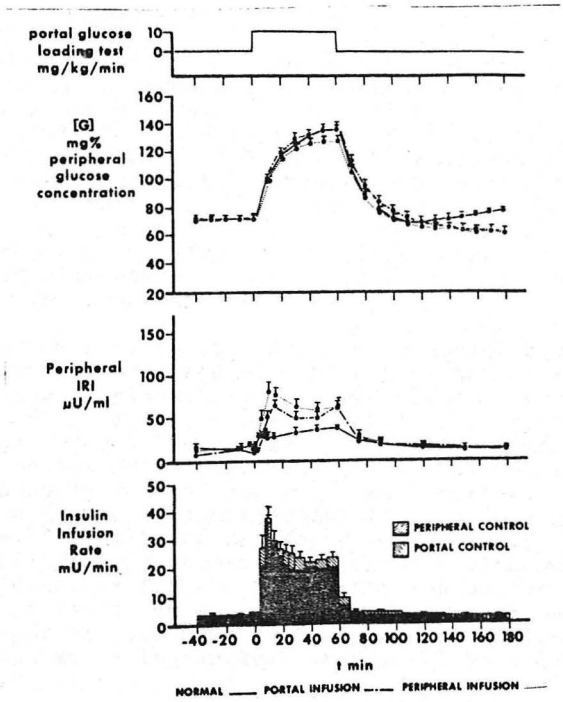


Fig. 21: The response to a glucose infusion (top) in (1) the healthy dogs (N=5), (2) the pancreatectomized dogs given insulin by the artificial pancreas into a peripheral vein (N=15), and (3) the same dogs given insulin by the artificial pancreas into a portal vein (N=16). IRI signifies immuno reactive insulin.[45]

Additional promising results have been obtained using less complicated devices such as one which has only a computer operated insulin delivery system based on data provided by a continuous blood glucose monitor without provision for administration of a hyperglycemia substance[49], or even one which provides for only preprogrammed intravenous insulin administration without feedback control of delivery rates.[50]

Finally, Mirouze, et al[51] have recently shown that treatment with the artificial pancreas and subsequent complete normalization of the plasma glucose levels for several days resulted in a sustained remission (3-14 months duration) in 9 out of 12 (75%) recent onset ketosis prone juvenile diabetics. Whereas, only 11% of a similar group in whom conventional subcutaneous insulin treatment was used underwent remission. The authors interpreted this finding as suggesting that early effective treatment of recent-onset juvenile diabetes may break the vicious circle of hyperglycemia-insulin depletion-hyperglycemia and lead to frequent and sustained remissions in these patients.

Development of a miniature implantable device has progressed slowly. Dr. Soeldner and his colleagues have developed a miniature, totally tissue implantable, "glucose sensor" and a miniature power supply-radiotelementry system to which this "glucose sensor" could be attached. The entire unit ("glucose monitor") is small enough to be implanted subcutaneously. This unit can automatically and continuously communicate to a small portable receiver for the translation of the transmitted information to a glucose concentration value.[52] Yet to be developed is a totally implantable insulin delivering system consisting of a glucose sensor, a miniature computer interface for the interpretation of the glucose levels and rates of change as sensed by the miniature sensor, and an insulin reservoir and pump delivery system. Figure 22 shows a diagram of what an implantable artificial beta cell might look like.

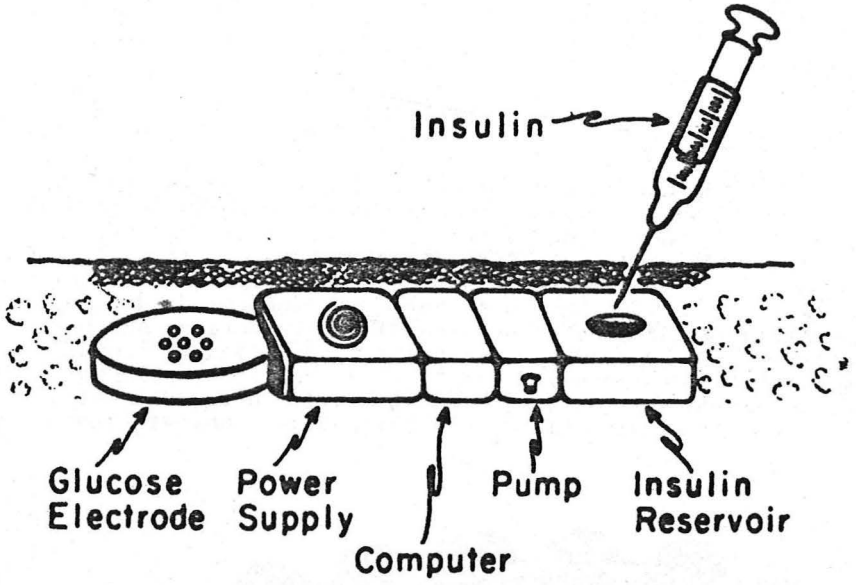


Fig. 22: The "Artificial Beta Cell"
This diagram depicts what an implantable artificial beta cell might look like. It is positioned just under the skin in the subcutaneous tissue. An insulin syringe showing how the reservoir is to be refilled is also shown. [52]

The glucose sensor used by this group consists of two platinum electrodes, shielded by special membranes to protect them from interfering substances found in biological fluids and from overgrowth of tissue when implanted. An electrical current produced is derived from the reaction of glucose with oxygen on the surface of the platinum electrode, the change in electrical current being proportioned to the glucose concentration in the fluid in which it has been implanted. This type of sensor has functioned when implanted into Rhesus monkeys for up to 117 days. Another electrode under development is one which uses glucose oxidase and an oxygen electrode to measure the surrounding glucose concentration.[53]

However exciting the idea of a miniaturized artificial pancreas may be, there are many problems that must be overcome before any clinical application will be forthcoming.

SOMATOSTATIN

The discovery of somatostatin is an example of how careful investigation following an unexpected observation can lead to far reaching and important scientific advances. Dr. Roger Guillemin and his colleagues at the Salk Institute were searching for a proposed hypothalmic stimulator of growth hormone secretion. Much to their surprise, the hypothalmic extracts inhibited the release of growth hormone rather than stimulating its release.[54] Shortly thereafter, this substance was sequenced and reproduced synthetically.[55 56] Its structure was determined to be a 14-amino acid polypeptide with a disulfide chain linking the cysteine residues at positions 3 and 14 (Figure 23).

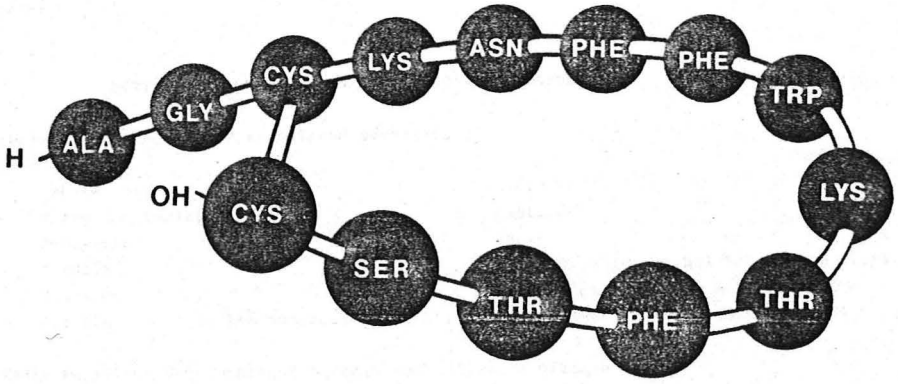


Fig.23: Amino Acid Sequence of Somatostatin

The synthetic substance was as biologically active as the natural compound, inhibiting growth hormone release in an *in vitro* system at concentrations as low as 10^{-10} M.[57] Because it inhibited somatotropin hormone release it was given the name somatostatin.[54]

It was soon discovered that this substance had widespread biological activity. It not only inhibited the growth hormone response to all physiological and pharmacological stimuli[57,58,59] but seemed also effective in lowering growth hormone levels in patients with acromegaly.[60,61] It was also realized that, in addition to its ability to suppress growth hormone release, somatostatin had widespread activity on other endocrine tissues as well as non-endocrine tissues. Listed in Table IV are the actions of somatostatin on endocrine tissues.

TABLE IV

ACTIONS OF SOMATOSTATIN UPON ENDOCRINE TISSUES

Inhibits resting and/or stimulated secretion of:

Growth Hormone	Secretin
Thyroid stimulating hormone	Gastrin
Prolactin	Renin
Insulin	Vasoactive Intestinal Polypetide (VIP)
Glucagon	Adrenocorticotrophic Hormone* (ACTH)
Gut Glucagon-like Immunoreactivity (GLI)	Cholecystokinin-Pancreozymin (CCK)

*Only in Nelson and Cushing Syndromes and Addison's Disease

In addition to inhibiting the release of growth hormone, somatostatin inhibits the release of thyroid stimulating hormone[62,63], prolactin[62,63], secretin[64], gastrin[65], and GLI.[66] Koerker and her colleagues first noted the effect of somatostatin on pancreatic islet cell function when they showed that somatostatin, when infused into baboons, lowered both insulin and glucagon levels.[67] In vitro rat pancreas perfusion studies demonstrated that the inhibition of insulin and glucagon secretion occurred at the islet cell level.[68 69 70 71] Other studies showed that this agent blocked the stimulated secretion of these pancreatic hormones as well as their basal secretion.[72 73 74] Somatostatin also has actions on non-endocrine tissues. Listed in Table V are some its actions on non-endocrine tissues. As can be seen, it has multiple effects on the gastrointestinal tract. It diminishes hydrochloric acid secretion[65], gastric emptying[75], duodenal motility[76], gallbladder contraction[77], pancreatic exocrine functions[77,78], and delays xylose absorption[79].

TABLE V

ACTIONS OF SOMATOSTATIN UPON NON-ENDOCRINE TISSUES

Diminishes the following processes:

Splanchnic blood flow	Gastric emptying
Xylose absorption	Duodenal motility
Glucose absorption	Gallbladder contraction
Amino acid absorption	Pancreatic exocrine function
Fat absorption	Food intake
HCl secretion	CNS neuron

Acetyl choline release from nerve endings

Krejs, et al[80], demonstrated marked inhibition by somatostatin of jejunal glucose, amino acid, oleic acid and water absorption. The most likely explanation for these multiple effects of the drug on absorption is that somatostatin must effect changes in mucosal surface areas. Many of these gastrointestinal actions of somatostatin may contribute to the hypoglycemic action of somatostatin when used as adjunct treatment in diabetes.

Although somatostatin was originally discovered in the hypothalmus, it has been shown to have widespread distribution in various tissues. Within the hypothalmus somatostatin has been demonstrated in highest concentration in the median eminence and arcuate nucleus. although all hypothalmic nuclei contain some of this substance. Other areas in the central nervous system such as the cerebral cortex and cerebellum contain somatostatin as well.[81 82]. In addition, other tissues such as the stomach[83], small intestine[84], and pancreas[85 86], have been shown to also contain somatostatin. This has been demonstrated in several species including man. Somatostatin has been localized to the D-cells of the pancreatic islets[87] which make up some 10% of the islet cell mass. In man and the rat the D-cells are located between the outer A-cell mantle and the inner mass of B-cells.[87] Since recent studies have clearly shown secretion of pancreatic somatostatin into the venous effluent to various stimuli including glucagon administration in in vitro perfused pancreas preparations[88.89.90 91 92], it has been suggested that all three cell types of the pancreatic islet function as a "paracrine" unit in which the secretory products of the three cell types influence the secretion of one or more of the nearby cell types.[93 94] The vast tissue distribution and varied biologic action, coupled with the fact that pancreatic secretion of this substance can be stimulated under various experimental circumstances, have lead some to postulate a possible function of somatostatin as a regulator of growth hormone and TSH release[95] as well as a modulator of both endocrine and exocrine gastrointestinal function.[96]

The exact mechanism by which somtostatin inhibits the release of hormones is not clear. It was originally thought that hormone release was inhibited by somatostatin via its effect on calcium transport[97 98] however, this concept has recently been challenged.[99]

THERAPEUTIC CONSIDERATIONS

The notion that somatostatin might be useful in the treatment of diabetes mellitus is based almost entirely on the bihormonal theory of diabetes developed over the past several years by Unger.[100] According to this thesis, the metabolic abnormalities of diabetes, i.e., the hyperglycemia and the hyperketonemia are not the consequence of insulin deficiency alone, but result from the combination of both insulin deficiency and glucagon excess. The traditional view that insulin deficiency alone is responsible for the full expression of the diabetic syndrome is derived from the experiements of Von Merring and Minkowski[101], who showed that pancreatectomy resulted in the development of severe diabetes, and those of Banting

and Best[102], who demonstrated that the diabetes produced by pancreatectomy could be ameliorated by the injection of insulin containing pancreatic extracts. It is now clear that in almost all forms of human and experimental diabetes, the insulin deficiency[1] is, with very rare exception, associated with relative or absolute hyperglucagonemia.[103] Glucagon is a potent glycogenolytic, gluconeogenic, lipolytic and ketogenic hormone, whose actions are opposed by insulin and intensified by insulin deficiency. Therefore, glucagon excess might be expected to make a deleterious contribution to the metabolic syndrome of diabetes when coupled with insulin deficiency. The potential therapeutic benefit of reducing diabetic hyperglucagonemia should be obvious.

Since the discovery of insulin by Banting and Best, all therapeutic interventions in the treatment of diabetes mellitus have been directed at either rigid dietary management and/or aggressive insulin therapy. It is clear that present methods for controlling the plasma glucose levels in most diabetic patients leave much to be desired. Whereas, in non-diabetic subjects, the plasma glucose concentration, as shown in these studies where it was measured every two hours over a 48 hour period, rarely exceeds 150 mg/dl at anytime during the day even when blood samples are taken one hour after breakfast and lunch.[104] Few, if any, diabetics studied in a similar fashion are maintained at this level of glycemia throughout the day. Most exhibit hyperglycemia for 18-16 hours per day despite careful management with diet and multiple daily injections of insulin[104]. Although in these studies, the high dose of insulin used did reduce the level of diabetic hyperglucagonemia, such large quantities of insulin might be expected to markedly increase the risk of hypoglycemia. It therefore seems reasonable that since hyperglucagonemia contributes significantly to the metabolic abberations of the diabetic syndrome and that even meticulous treatment with diet and insulin is insufficient to restore glucose homeostasis to normal in the diabetic patient, that pharmacological suppression of the hyperglucagonemia characteristic of the disease might be of therapeutic benefit.

As early as 1974 it was clearly demonstrated that the infusion of somatostatin with resulting lowering of plasma levels of both insulin and glucagon caused a fall in plasma glucose levels in fasting non-diabetic individuals. This was shown by Koerker, et al in baboons[67], Sakurai, et al in dogs[105], and Gerich et al in man.[106] Likewise, Sakurai, et al showed that somatostatin infusion could lower basal plasma glucose concentration to 77% of pre-infusion levels in fasting insulin deprived alloxan diabetic dogs. When the somatostatin infusion was terminated, glucagon rose and the plasma glucose concentration increased 21% in 30 minutes.[107] Similar results were obtained in human diabetics by Gerich and his colleaques. When these workers infused somatostatin for two hours in 10 insulin dependent diabetic subjects, plasma glucagon levels fell significantly from 105 $\pm$ 15 pg/ml to 77 $\pm$ 10 pg/ml, and plasma glucose

[1]either absolute or relative to the ambient plasma glucose concentration

from 260 ± 20 mg/dl to 191 ± 21 mg/dl.[108] Finally in this study it was demonstrated that somatostatin infusion combined with subcutaneous insulin administration abolished post-prandial hyperglycemia in diabetic subjects and clearly was more effective in this regard than subcutaneous insulin alone. Furthermore, Meissner, et al, showed that the used of somatostatin in insulin dependent juvenile diabetic patients reduced the insulin requirements from 38 to 79 percent when the artificial pancreas was used to normalize blood glucose levels following meals or oral glucose loads.[109]

Perhaps the most elegant study which relates not only to the possible practical importance of glucagon suppression but to theoretical matters as well, was also conducted by Gerich and his co-workers. In these studies the development of diabetic ketoacidosis, which follows the acute withdrawal of insulin within 10 hours, was prevented for 18 hours by the infusion of somatostatin and the resultant suppression of glucagon secretion.[110] These studies clearly showed, in addition to the potential therapeutic benefit of glucagon suppression in preventing the development of diabetic ketoacidosis, that insulin deficiency alone does not lead to the development of fulminant diabetic ketoacidosis in man and that glucagon is a prerequisite for the full expression of the condition. It must be pointed out, however, that somatostatin has been shown to be of no benefit in the treatment of manifest diabetic ketoacidosis.[111]

It seems clear from these studies that reduction of the diabetic hyperglucagonemia with somatostatin can improve the metabolic abnormalities of diabetes when used in combination with insulin for periods of time up to 20 hours. But, would somatostatin be beneficial in this regard when used for longer periods of time? Figure 24 shows one of our studies on the use of somatostatin as adjunct to insulin treatment in a juvenile type diabetic patient.[112] In this patient we studied the effect of a continuous somatostatin infusion for three days in combination with insulin therapy upon the profile of plasma glucose and immunoreactive glucagon and 24-hour excretion of glucose as compared with conventional split dose insulin therapy alone. As can be seen, insulin therapy alone, although resulting in reasonably normal fasting plasma glucose levels, was ineffective in controlling post-prandial hyperglycemia. The continuous infusion of somatostatin in a dose of 2.0 mg/day for three days was accompanied by a reduction in the plasma glucose profile to normal and a significant reduction in plasma IRG levels. Termination of the somatostatin infusion resulted in an elevation of the plasma glucagon level and a return of post-prandial hyperglycemia.

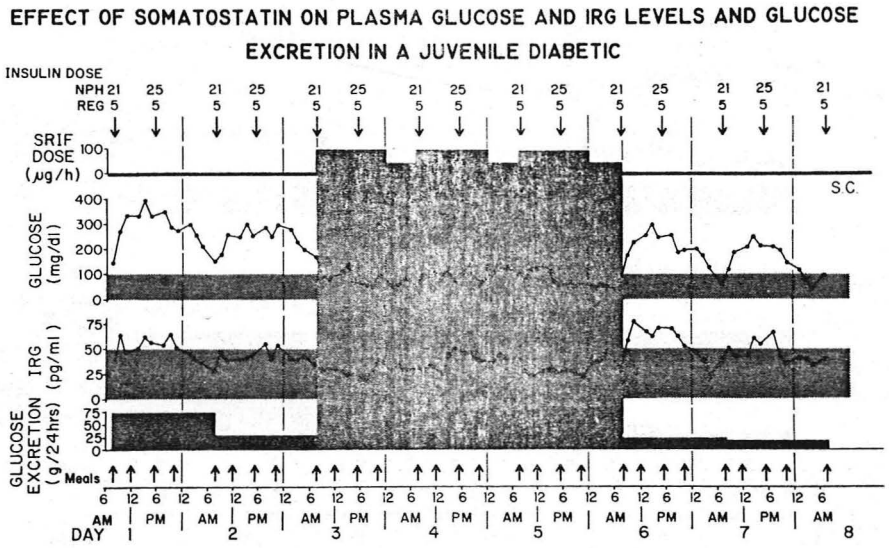


Fig. 24: Effect of a continuous somatostatin infusion for 3 days on the daily profiles of plasma glucose and IRG levels and 24-hour excretion of glucose in a juvenile type diabetic. Arrows indicate meal times.

Similar results have also been reported by Gerich [113]. During a three day continuous somatostatin infusion in six insulin dependent patients he was able to achieve improved blood glucose control on doses of insulin less than one half as much that was unsuccessful in controlling post-prandial hyperglycemia in the absence of somatostatin.

In recent studies we have extended these observations.[114] Figure 25 shows the results of experiments carried out in four juvenile onset diabetic patients receiving a continuous insulin infusion and a diabetic diet containing 150 g per day of carbohydrates. When a constant infusion of somatostatin was added to the insulin infusion lowering plasma mean IRG levels from 182 $\pm$ 34 pg/ml to 60 $\pm$ 13 pg/ml mean plasma glucose levels fell into the non-diabetic range and post-prandial hyperglycemia was eliminated. A replacement infusion of glucagon for 48 hours during the somatostatin infusion raised the mean plasma IRG level to 272 $\pm$ 30 pg/ml and plasma glucose levels also increased to 202 $\pm$ 20 mg/dl.

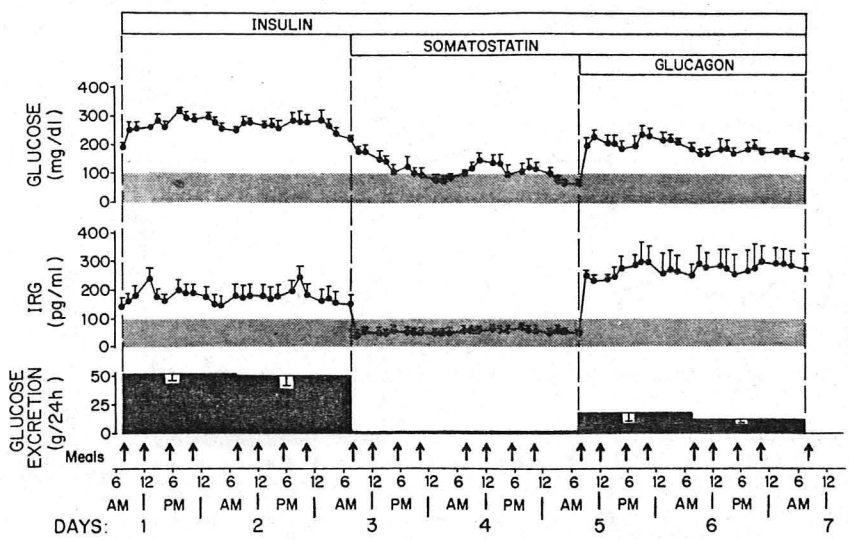


Fig. 25: Effects of somatostatin and somatostatin plus glucagon upon the daily profiles of mean (± SEM) glucose and IRG levels and upon glucose excretion in 4 juvenile-type diabetic subjects receiving a continuous insulin infusion and a diet containing 150 g/d of carbohydrate. Arrows indicate meal times.

Similar results were obtained when these studies were repeated in juvenile diabetics on a carbohydrate free diet (Figure 26), indicating that the hypoglycemic effect of somatostatin in these studies was not the result of a reduction in gastrointestinal glucose absorption.

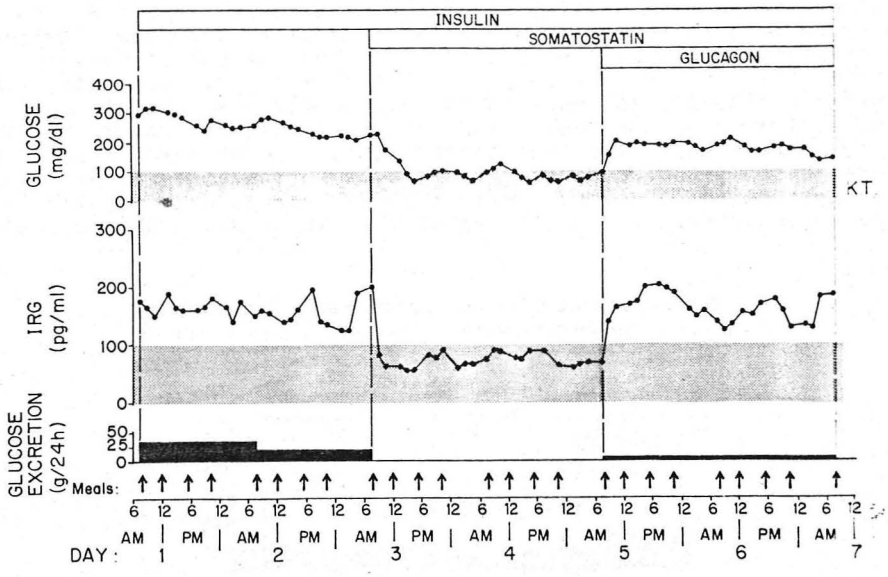


Fig. 26: Effects of somatstatin and somatostatin plus glucagon on the daily profiles of plasma glucose and IRG levels and upon glucose excretion in a juvenile type diabetic receiving a continuous insulin infusion and a diet containing only 30 g/d of carbohydrate. Arrows indicate meal times.

Although it is possible that some of the hypoglyemic actions of somatostatin are related to its effects on decreased splanchnic blood flow and reduced carbohydrate absorption that have been reported[79 80 115] it seems likely that most of the metabolic improvement is a direct result of glucagon suppression and the resulting decrease in hepatic glucose production. Some workers have suggested an effect of somatostatin on the liver, claiming that the drug itself could decrease hepatic glucose production either directly or by reducing the sensitivity of the liver to the glycogenolytic action of glucagon independent of its effects on glucagon secretion[116;117,118]. It has been clearly shown that somatostatin has no direct effect on peripheral glucose utilization.

It is important to remember that all these studies were done in patients with the insulin dependent form of diabetes and the somatostatin was used only as adjunct to insulin therapy. It is clear that the administration of somatostatin alone, to diabetic patients who have residual insulin secretion, which also would be suppressed by the somatostatin, will result in a deterioration of diabetic control. This fact has been clearly documented[61 120 121] and no claims for the possible use of somatostatin in the absence of concomitant insulin treatment have been made. In preliminary studies we have demonstrated a beneficial effect of somatostatin when used in combination with insulin on the blood glucose levels in patients with adult onset diabetes (Figure 27) suggesting that somatostatin may have utility in this form of human diabetes as well.

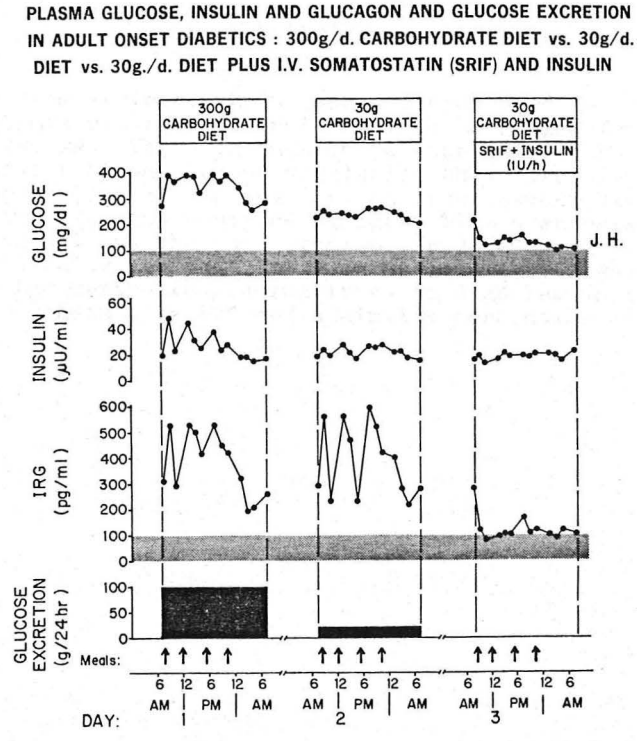


Fig. 27: Effects of somatostatin and insulin on the daily profiles of plasma glucose, insulin and IRG levels in an adult onset diabetic. Arrows indicate meal times.

Although somatostatin causes abnormal platelet function when administered in very large doses to animals[122,123], there has been no indication of this occurring in man[124,125]. To date the only reported untoward effect of long-term somatostatin administration in man has been occasional nausea, vomiting, mild diarrhea and abdominal pain.[126,127,128]

The true efficacy of somatostatin in the treatment of diabetes mellitus remains unknown. However, many experiments conducted evaluating the use of this drug for this purpose have yielded promising results. Whether somatostatin or one of its analogs will turn out to be the final therapeutic modality, it seems clear that attempts at pharmacologic suppression of hyperglucagonemia can be of tremendous value in the treatment of diabetes mellitus.

CONCLUSION

Although far from perfect, the present methods used in treating the hyperglycemia of diabetes must continue unchanged for the foreseeable future. There is much to be done before the promising methods for blood glucose regulation that were discussed today will have clinical application. Of the methods discussed, it seems that a glucagon suppressing agent like somatostatin or one of its analogs has the most promise for developing into something clinically useful. There is no question, however, that a "cure" for the metabolic abnormalities of diabetes mellitus is not eminent. Others have reached a similar conclusion.[129,130,131,132,133,134]

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