Pancreatic islet transplantation: studies on the technique and efficacy of islet isolation and transplantation

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1 Introduction

The ultimate goal of pancreatic islet transplantation is to reinstate normal blood glucose regulation in patients with insulin-dependent diabetes mellitus to improve the quality of life, and prevent, postpone or ameliorate the long-term complications of the disease and, thereby, promote longevity.

Diabetes mellitus

Diabetes mellitus is a disease characterised by chronic high levels of blood glucose (hyperglycemia) which affects 2-4% of the population of industrialised countries. Two primary types of diabetes are recognised. By far the most common form of diabetes is non-insulin-dependent diabetes mellitus (NIDDM). NIDDM usually begins in middle age, but can occur earlier or later, and patients are frequently overweight. These patients — who still have endogenous insulin production, though quantitatively or qualitatively insufficient to maintain normal glucose levels — may be treated by diet or oral drugs (sulfonylureas). Some patients are treated with insulin to improve glucose control, but insulin administration is not of vital importance. NIDDM is also called type-2 diabetes. Roughly 5-10% of all diabetic patients depend, however, entirely on insulin administration to prevent life-threatening ketoacidosis [1–3]. In these patients with so-called insulin-dependent-diabetes-mellitus (IDDM) — also called type-1 diabetes — the hyperglycemia is a consequence of the inability to produce insulin, due to the loss of the insulin producing ß-cells within the pancreatic islets of Langerhans.

Both genetic and environmental factors — resulting in autoimmune destruction of the pancreatic ß-cells — are involved in the development of IDDM. The conventional treatment with daily subcutaneous insulin injections is a life-saving intervention for patients devoid of ß-cells, but this approach falls short of the glycemic control achieved in normal individuals by the continuous

moment-to-moment adjustion of insulin secretion by the ß-cell in response to the small physiological changes of blood glucose levels — therefore, in general, chronic hyperglycemia cannot be prevented. IDDM usually starts in childhood, and 20–30 years later two-thirds of the patients end up with proliferative retinopathy, which impairs and threatens vision, and one-third of the patients end up with overt nephropathy, which leads to kidney failure requiring dialysis or a renal transplant — further, neuropathy, cardiovascular disease, and lower limb amputations are well-known complications [4–6]. The overall morbidity accounts for a one-third reduction of the life expectancy of the insulin-dependent diabetic patients [7–9].

Glucose control

It has become evident that the long-term complications of diabetes are to be considered as caused by derangement of glucose control instead of being manifestations of type-1 diabetes as such [10, 11]. The importance of tight glucose control [12] has recently conclusively been shown by the Diabetes Control and Complications Trial Research Group, which demonstrated that long-term tight control by intensive insulin treatment delays the onset and slows the progression of long-term diabetic complications [13]. Based on these data a strong case can be made for close to normal glucose regulation. The message for clinical practice is clear: near-normoglycemia must be the aim in all type-1 diabetic patients from diagnosis; however, new strategies are required for broad implementation of tight control in the diabetic population, while minimising the increased risk of hypoglycemic episodes concomitant with intensive treatment [12–15]. Now, considerable attention is focused on the education of patients for self-control [15, 16], and new techniques for the optimal delivery of insulin (and analogues) are currently examined [17] — such as, nasal insulin administration, polymeric capsules, and implantable pumps with glucose control by intermittent feedback from a glucose sensor ('artificial ß-cell'). The refinement of these methods is anxiously awaited. As yet, technical problems hamper closed-loop insulin pumps, and current exogenous insulin delivery methods do not mimic the exquisite glucose control provided by the release of insulin and the counterregulatory hormone glucagon from native pancreatic islets [18].

In order to deliver insulin (and glucagon) at the right places, in right doses, and at right times, a rational choice would be transplantation of a normal pancreas or transplantation of only the islets in type-1 diabetic patients [19, 20]. At present pancreas transplantation is the only reliable method available to

realise long-term near-normoglycemia in diabetic patients [21, 22]. Pancreas transplantation, however, is a major operation [19, 23] and requires life-long immunosuppression — with its toxic side effects. This procedure will therefore probably never be applicable to diabetic children or young adults. Pancreas transplants are now generally restricted to a minority of type-1 diabetic patients who already have or simultaneously will have a kidney transplant for end-stage renal failure [24, 25]. Considering the usually advanced complications in these patients, no dramatic effects of the pancreas graft would be expected. Beneficial effects both in terms of morbidity, longevity, and quality of life have, nevertheless, been demonstrated. Morphological changes of the kidney graft are prevented or reversed, the nerve function improves, and the patient's quality of life considerably improves by the elimination of both the acute diabetic complications, the need for frequent insulin injections and glucose measurements, and other restrictions. [19, 21, 23, 26–31]

The transplantation of purified isolated islets potentially offers many additional advantages over pancreas transplantation:

- Because of the small mass of a purified adult human islet graft (~ 1 millilitre) and the small size of the individual islets (with diameters from 0.05–0.5 millimetre) islet implantation is simple and safe, consisting of little more than an injection of a few millilitres of an islet suspension.
- Islet 'banking' by cryopreservation or storage of islets in tissue culture [32, 33] allows (i) the simultaneous or repeated transplantation of the islets of multiple donors [32], (ii) sophisticated prospective matching [32, 34], (iii) in vitro manipulation prior to implantation to lower the immunogenicity [35–37], or (iv) in vitro encapsulation in semipermeable artificial membranes ('bioartificial pancreas') to eliminate the need for immunosuppressive therapy [38–40], and potentially xenotransplantation to obviate donor shortage [41].
- Islets can be implanted at immunoprivileged sites [42–46], and sites allowing portal physiological drainage of hormones [47–50]

Thus in contrast to transplantation of the 'big dirty island' (i.e. the whole pancreatic organ), transplantation of isolated islets could become available to patients before the onset of devastating complications and thereby prevent them. Recently, insulin independence following adult human islet allografting in insulin-dependent patients has demonstrated the feasibility of this approach [32, 51]. As yet, however, many problems still prevent the large-scale clinical application of this technique, although potential solutions are appearing in outlines [32]. Past accomplishments and a historical perspective of important

concepts and methods in the islet transplantation field are discussed below.

The pancreatic origin of diabetes mellitus

As far back as Egyptian times, 1500 BC, the syndrome of diabetes has been recognised, due to its remarkable symptoms of frequent and voluminous urination and unquenchable thirst [52]. For a long time the high production of urine was the major criterion of diagnosis. In 1685, Brunner was the first to observe these symptoms after partial pancreatectomy and duct ligation of the remnant pancreas in dogs — however, he did not think of the diagnosis 'diabetes' [53]. The sweet taste of urine was reported first in the Western world by Willis in 1674 [52]. Next, when chemical methods for glucose assessment became available in the early 1800s, sugar in the urine and later in blood became the criterion. In 1869, Paul Langerhans was the first to describe the islets — which were later named after him by Laguesse — as "groups of cells ... appearing as vivid yellow specks...with a diameter of 0.1 to 0.24 mm ... scattered throughout the pancreas" ; but Langerhans offered no explanation as to the nature of these 'groups of cells' [54]. The pancreatic origin of diabetes was first established in 1889 by Von Mering and Minkowski who observed glucose in the urine of dogs after pancreatectomy [55]. From 1890–1892 Hédon confirmed these experiments, and performed the first subcutaneous segmental pancreatic autograft (without interruption of the blood supply) in pancreatectomised dogs, and demonstrated that diabetes was prevented by some internal secretion prior to graft removal [56]. This was confirmed by Minkowski [57] in 1892, and by Thiroloix, who demonstrated by obliterating the pancreatic ducts with oil and lamp-black, that the fibrosed pancreas could likewise maintain normoglycemia in dogs [58]. Laguesse in 1893 not only proposed the name "islets of Langerhans" but also voiced for the first time the opinion that the islets were the seat of the internal secretion that served a regulatory function in carbohydrate metabolism [59]. Finally, in the early 1900s, after the link between diabetes and an abnormal histology of the human islets had been established by Opie and anatomical studies by Ssobolew had demonstrated that no diabetes develops after duct ligation induced atrophy of the acinar tissue as long as the islets remained intact [60], it was generally accepted that the islets produced a hormone — termed "insulin" by De Meyer in 1909 or "isletin" initially by Banting and Best [61, 62] — that plays an essential role in the regulation of glucose homeostasis.

Towards a cure of diabetes

Insulin administration

After initial unsuccessful attempts, from 1892 by Minkowski [57] and a host of other investigators, to treat diabetes by injection of pancreatic extracts, promising blood glucose lowering effects were finally obtained in animals and man by Zülzer in 1904, Paulesco in 1921, and Banting and Best in 1921 [55, 62, 63]. Toxic side-effects of these extracts, however, clouded the interpretation of data, and denied further clinical application, until Collip, Banting, Best, and McLeod discovered the purification of insulin which allowed the wide-spread successful treatment of diabetes from 1923.

Free grafts

The first attempt to treat insulin-dependent diabetes with pancreatic islet transplantation was performed by Williams and Harsant in 1893, who transplanted three pieces of a freshly slaughtered sheep's pancreas subcutaneously in a 15-year-old boy [64]. From this and similar experiments with free pancreatic grafts it became evident around the turn of the century that the survival of free grafts was threatened by necrosis due to the bulk of tissue and by autolysis due to the digestive enzymes [65–67]. Because the exocrine pancreas appeared unnecessary for treatment of diabetes, other approaches were adopted that aimed at transplantation of the islets without the potentially harmful exocrine pancreas. These new approaches comprised transplantation of either (i) islet-rich tissue such as the fetal or neonatal pancreas — and an allograft of cultured insulinoma tissue in one patient with transient success, by Gaillard at our institution (Lab. of Cell Biology and Histology) in 1944 [67] — , or (ii) islet-enriched, so-called, "modified" pancreatic grafts by duct obstruction of the pancreas, which results in atrophy of the exocrine tissue whilst largely preserving the islets [66, 67] — a method first proposed by Ssobolew in 1902 as "a means of isolating the islets ... and organotherapy for diabetes in a rational manner" [20]. An appropriately aged fetal or neonatal pancreas will provide islet-rich tissue that may continue to develop without the potential of autolysis. An initial intramuscular allotransplant attempt in man in 1928 was unsuccessful, but from the 1950s experiments in rodents have established the selective long-term survival of islets in these free grafts at several sites together with amelioration of the diabetic status of the recipient [66]. Over the past decade human fetal tissue has been shown to survive for up to a year in insulin-dependent patients and some graft functioning has been demonstrated; however, no substantiated cases of reversal of diabetes have been reported.

Progress in this field of transplantation has been slow, probably because only a few groups have committed themselves to this field of islet transplantation, or perhaps for reasons of lack of tissue supply and ethical problems [68, 69]. Attempts to transplant the "modified" pancreatic grafts were successful, provided they were either finely minced to the size of large islets [65], or vascularised [58, 66, 67].

Vascularised grafts

The first successful transplants of vascularised pancreatic grafts were made in dogs in the 1920s. Further refinement of pancreas transplantation techniques worked out in the 1950s and 1960s led to the first clinical attempts at vascularised pancreas allotransplantation from 1966 [67]. Most of these first attempts with pancreaticoduodenal grafts failed, mainly due to exocrine leakage that led to poor graft survival and high mortality, and this subdued the initial enthusiasm about clinical pancreas transplantation. Pancreas transplantation revived following the introduction in 1977 by Dubernard and coworkers [70] of the new method of obliterating the ductal system of a segmental pancreas with a solidifying polymer (neoprene), which induces a chronic pancreatic inflammation, leading to atrophy of the exocrine pancreas. This technique avoided most of the complications after ligation of the ducts such as possible leakage of the pancreatic enzymes through dissected lymph vessels, and also avoided the need for an anastomosis of the ductal system for the drainage of acinar enzymes to a hollow organ such as the small bowel — and thereby similar risks of leakage of the enzymes. Segmental duct-obliterated pancreas transplantation evolved to the first widely applied islet replacement technique in diabetic patients. The safety of this method is the most important advantage, but the long-term glucose control is subject of much discussion [71–80], and this modality of vascularised pancreatic islet transplantation has recently been superseded by an improved method of transplantation of the vascularised whole organ with bladder drainage of the exocrine secretion [25]. According to the International Pancreas Transplant Registry data close to 5000 pancreas transplants were performed since the revival of pancreas transplantation from 1977, and experienced centres currently report patient survival of over 90% and graft survival rates of 80-90% [25, 71].

Transplantation of collagenase-isolated islets

Basics. In mammals including man, the islets of Langerhans vary in size from roughly 0.05–0.5 millimetre in diameter, and are scattered throughout the

pancreas. The total number of islets in the human pancreas varies from about 0.5 to 2 million, and the total weight of these islets roughly amounts to 1 gramme — corresponding to approx. 1.5% of the adult human pancreas of ~70 gramme. An islet comprises from a few cells to several thousands cells of mainly four types, i.e.: glucagon secreting alpha cells, pancreatic polypeptide secreting PP cells, somatostatin secreting D-cells, and mostly (60–80% of all cells) the insulin secreting ß-cells. The islets and the acinar structures in the pancreas of most mammals are surrounded and held together by a fine network of collagen-containing fibrous tissue and large inter-lobular septae, which can be degraded at 37°C using a collagenase-containing enzyme complex. After collagenase digestion of the pancreas the tissue is further dissociated mechanically e.g. by gentle shaking, or aspiration through needles to release the isolated islets. Large non-digested structures such as the capsule of the pancreas, ducts, and vessels, are retained by sieving. Because the density (specific gravity) of islets usually differs from the density of non-islet tissue, the islets may be separated by centrifugation in density solutions to obtain a pure islet suspension for transplantation by infusion in organs such as the liver and spleen.

Rodent islet isolation and first clinical attempts. Tedious microdissection techniques to isolate a small number of large islets for chemical and physiological experiments were reported by Bensley as early as 1911 [81], and Hellerström in 1964 [82], but Moskalewski's finding in 1965 at our institution (Lab. of Cell Biology and Histology) that the islets are released in large numbers from the chopped rodent pancreas after exposure to the digestive action of collagenase [83], opened the door to transplantation experiments. Two years later, Lacy and Kostianovsky [84] refined this method by (i) intraductal infusion of a saline solution resulting in distension of the rat pancreas prior to the chopping and collagenase digestion steps, and (ii) introduction of the concept of density gradient purification of islets — albeit using rather unsuccessful sucrose gradients. By intraductal distension, the mechanical disruption of tissue was improved, and thereby the exposure of tissue to the digestive collagenase action, which resulted in higher islet yields. Moskalewski demonstrated in 1969 the survival of collagenase-isolated islets from guinea pigs in culture and after transplantation in the pancreas and at other sites [49]. Next, in the early 1970s, a surge of interest developed in islet transplantation following reports of the amelioration of diabetes after transplantation of collagenase-isolated islets in the rat by Younoszai and coworkers in 1970 [85], and Ballinger and Lacy in 1972 [86] — who further introduced the now widely

used Ficoll density gradient technique for islet purification [81]. The several hundreds of islets needed to normalise the glycemia in diabetic rats for these and subsequent isogenic transplantation experiments were obtained from multiple donors (~3-6 animals). Besides separation of the islets by centrifugation in Ficoll density gradients, hand-picking of islets by pipette was used — and still is used [48, 87–89] — if high quality purification of islets is required, to exclude either contamination with non-islet tissue, or possible detrimental effects of density gradients. This protocol became the standard for rodent islet isolation during the next decade [81]. Subsequent application of this method to the pancreas of large animals and man was far less successful [81], due to the different more compact and fibrous nature of large mammalian pancreases. Since after collagenase dissociation of the large mammalian pancreas, islet-containing pancreatic microfragments rather than free isolated islets were obtained no efficient density purification was possible, but abandonment of the purification step nevertheless allowed successful intrasplenic autotransplantation of these pancreatic microfragments in pancreatectomised dogs from 1976 [90, 91]. In the late 1970s, several investigators transplanted non-purified human pancreatic microfragments into patients with diabetes [92]. As a result one IDDM patient was reported in 1979 in Zürich by Largiader to have stopped daily insulin therapy after receiving an intrasplenic allotransplant of pancreatic microfragments simultaneously with a kidney [93]. Generally, however the outcome of these first clinical islet transplantation attempts had been disappointing. The problems included apart from an insufficient islet mass, inadequate immunosuppressive therapy and complications of portal hypertension and disseminated intravascular coagulation from inadequate graft purity after allo- and autotransplantation [92, 94]. It was obvious that new methods tailored to the isolation of islets from large mammalian pancreases were essential.

New concepts for large mammal islet isolation. The major breakthrough for large animal islet isolation was the inclusion of collagenase in the intraductal perfusion solution — first introduced in dog experiments by Horaguchi and coworkers in 1981 [95]. By using the duct for collagenase delivery the dissociation of the large mammalian pancreas was improved to the point that first (i) a superior functional outcome after autotransplantation of pancreatic microfragments in dogs was obtained [96–99] and next (ii), after slight modifications, islets cleanly separated from acinar tissue could be obtained from the human pancreas by Gray and coworkers in 1984 [100]. Further refinement of several steps in large animal islet isolation procedures led to

more efficient isolation of islets cleanly cleaved from acinar tissue and thereby allowed efficient subsequent density gradient purification, which in the late 1980s led to successful transplantation of purified islets in large animals, and finally in man. These refinements comprised:

- new assessment methods that allowed direct comparison of the isolation outcome between centres [101–104]
- improved control over the temperature, pressure, and flow during intraductal collagenase delivery, either by injection or using a pump [92, 105, 106]
- the use of new collagenase preparations, and improved control over the conditions during digestion [106, 107]
- emphasis on a gentle approach to prevent fragmentation of islets [92, 106]
- substitution of solutions specifically designed for the hypothermic preservation of tissue, for the conventional physiological-salt type solutions during the cold phase of islet isolation — first introduced in 1989 by our group [108, 109]
- and, modifications by Ricordi and coworkers [110] of the continuous digestion-filtration method [81, 111] that allows the continuous collagenase digestion of large pancreatic fragments in a perfusion chamber equipped with a mesh screen and simultaneous release of isolated islets, which are saved from further digestion [92].

First in 1986, Alejandro and coworkers — who at that time used a rather complex method of collagenase perfusion via both the ducts and veins of the pancreas [112] — reported the isolation of a sufficient number of purified islet cells from one pancreas to allow autotransplantation in dogs, and soon other centres reported successful islet auto- and allotransplantation in dogs and monkeys [104, 113–116]. These experiments finally led in 1990 to the first well-documented report, from the St. Louis group [51], of insulin-independence in a type-1 diabetic recipient of a highly purified adult islet allograft.

Clinical islet transplantation. According to the International Islet Transplant Registry data [117] 214 adult islet allografts and one adult islet xenograft have been performed in humans between Dec 12, 1893, and Dec 31, 1993, at 30 different institutions (Table I.1). The total number of diabetic patients reported to be insulin independent (for at least 1 wk) after adult islet allotransplantation through Dec 31, 1993, was 25. Among these, two type-1 diabetic recipient of an islet allograft (after or simultaneous with a kidney allograft) have been insulin independent for ~2.5 years, and one pancreatectomised recipient of a

simultaneous islet and liver allograft has sustained euglycemia in the absence of exogenous insulin for more than 4 years now. Thus the feasibility of clinical islet transplantation has been proven.

Table I.1

Islet Transplant Registry summary of adult islet allografts (and one xenograft*) through December 31, 1993

Institution	Year of transplant	No. of cases
Bristol*	1893	1
Newcastle-upon-Tyne	1916	2
Padova	1927	2
New York	1935	1
Leiden	1944	1
Petah Tikva	1968	1
Minneapolis	1974–1993	38
Zurich	1977–1988	8
Genoa	1978–1979	13
Hannover	1978	2
Detroit	1980–1985	7
Giessen	1980–1993	8
East-Berlin	1982–1987	8
St. Louis	1985–1993	25
Miami	1985–1993	13
Paris	1988–1991	7
Perugia	1989–1991	5
West-Berlin	1989	1
Edmonton	1989–1993	5
Milan	1989–1993	15
St. Louis/London, Ontario	1990–1992	4
Pittsburgh	1990–1993	30
Leicester	1991–1992	3
Oxford	1991–1993	3
Charlestown	1991	2
Los Angeles I	1992–1993	4
Madrid	1992–1993	3
Los Angeles II	1993	1
Verona	1993	1
Homburg/Saar	1993	1

In total 215 islet transplants were performed in 30 institutions.
Data from ITR Newsletter No. 5 Vol.4(No. 1), Hering et al. (eds) Giessen, 1994

Rationale of the studies in this thesis

Although it can be concluded, as noted above, that clinical islet transplantation is technically feasible and insulin-independence can be attained, as yet many problems still prevent the large-scale clinical application of this technique [21, 32, 94, 118–121] — but potential solutions are appearing in outlines.

Problematical issues include:

- the condition of donor tissue, control of the conditions during islet isolation, the variability of islet isolation outcome
- the quality control of yield, purity, integrity, viability, and sterility of islets
- the diagnosis and prevention of rejection of the grafts
- ß-cell toxic side-effects of immunosuppressive drugs such as CsA
- the beneficial or detrimental effects of exposure of the islet tissue to unnatural environments after implantation in e.g. the liver or thymus
- the metabolic sequelae of isolating the islets from their natural environment
- the impact of the metabolic state of the recipient on graft function and survival.

The studies in this thesis addressed some of the aspects of: (i) the variability of islet isolation outcome, (ii) the conditions during islet isolation, and (iii) glucose regulation after islet transplantation — with special attention to the importance of insulinotropic gut hormones for isolated islet function.

These issues were studied in a dog model, because (i) previous metabolic data and pancreas transplantation related experience were obtained in this model (ii) the anatomy of the dog's pancreas is similar to the human pancreas regarding the technical isolation-related problems, and (iii) the logistics of large-scale islet isolation and transplantation can be developed in this large animal.

Donor-related and isolation-related variability

The variability of islet isolation outcome is considered a major obstacle for large scale clinical application. Both donor-related variables and conditions during islet isolation may determine the pretransplant quality and quantity of isolated islets. In order to study the efficacy of islet isolation, however, new methods and parameters for assessment are needed.

In previous studies morphometric assessment of the total volume of isolated islets was reported, but the efficacy of islet isolation was indirectly and not directly addressed, because the isolation outcome was not compared with the native volume and size of islets in the pancreas before isolation. Therefore a

new approach in islet isolation assessment was adopted: the morphometric assessment of the islets in the native pancreas, and after collagenase isolation of the islets, in order to quantitate (i) the efficacy of islet isolation, and (ii) the variance due to interindividual differences in donor characteristics such as age and body weight and differences in islet content of pancreases. This study is reported in Chapter 2.

Assessment of islet isolation outcome

Assessment of islet isolation outcome is generally restricted to the measurement of the volume of isolated islets, and rather subjective measures of the purity of the islet preparation after collagenase dissociation of the pancreas and subsequent islet purification in density gradients. In addition, convenient biochemical assessment of insulin and amylase yield has been reported in the past. These biochemical parameters are generally no longer reported, because many factors during organ procurement and subsequent islet isolation are considered to potentially affect the viability or quality and thereby the insulin and amylase content of pancreatic tissue. Actually, both the outcome per se and the assessment of the outcome of large mammalian, especially human, islet isolation is generally considered uncertain. Therefore, the islet and acinar components of the intact pancreas and isolated tissues were examined using both morphometric, biochemical, and histological parameters to, (i) study the outcome of collagenase isolation and density gradient purification with respect to the yield, integrity, and purity of islets; (ii) to determine the impact of the donor pancreas, and the isolation and purification steps on the variability of outcome; (iii) for comparison of both (morphometric and biochemical) assessment methods, and (iv) to study new parameters based on both these methods. This study is reported in Chapter 3.

Islet preservation in the University of Wisconsin solution

All previous islet isolation methods used conventional physiological salt-based solutions during islet isolation. After collagenase digestion of the pancreas the islets are generally, however, isolated in the cold (a non-physiological condition), and the tissue is thus in a situation analogous to the cold stored

Table I.2

Comparison of the RPMI solution and the University of Wisconsin solution (UWS)

Components (mM)	RPMI	UWS
Cations		
Na^+	114	30
K^+	5	120
Mg^{2+}	0.4	5
Ca^{2+}	0.4	-
Anions		
Cl^-	110	-
lactobionate$^-$	-	100
HCO_3^-	5	-
SO_4^{2-}	0.4	5
PO_4^{2-}	6	25
Saccharides		
glucose	11	-
raffinose	-	30
Specific components	metabolites/Hepes	antioxidants/ Pentastarch

The mechanism of hypothermia-induced cell swelling is well-known. In the cold the ionic pumps in the cell membrane are suppressed, which leads to cell swelling — due to intracellular accumulation of sodium, chloride, and water — when the cell is bathed in a solution similar to the extracellular fluid (containing high sodium and chloride). The UWS has been designed to minimise cell swelling during cold storage of the pancreas — essentially by replacing chloride with lactobionate, which due to a larger molecular weight is relatively impermeable across the cell membrane. Likewise glucose is replaced by the larger raffinose molecule, and the impermeant Pentastarch also counteracts cell swelling.

organ. Therefore, it seemed more logical to use the University of Wisconsin solution (UWS) — a solution originally designed specifically for preservation of the pancreas during cold storage prior to pancreas transplantation [122–124] — for islet isolation, and the effect of substitution of the UWS for the conventional RPMI tissue culture solution was explored. Next, after finding that islet isolation in the UWS greatly improves the outcome of density gradient purification [108–109]: the hypothesis was formulated and examined that (i) the UWS prevents cell swelling at low temperature during islet isolation and purification, and (ii) thus, preserves the normal density difference between islet and non-islet tissue, and, thereby, allows complete purification in density gradients, and further (iii) may improve the viability of islets. This study is presented in Chapter 4. The components of the UWS and the RPMI tissue culture solution that are important for cell volume regulation are illuminated, in Table I.2.

Metabolic control by isolated islets

The thesis that diabetic complications are halted or prevented by continuous precise glycemic control is central to the concept of islet transplantation. Therefore, it is essential to establish whether islet transplantation can produce adequate long-term metabolic control. The main focus in the islet transplantation field has been on technical and immunological issues, and metabolic control generally received scant attention. The clinical work is still anecdotal, and experimental studies in preclinical models are generally confined to intravenous glucose tolerance testing — thus, detailed studies are needed, that address the insulin secreting capacity, insulin action, and particularly the performance of transplanted islets under normal physiological conditions [125].

The normal native islet function is a result of the anatomical and physiological neural, hormonal, and other interrelations of the gastro-entero-pancreatic axis, at several levels.

- At the bottom level of the individual islet, the islet architecture determines (i) the path of the intra-islet blood flow, (ii) intercellular communication via gap-junctions, and (iii) paracrine effects of the islet hormones, insulin, glucagon, pancreatic polypeptide, and somatostatin [18, 126, 127].
- At the next level within the pancreas: the islets are interrelated via an intramural network of the intrinsic nerves, which co-ordinates the pulsatile delivery of islet hormones [128].
- At the top level of the gastro-entero-pancreatic axis (and interrelated

systems, e.g. the central nervous system) the islets communicate via nutrients and both neural and hormonal interrelations with other organs and the exocrine pancreatic component [129–132].

Pancreatic islet isolation and transplantation leads to destruction or disturbance of at least some of these interrelations. The issue of the extent of isolation of islets after pancreatic islet transplantation and the repercussions for glucose regulation are addressed in the next chapters.

Metabolic sequelae after duct obliteration induced 'isolation' of the islet cells. Our previous studies have demonstrated that duct obliteration induces complete atrophy of the exocrine tissue, reduces the islet mass to roughly 50% of the normal endocrine mass, and disrupts the normal native islet architecture. These histological changes are accompanied with a deterioration of glucose tolerance and insulin secretion during intravenous glucose stimulation tests within the first posttransplant month [72–76]. A study of the concurrent interruption of the enteropancreatic axis is reported in Chapter 5. The functional data in this study demonstrated that duct obliteration not only eliminates the exocrine pancreas but also results in the in vivo intrinsic denervation of the islets. Thus the duct obliterated pancreas graft eventually ends as a graft of 'isolated' islet cells. From the metabolic data in this study it was suggested for the first time that following intrinsic denervation after pancreatic islet transplantation non-pulsatile delivery of the islet hormones may lead to postprandial hyperglycemia, and a hyperglycemia-enhanced enteroinsular axis may contribute to postprandial normo- or hyperinsulinemia after transplantation.

Physiological, insulinotropic effects of gut hormones during in vitro perifusion of isolated islets. The duct obliteration study suggested the importance of hyperglycemia-enhanced insulinotropic effects of gut hormones after pancreatic islet transplantation. However, direct effects of physiological ('circulating') levels of potential insulinotropic gut hormones such as cholecystokinin (CCK), gastric inhibitory polypeptide (GIP) and the recently discovered incretin glucagon-like peptide (GLP-1) on isolated islets had not been reported. Therefore, the insulinotropic effects of these hormones were tested during in vitro perifusion of collagenase isolated islets at glucose levels as observed postprandially after pancreas and isolated-islet transplantation. This study is reported in Chapter 6.

Metabolic control after autotransplantation of isolated islets. Two consecutive series

of detailed metabolic studies after autotransplantation of collagenase-isolated islets in pancreatectomised dogs were performed. The first series of metabolic studies compared preoperative and postoperative data in dogs (that were their own controls) with emphasis after islet autografting on the natural history of the grafts, the comparison of intravenous versus oral stimulation tests, functional assessment of possible re-innervation, the metabolic contribution of the four major islet hormones (insulin, glucagon, pancreatic polypeptide, and somatostatin), the graft's insulin secreting capacity, and the predictive value of early posttransplant metabolic parameters for graft's life expectancy. This study is reported in Chapter 7. The second series of metabolic studies was performed in dogs with established islet autografts at 6 mo posttransplant (*vs* normal controls) with emphasis on the contribution of the insulin secreting capacity and intrinsic denervation of the islets to insulin resistance after grafting, the correlation of the insulin secreting capacity and action with postprandial performance, and the in vivo incretin effect during infusion of the major gut hormone GLP-1 at hyperglycemia after islet transplantation. This study is reported in Chapter 8.

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