17
Diabetes Mellitus and Closed-Loop Insulin Delivery

GARY ADAMS[1], JAN CLARK[2], TARSEM SAHOTA[2], SANGEETA TANNA[2] AND M. JOAN TAYLOR[2]*

[1]*School of Nursing, University of Nottingham, Dukeries Centre, The King's Mill Centre, Mansfield Road, Sutton-in-Ashfield, Nottinghamshire NG17 4JL, U.K. and* [2]*School of Pharmacy & Pharmaceutical Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, U.K.*

Introduction

Diabetes mellitus is presently one of the major health problems of the European and North American continents affecting at least 6% of the population. Of these, about 10% suffer from type 1, insulin-dependent disease. Diabetes, whether insulin-dependent or not, is a leading cause of death in developed countries (Cotran *et al.*, 1994; McGee *et al.*, 1992). In 1985 the World Health Organization (WHO, 1985) expert committee on diabetes defined this disease as a state of chronic hyperglycaemia, that is to say, the state of having an excessive concentration of glucose in the blood. The causes of hyperglycaemia are insulin deficiency or ineffectiveness, which leads to defective carbohydrate utilization and resultant aberrations in lipid and protein metabolism. The clinical manifestations of a patient presenting with this disease are thirst, hunger, fatigue and glucose in the urine. Depending on the stage and type, this may progress to stupor, coma and death. The symptoms may be less evident in type 2 patients in whom the secondary complications of diabetes may give rise to the presenting symptoms.

The incidence of diabetes is rising sharply worldwide (Amos *et al.*, 1997) but this is a disease that has affected man for millennia, the symptoms being described in the Ebers Papyrus of Egypt which date back to 1550 B.C. In about 200 A.D., Aretaeus of Cappadocia named the disease diabetes, the Greek word meaning to flow through a siphon (Pickup and Williams, 1997). Aretaeus described diabetes as leading to a 'moist and cold wasting of the flesh and limbs into urine'. He described the disease as chronic in character, and slowly engendered, though the patient 'does not survive long when it is completely established, for the marasmus produced is rapid and death speedy'. In the 6th century, Hindu physicians recognized that the urine from the

*To whom correspondence may be addressed (mjt@dmu.ac.uk)

Biotechnology and Genetic Engineering Reviews – Vol. 17, August 2000
0264–8725/00/17/455–496 $20.00 + $0.00

diabetic patient was sweet, although it was eighteenth century physicians who described it as tasting like honey (Montague, 1983).

In 1889, von Mering and Minkowski carried out experimental work regarding the pathology of diabetes (von Mering and Minkowski, 1890) and in 1869, Langerhans described an unknown cell type of the pancreas as consisting of 'tiny heaps'. Later Laguesse suggested that these cells be named the islets of Langerhans (Laguesse, 1893).

The histological examination of distinct cell types followed, as did the extraction and identification of an antidiabetic substance. Banting and Best, in 1921 successfully produced insulin preparations that gave rise to fairly reproducible euglycaemic states in diabetic patients (Banting and Best, 1922). Insulin replacement therapy has formed the basis of treatment for the more extreme form of diabetes ever since, preventing countless premature deaths in the process. Increasingly, it has also been used to improve the health of type 2 diabetics where necessary. The structure of insulin was elucidated in 1953, for which Sanger won the Nobel Prize in 1958 (Sanger, 1959), and this has been followed by increased understanding of the procedures by which insulin therapy could be improved with genetic engineering, synthetic chemistry, formulation and administration techniques. Despite these advances, however, there are serious long-term implications for patients treated with either insulin itself or oral antihyperglycaemic agents. The problems relate to the clear vulnerability of diabetics to cardiovascular conditions, renal disease, gangrene and blindness, among other well-recognized complications. Diabetes remains a serious health hazard and a severe drain on health services throughout the world for this reason.

The purpose of this article is to explain the normal feedback mechanisms in glucose control, to describe the aetiology and conventional treatment of diabetes and, finally, to show how specific improvements in treatment regimens could improve long-term outcomes for sufferers of this disease.

Structure of the pancreas and islet cells

The pancreas is a fleshy, wedge-shaped organ located just below the diaphragm. The duodenum curves around its wider part and the remainder lies behind the stomach extending laterally so that the narrower end (the 'tail') contacts the spleen. It has a mixed function, such that ducts carry digestive enzymes from it to the small intestine, in addition to the endocrine activity associated with the islets of Langerhans, of which there may be up to two million in the adult pancreas. The islets are discrete, rounded clusters of cells most numerous in the tail of the organ, varying in size and in the number of cells they contain (Williams and Pickup, 1998). They comprise several different endocrine cells, each of which is responsible for the secretion of one or more hormones. Approximately 70% of the main cell types are insulin and amylin-secreting cells (B or β) which form the centre of each islet, 20% are glucagon-secreting cells (A or α) and 5–10% are somatostatin-secreting cells (D, δ or type III). The hormones are all implicated in glucose regulation, but insulin and glucagon are the major players. The remaining 1–2% of islet cells (PP or F) secrete pancreatic polypeptide (Montague, 1983) which is concerned with the control of pancreatic exocrine function. Other minor populations have also been identified.

Chemistry of insulin

Sanger (1959), showed that bovine insulin consists of two chains, an A chain of 21 residues and a B chain of 30 residues, which are covalently joined by two disulphide links (A7–B7 and A20–B19). A third such bridge links 6–11 on the A-chain.

Insulin has a monomeric molecular weight of approximately 6 kDa and is normally present in the circulation in this form. The monomer predominates in low concentration and in alkaline zinc-free or organic environments (Brange *et al.*, 1997). Insulin is, however, capable of existing in solution as equilibrated concentrations of the monomer, dimer and hexamer. All the species are soluble at pH values above and below the isoelectric point at pH 5.4, providing no destructive fibrillated forms have developed. Fibrillation is a problem associated with the monomer and its hydrophobic interface. It occurs irreversibly as a result of shear or exposure to hydrophobic surfaces and causes loss of biological activity (Brange *et al.*, 1997; Quinn and Andrade, 1983). The dimer and hexamer associations are, by contrast, reversible (Ege, 1986), do not interfere with bioavailability and occur also in the pancreatic β-cell during secretion and storage processes. Bovine insulin differs from porcine and human insulin in the order and identity of certain amino acid residues, the variations affecting the crystalline form and the solubility of the protein (Brange *et al.*, 1987; Smith *et al.*, 1984; Trehan and Ali, 1998; Yip *et al.*, 1998). The more recently introduced synthetic lispro insulin has yet a further variation of its amino acid sequence which in fact precludes the formation of the hydrophobic surfaces which foster dimer formation. Its exclusively monomeric form makes it faster acting than conventional soluble insulin forms because of the raised diffusion coefficient (Burge and Schade, 1997, Burge *et al.*, 1997; Hoffman and Ziv, 1997; Holleman and Hoekstra, 1997; Kucera and Graham, 1998; Wilde and McTavish, 1997).

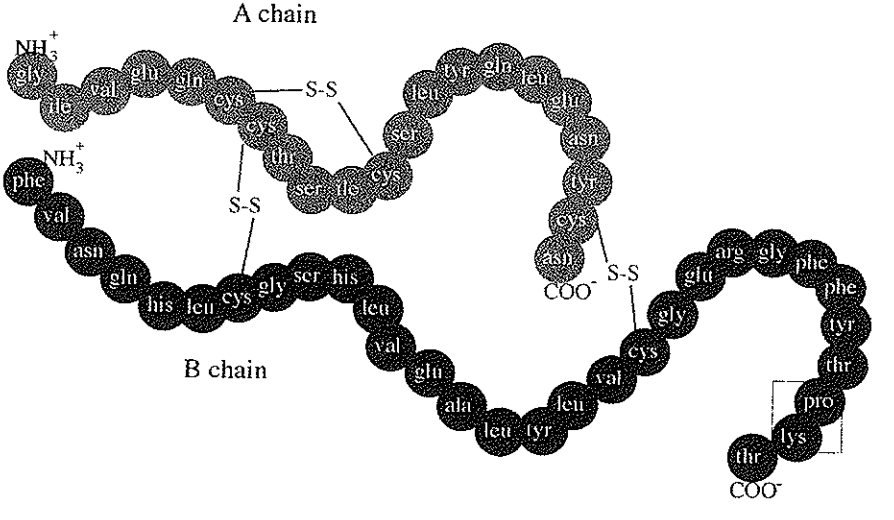

Figure 17.1. Human insulin comprises two polypeptide chains and three disulphide bridges. Changing the order of lysine and proline in the B chain, disables the formation of hexamers.

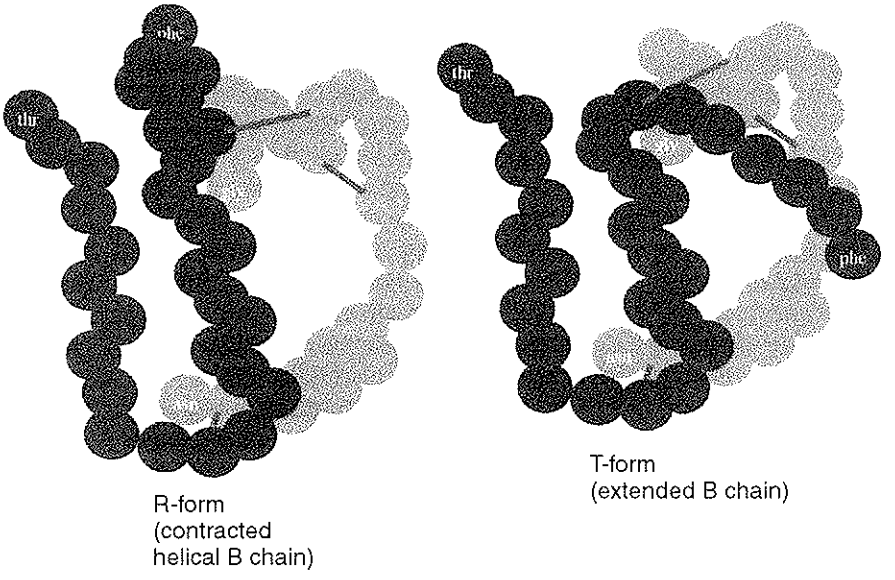

Figure 17.2. The chains can be arranged in more than one three-dimensional shape, such that the B chain may either be extended (T form) or contracted into a helix (R form) which allows access to the interior.

In the three-dimensionally folded state, the B chain of each monomer can be either extended or contracted into an α-helix.

These two possibilities give rise to the so-called T and R states respectively (Chang *et al.*, 1997; Rahuel-Clermont *et al.*, 1997) in which the N terminus of the B chain moves through more than 30Å. This flexibility has relevance for the association behaviour of insulin and thus for the design of non-associating variants (Bakaysa *et al.*, 1996; Chang *et al.*, 1997; Jacoby *et al.*, 1996). Two trimeric structures with the B chain in T or R position, interdigitate to produce the dimer structures within each hexamer. The hexamers thus have the three possible forms T6, T3R3 and R6.

Two zinc molecules are held within the longitudinal axis of this globular structure. Histidine residues from three monomer B chains act as coordination contributors to each zinc. During the transition from the T shape to the open R shape, the octahedral coordination of the zinc gives way to a four or five membered coordination involving and provoked by anions such as chloride and other small molecules such as phenols which locate in hydrophobic axial pockets. Phenolics and chloride are present in insulin formulations for preservation, buffering and isotonicity. They contribute, therefore, to the physicochemical state of the insulin in addition to their intended effects. Most formulations therefore involve T3R3 and R6 configuration (Rahuel-Clermont *et al.*, 1997) and this arrangement is favourable for hexamer formation, thus shifting the equilibrium away from monomers and consequent fibril formation. A similar molecular rearrangement may occur during the physiological interaction of insulin with its cellular receptor, presumably involving biochemical agents acting similarly to the *in vitro* phenols.

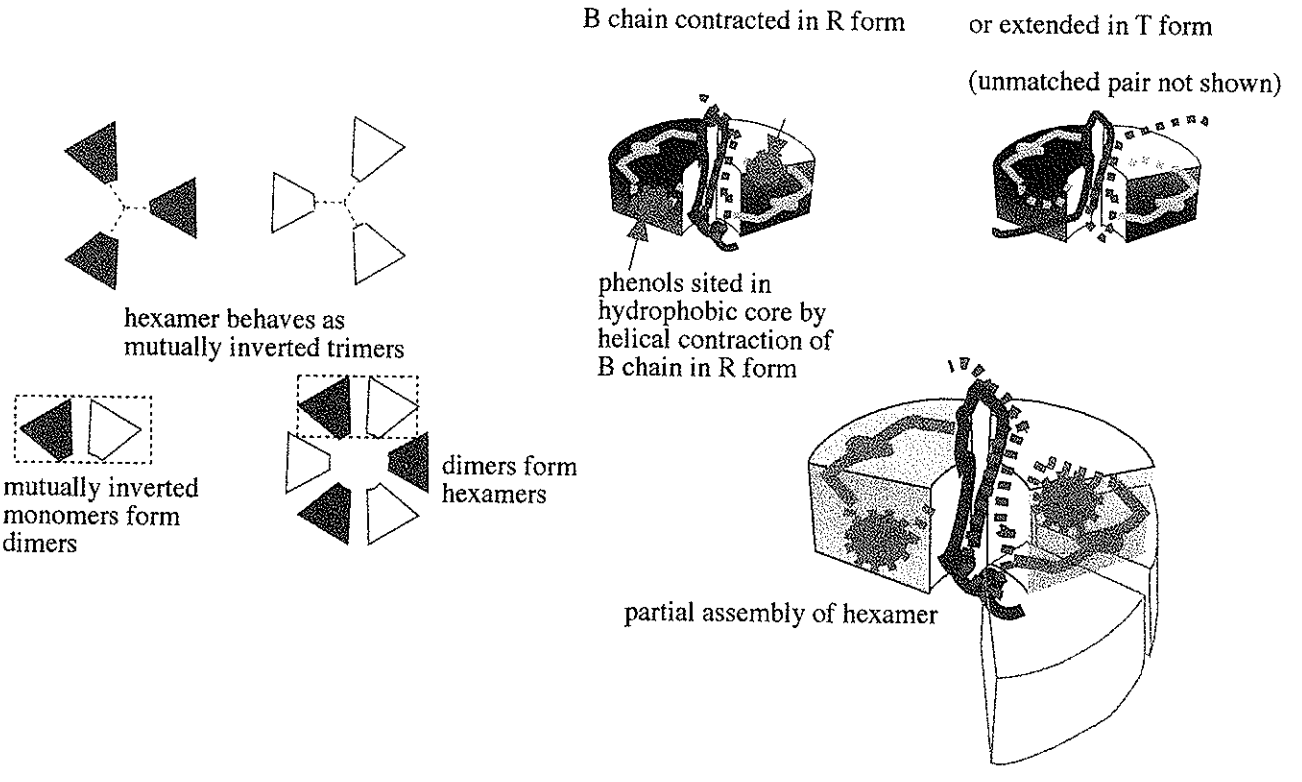

Figure 17.3. Dimers form as if paired by the superimposition of two mutually inverted trimers which may each be in T or R state. The hexamers therefore have a structure, when fully assembled, in which the monomers alternate in orientation, rounding the complex and leaving an axial space for zinc. The monomeric R form can accommodate phenol, so the resulting hexamer can contain phenol to an extent depending on the form of each of the contributing trimers.

Biosynthesis, secretion and catabolism of insulin

Glucose induces protein synthesis on the rough endoplasmic reticulum of the islet β-cell to yield pre-proinsulin (Brook and Marshall, 1996). The hydrolysis of pre-proinsulin yields proinsulin, which is then transferred to the Golgi apparatus approximately 20 minutes after the initiation of protein synthesis (Pickup and Williams, 1997). Human proinsulin is a single chain polypeptide composed of 86 amino acids and with a molecular weight of approximately 9 kDa. It contains the A and B chains of insulin linked by a connecting peptide of 35 amino acids which joins the C-terminus of the B chain to the N-terminus of the A chain. The proinsulin molecule is enclosed in vesicles or granules which carry specific proteases bound to the membrane (Greenstein, 1994). Within the next 30 minutes to 2 hours of proinsulin formation, there is proteolytic cleavage of the proinsulin to release the connecting C-peptide and insulin within the granule.

The secretion of insulin, unlike its synthesis, is stimulated by a range of secretagogues in addition to glucose, including glucagon, fatty acids and ketones. The effect of these is to induce the β-cell to take up extracellular calcium that causes microtubules to propel the insulin-containing granules towards the cell membrane. This is followed by fusion of the granule structures with the cell membrane and release of the insulin by exocytosis. A further slower release of newly synthesized insulin follows this in cases where levels of glucose (but not other secretagogues) has provoked the response. The consequent reduction of blood glucose inhibits this secretory process. Insulin release is additionally controlled by the sympathetic and parasympathetic nervous system providing a range of counter-regulatory effects which, in the healthy state, protects against hypoglycaemia among other effects. Regulation of circulating insulin levels is also maintained by the enzyme glutathione-insulin transhydrogenase which reduces the disulphide (S-S) bonds to sulphydryl (SH) groups of the insulin molecule with the separation of the A and B chains and loss of biological activity (Keele *et al.*, 1982).

Action of insulin, glucagon and somatostatin in health

Under normal physiological conditions, glucose levels are maintained between 3.5 and 6.5 mmol/L by a negative feedback system which incorporates two of the main processes, glycogenesis and glycogenolysis (Frayn, 1997) regulated mainly by the interplay of the secretion and activity of insulin and glucagon.

Insulin itself has at least six important physiological functions which are mainly centred on its role in removing glucose from the circulation after absorption from the gut and increasing glucose transport into striated and myocardial muscle cells, fibroblasts and adipose tissue cells. First, insulin facilitates the conversion of glucose into the polymer glycogen which is then stored in the liver and muscle cells from which glucose can be mobilized rapidly when required. Second, it moves glucose from plasma to peripheral cells, where glucose metabolism occurs. Third, insulin facilitates cell uptake of amino acids, increasing protein synthesis. Fourth, it fosters the conversion of glucose to triglycerides (lipogenesis) for storage in adipose tissue accounting for about 90% of stored glucose. Fifth, insulin suppresses the breakdown of glycogen to glucose (glycogenolysis) and the production of glucose from other

Table 17.1. Effects of insulin on target tissues (from Williams and Pickup, 1998)

Tissue	Effect
RAPID	
Muscle/Adipose	Increased membrane transport of glucose
Muscle/Adipose/Liver	Increased membrane transport of amino acids
INTERMEDIATE	
Carbohydrate metabolism	
Muscle/Liver	Increased glycogen synthesis
Muscle/Liver	Decreased glycogenolysis
Muscle/Liver/Adipose	Increased glycolysis
Liver	Decreased gluconeogenesis
Lipid metabolism	
Muscle/Liver	Increased lipogenesis
Muscle/Liver	Increased esterification
Adipose	Decreased lipolysis
Liver	Increased cholesterol synthesis
Liver	Decreased ketogenesis
Liver/Adipose	Increased dietary lipid utilization
Liver/Adipose	Decreased oxidation of fatty acids
Protein metabolism	
Liver/Muscle/Adipose	Increased protein synthesis
Liver/Muscle	Decreased proteolysis
LONG-TERM	
	Promotion of cell growth
	Promotion of cell division
	Promotion of DNA synthesis
	Promotion of RNA synthesis

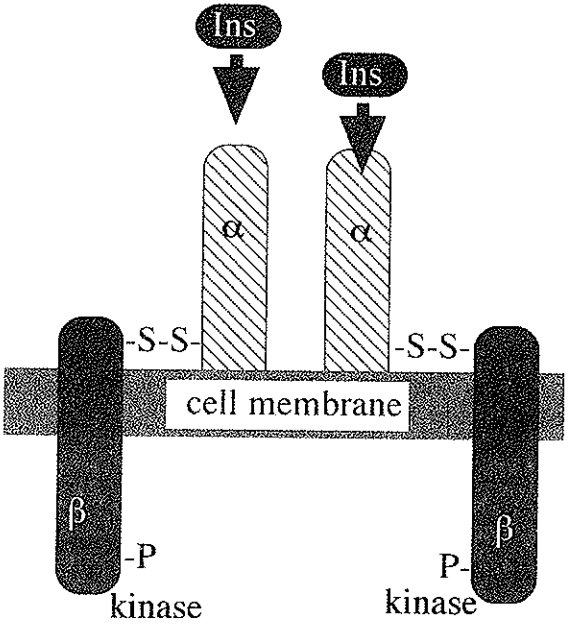

Figure 17.4. The cellular receptor for insulin comprises two surface bound alpha units and two beta units which contact the cell interior. connected by disulphide bridges.

sources such as amino acids and lactic acid (gluconeogenesis). Finally, nucleic acid synthesis and the growth and differentiation of certain cells depend on the actions of insulin (Cotran *et al.*, 1994).

Insulin action is mediated by a specific receptor on the plasma membrane of insulin-sensitive cells. The receptor consists of two alpha (135 kDa) and two beta (95 kDa) sub-units of glycoprotein structure, linked covalently to each other by disulphide bridges.

Insulin binds to those parts of the alpha sub-units which project from the cell surface into the extracellular space. The beta units project into the cytoplasm and transmit the effects of the allosteric changes induced in the receptor by the insulin binding. This results in the triggering of an intracellular cascade of phosphorylation and dephosphorylation reactions, involving ATP and magnesium, beginning with the phosphorylation of the beta sub-unit, via its associated kinase. These are translated into the observable actions of insulin, beginning with the facilitated uptake of glucose by glucose transport protein units (GLUTs). These travel from the Golgi complex to the surface where they complex with and transport glucose to the cell interior. In the

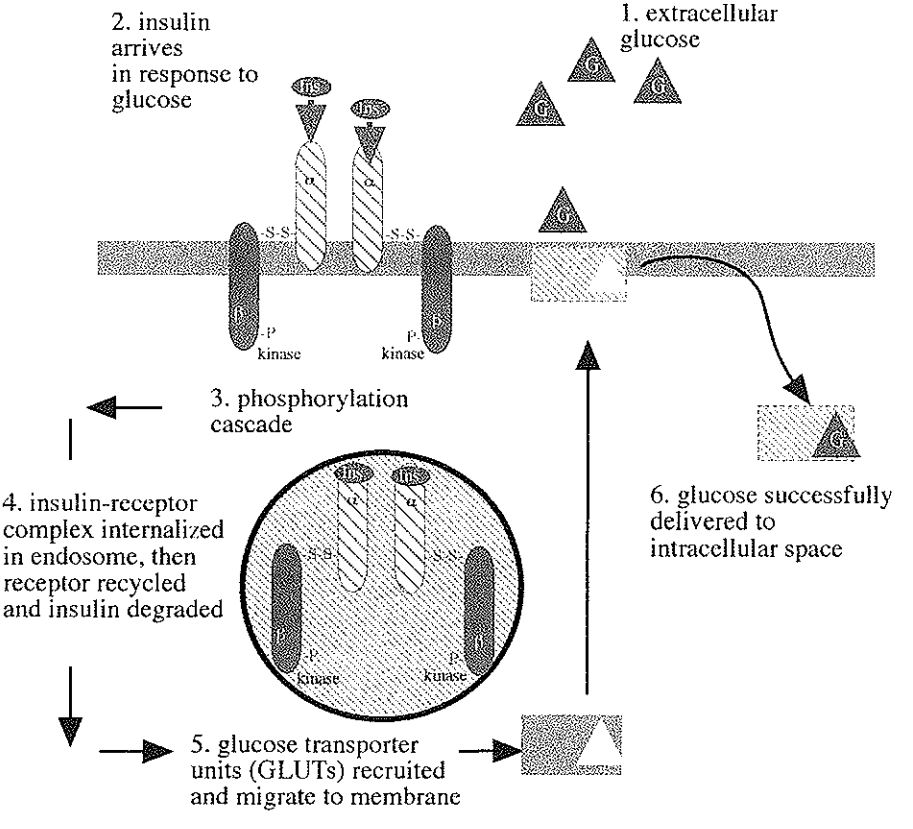

Figure 17.5. Insulin triggers a cascade of phosphorylations which result in the transport of glucose to the cell interior thus making glucose available to the cell and preventing hyperglycaemic levels extracellularly.

case of muscle and adipose tissue, the GLUTs are insulin dependent. In the β-cell of the pancreatic islets, however, they transport glucose to the cell interior rapidly and independently of insulin so that they act as a monitoring system for the extracellular glucose levels, thus governing the insulin synthesis process in those cells. Brain cells are also independent of insulin in terms of their glucose uptake.

The insulin-receptor complex formed on binding enters the cell in an endosome, after which the receptor is recycled to the membrane and insulin is degraded. Receptor internalization may limit the number of receptors available to the hormone, thus contributing to the loss of sensitivity or down regulation when circulating insulin levels are high.

Glucagon has essentially opposite actions to those of insulin, and therefore acts as a hyperglycaemic agent, stimulating glycogenolysis and gluconeogenesis (of which the former is a more important fast energy source). It causes fat breakdown from adipose tissue, releasing fatty acids and glycerol from the triglyceride stores. A reduction in the blood glucose levels below 3.5 mmol/L initiates the secretion of glucagon from the β–cells of the pancreas, mediated by adrenergic β-receptor stimulation (Frayn, 1997). Glycogen mobilization from the liver is regulated by glucagon, which activates adenylate cyclase to produce cyclic adenosine 5'-monophosphate (cAMP) from adenosine 5'-triphosphate (ATP). The cyclic AMP activates a protein kinase which converts inactive phosphorylase b to the active phosphorylated enzyme, phosphorylase a (Stryer, 1988). This results in glucose-1-phosphate being formed from glycogen.

Biochemical aberrations in diabetes

In diabetes, insulin is absent or ineffective and glucose is no longer removed from plasma to cells. Despite hyperglycaemia, the insulin-dependent cells are deprived of glucose and other pathways capable of generating glucose start to predominate. The relative concentration of glucagon in the portal supply is inappropriately high and this decreases the hepatic concentration of fructose 2,6-bisphosphate. As a consequence, phosphofructokinase is inhibited, thus stimulating gluconeogenesis (Brosnan, 1999; Greenstein, 1994). Subsequently, the catabolism of glycogen is induced and its synthesis inhibited. This concert of activity contributes further to the hyperglycaemia (McGee *et al.*, 1992) and leads directly and indirectly to effects which explain the symptoms and complications of diabetes.

Where residual insulin is present, as in the type 2 insulin-resistant states described below, hyperglycaemia may develop insidiously but to a severe degree prompting diuresis and consequent dehydration and electrolyte derangement. This may impair renal flow, which further raises blood glucose, finally provoking a hyperosmolar nonketotic crisis.

The hyperglycaemia also fosters chemical interactions leading to the formation of glycosylated products. A sugar molecule in its aldehydic form reacts non-enzymatically with protein lysines, for example, to produce Schiff's bases (Marshall, 1992). These are reversible at first but eventually undergo irreversible rearrangement and reduction and may disrupt the protein activity (Obsil and Pavlicek, 1997; Tsukushi *et al.*, 1999; Varma *et al.*, 1997) among other pathogenic effects, especially when subsequent crosslinking occurs. A variety of structural and functional proteins can be glycosylated,

such as haemoglobin and membrane proteins, particularly in vasculature, nervous tissue, renal tubules and placenta.

In insulin-independent cells, the aldehydic form of glucose is converted to sorbitol via the enzyme aldose reductase and thus sorbitol may accumulate to exert abnormal osmotic effects within the cell as a direct result of the hyperglycaemia. This is widely held responsible for nerve cell membrane damage and for ophthalmic pathologies.

Secondary metabolic effects include the mobilization of fats and proteins as sources for glucose. When fat breakdown predominates over carbohydrate metabolism abnormally like this, acetyl coenzyme A in the liver cannot enter the citric acid cycle because the concentration of oxaloacetate is low as a result of carbohydrate being unavailable (Stryer, 1988). The subsequent increase in ketone metabolites such as acetoacetic acid, beta-hydroxybutyric and acetone (Williams and Pickup, 1998) is characteristic of uncontrolled type 1 diabetes. Although ketones are an available energy source, ketoacidosis occurs when their production by the liver exceeds cellular utilization and renal excretion. The metabolic acidosis is caused by the excessive ketoacids that require buffering by bicarbonate ions consequently leading to, in turn, a lowered serum bicarbonate (Willatts, 1992). In addition, when protein is moved from muscle and used in gluconeogenesis, producing significant weight loss (in addition to that caused by fat catabolism), more ketones are produced in some cases, contributing to the problem. Lipoprotein lipase is insulin-dependent and thus lipoproteins accumulate in the blood.

Classification of diabetes mellitus

In the United States, the guidelines issued by the National Diabetes Data Group in 1979 have been used largely unaltered until recently when The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus made its report in 1997. Its recommendations were to move away from classification by pharmacological treatment to one based on aetiology, while nevertheless recognizing that the evidence for the primary cause of hyperglycaemia in a patient may often be equivocal (Alberti and Zimmet, 1998; Gavin, 1998; Krans, 1998; Wood, 1998). The committee has therefore proposed the elimination of the terms insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus (and their acronyms) but retained the descriptions type 1 and type 2 to refer to mainly auto-immune and insulin-resistant disease respectively. A malnutrition-related category has been dropped as cases were found to fall into other types, but gestational diabetes (GDM) is still recognized. The impaired glucose tolerance condition (IGT) is retained and added to it is impaired fasting glucose (IFG). It is recognized that the process causing these two conditions may be identical with that in other categories and that the demarcation between types is often not absolute or permanent.

In this report, the criteria for diagnosis of diabetes mellitus have also been changed from those used by NDDG and WHO. They rely on three diagnostic points, namely:

(1) the fasting plasma glucose above 7.0 mmol/L,
(2) the plasma glucose levels of over 11.1 mmol/L two hours after an oral glucose challenge,
(3) a combination of observed symptoms with casual plasma glucose levels greater than 11.1 mmol/L.

The finding must be confirmed by a second result on a subsequent day. The fasting plasma glucose is the recommended standard for use in epidemiological studies because it is easier and less costly to conduct than glucose tolerance tests but may slightly underestimate the prevalence of the disease compared with the oral glucose test method. The glycosylated haemoglobin test, widely used for the estimation of long-term glycaemia and compliance, is not yet recommended for diagnosis because of a lack of standardization of method and some difficulties in correlation with existing criteria.

Pathogenesis and symptoms of diabetes mellitus

Type 1 or insulin-dependent diabetes mellitus is widely accepted to be caused by a reduction in the number of insulin-secreting β–cells because of insulitis, a destructive inflammation of the islets. This is typically an auto-immune condition which may, however, be provoked by an environmental factor such as an infection or toxin (Giannoukakis *et al.*, 1999; Imagawa *et al.*, 1999; Jaeger *et al.*, 1999; Knip, 1998; Moriwaki *et al.*, 1999; Mysliwiec *et al.*, 1999; Pickup and Williams, 1997; Shimada *et al.*, 1999; Signore *et al.*, 1999). Autoantibodies are produced against targets including islet cell components, insulin and enzymes such as glutamic acid decarboxylase and tyrosine phosphatases.

As a group, patients with type 1 disease are vulnerable to other auto-immune syndromes. Sometimes it is clear that such immunological traits are inherited but there are also inherited idiopathic forms of type 1 diabetes which show no auto-immunity characteristics. This often concerns patients of Asian or African racial origin.

Type 1 disease can occur at any age but is typified by rapid onset diabetes in non-obese juveniles in which acute ketoacidosis is the presenting symptom. Even in these cases, however, there is often a recognition of a brief history of polydipsia (extreme thirst), polyphagia (constant hunger), nausea and fatigue. Glycosuria will be demonstrable but will not usually be the alerting symptom. Blood glucose levels are elevated and range from 14 mmol/L to values greater than 50 mmol/L (Porth, 1998). Once treatment has been started, there is sometimes a so-called 'honeymoon' period when the disease reverses temporarily as the pancreatic cells recover some function in the presence of administered insulin.

Progression is often slower in adults developing the type 1 disease. For this reason, the ketoacidosis with which it is associated, may not occur until the disease has been established for a long time. Ketoacidosis may sometimes also develop as a result of treatment failure (such as with blockage of automatic dosage pumps) or illness.

Type 2 diabetes is not insulin dependent, although in some cases it may eventually become so. The cause is thought to be the resistance of cells to the effects of insulin, thus producing a less than normal biological response (Montague, 1983). Insulin travels from the β-cell to a target cell through the circulation, and dysfunction at any one of these loci could influence the ultimate action of the hormone as indicated in *Table 17.2*.

Patients are typically obese (or may carry a disproportionate percentage of body fat in the abdominal region). Obesity itself is a contributing factor to the insulin resistance and the planned loss of even small amounts of weight usually improves the

Table 17.2. Possible causes of insulin resistance (from Bloom and Ireland, 1992)

Circulating insulin antagonists	Target tissue defects
Raised levels of counter-regulatory hormones: growth hormone; placental lactogen; cortisol; glucagon or catecholamines	Insulin receptor defects; decreased affinity; decreased number
Anti-insulin antibodies	Post-receptor defects; defective coupling; defective response mechanism
Anti-insulin receptor antibodies	

type 2 condition significantly. The measured insulin levels are often normal and even raised (hyperinsulinaemia) compared with non-diabetics. However, in the presence of the raised glucose levels that are typical for this disease, the insulin output should actually be very much higher still. The insulin secretory process must therefore be defective in addition to there being resistance to insulin effects. This form of diabetes is not usually associated with ketoacidosis and therefore the disease, which is mild in terms of the symptoms experienced in the early stages, may remain undiagnosed for years. However, there is a significant mortality risk associated with hyperosmolar hyperglycaemic nonketotic crises which may present with very high blood glucose levels, often in association with an intercurrent illness. The absence of the severe symptoms of ketoacidosis may mask this until too late. It is estimated that half the type 2 diabetics in the United States, for example, are unaware of their disease and so an insidious worsening can easily go unnoticed.

Certain ethnic groups face increased risk of type 2 disease and, as with type 1 diabetes, there is evidence of the disease being heritable. Type 2 diabetes has been associated with middle age and indeed the incidence rises sharply over 45 years. However, recent findings reveal an increase in the incidence of type 2 diabetes in children (Alberti and Zimmet, 1998). The typical diagnosis for maturity onset diabetes in the young (MODY) is based on diabetes in an obese, African, Hispanic or Asian pubertal individual, usually showing no ketoacidosis and no relevant antibody titre.

Hypoglycaemia is not itself a symptom of diabetes, but a hazard of treatment. It is more common with insulin therapy than with oral antihyperglycaemics and happens if the dose is inappropriately balanced with calorie intake. It occurs when blood glucose levels fall below about 2.7 mmol/L, is sudden in onset and progresses rapidly (Novak and Handford, 1998). Symptoms involve the autonomic nervous system producing pallor, sweating and tremor, but can progress to depress cerebral function producing confusion and coma (Williams and Pickup, 1998). Effective treatment consists of rapid calorie intake (McGee *et al.*, 1992) but may require emergency glucose or glucagon injection.

Long-term complications of diabetes

The long-term complications (Swidan and Montgomery, 1998) such as nephropathy (Sakai *et al.*, 1996), neuropathy (Tomlinson, 1998), retinopathy (Ruggiero *et al.*, 1997), and arteriopathy (Clements *et al.*, 1998) are widely suspected to be related to the primary and secondary biochemical changes occurring in both the major forms of diabetes. However, they could conceivably also form part of a syndrome of which

primary symptoms of diabetes are the prelude. The difference between these two possibilities is important for the aims of treatment because the imposition of very strict glycaemic control, including the development of an artificial feedback mechanism, could control the hyperglycaemia but would be a pointless effort if the sequelae occurred despite it. However, the circumstantial evidence for the effectiveness of perfect control comes from several retrospective studies, which relate the extent of control to the incidence and delay of secondary symptoms. Indeed, predictions can be made for the likely number of years before complications will arise based on the current glycosylated haemoglobin reading (Anonymous, 1999; Anonymous, 1996; Baldeweg and Yudkin, 1998; Bilous, 1999; Brankin and Fisher, 1998; DeFronzo, 1999; Leslie, 1999; Nasr *et al.*, 1999; Orchard *et al.*, 1997; Schiel and Muller, 1999; Schifferdecker *et al.*, 1994; Schorr, 1999; Turner, 1999; Turner *et al.*, 1999). This information has recently become readily available to the public on interactive web sites intended to encourage compliance by education.

The neuropathies affect autonomic, motor and sensory nerves and lead to pain and loss of sensation. The damage may result from sorbitol accumulation in the Schwann cells followed by changes in myelin lipid and myo-inositol metabolism (Frayn, 1997). Protein glycosylation may also contribute (Swidan and Montgomery, 1998; Tomlinson, 1998; Tsukushi *et al.*, 1999).

The preponderance of lipids in the circulation in diabetes may predispose to atherosclerosis (Goff *et al.*, 2000; Williams and Pickup, 1998) which causes large vessel disease mainly in the lower limbs but also in the heart and brain. The atheromatous plaque that characterizes this complication also seems to involve collagen deposition as well as the adhesion of platelets in abnormal numbers. This results in impaired blood supply to the limbs, producing impotence, ischaemic pain and damage, among other effects. Gangrene is not uncommon and the incidence of lower limb amputation is therefore high among diabetics.

Damage also occurs to the capillary circulation, principally in the kidney and retina, although other tissues are commonly affected. The abnormalities include coagulation, microthrombi and the thickening of vessel epithelia. The latter is likely to result from glycosylation (Frayn, 1997) and subsequent crosslinking of protein and other materials (Cotran *et al.*, 1994) as referred to earlier. In the retina, new vessels proliferate and leak producing extensive damage (Clements *et al.*, 1998; Orchard *et al.*, 1997). These and other ischaemic changes lead to blindness, of which diabetes is one of the leading causes in the western world. Delays in diagnosis of type 2 disease is one of the contributory factors to the prevalence of this complication (Roysarkar *et al.*, 1993), although sight deterioration may prompt the investigations leading to the identification of the cause. Lens damage also occurs in diabetes resulting from osmotic swelling from sorbitol, as described above. Both protein glycosylation and sorbitol induced disulphide bond formation may predispose to the formation of cataracts (Montague, 1983).

Diabetes is a leading cause of end stage renal failure, the preliminary symptoms of which are proteinuria and decreased filtration rate. Basement membrane thickening of both capillaries and tubules account for much of the damage, since this destroys the pore structures and therefore interferes with the exchange mechanisms in the capsule, glomerulus and tubule (Sakai *et al.*, 1996). It is also responsible for the occlusion of vessels in the glomerulus. The causes are as previously discussed for other mani-

festations of microangiopathy, namely abnormal glycosylation, which in this case is likely to change the packing and proteolysis of membrane protein components.

The treatment of diabetes mellitus and its limitations

The four main objectives in the management of diabetes (McGee *et al.*, 1992) are to

(1) preserve the life of the patients and control symptoms
(2) maintain as normal a life as is possible (in terms of quality and style)
(3) re-establish and maintain good metabolic control
(4) avoid and possibly mitigate complications

The emergency treatment for coma of either the ketoacidotic or hyperosmolar nonketotic type focuses on cautiously correcting fluid and electrolyte imbalances since the dehydration is more threatening than the hyperglycaemia. For ketoacidosis, the pH may be normalized with bicarbonate if intravenous soluble insulin administration does not accomplish this, although this is controversial in view of the effect on oxyhaemoglobin dissociation. Insulin resistance accompanies severe acidosis and thus small repeated doses of insulin are used instead of large bolus doses, until the crisis has resolved (Porth, 1998). The prognosis for hyperosmolar nonketotic coma is not as good as with ketoacidotic crises because it is often associated with a second serious illness which has created the loss of glycaemic control.

The routine control of blood glucose, however, usually differs for type 1 and type 2 patients, although it has common components. For some type 2 diabetics, the treatment consists entirely of dietary adjustment, exercise and blood glucose monitoring. However, these components are an important part of treatment for all diabetics. The tenets of good diet in this context are to manage calorie intake, avoiding carbohydrate and fat excess and aiming for regularity. Carbohydrates are better taken in polymeric form, such as starch, than as rapidly available simple sugars. Other type 2 patients will have one or more oral anti-hyperglycaemic agents added to this regimen. These may include the sulphonylurea glibenclamide, which boosts insulin secretion, and the biguanide metformin, which increases insulin sensitivity. Type 2 patients are sometimes advised to use insulin to improve the control of their condition and occasionally their disease progresses to insulin dependence.

Conversely, type 1 patients are treated only with insulin since there is no β-cell activity to stimulate with drugs such as those used in type 2 treatment. Insulin is inactivated by gastro-intestinal enzymes and must, therefore, be given parenterally. It can be injected into the upper arms, thighs, buttocks, or abdomen via the subcutaneous route. Unfortunately, peripheral circulatory flow, capillary permeability and enzymatic action may modify the availability of insulin from subcutaneous sites. All of these factors may differ between injection sites and times. The depth of injection is also an important variable, as are the local skin movement and generalized exercise afterwards, and all these can affect glycaemic control. The injection sites are used in rotation to avoid such conditions as fatty hypertrophy. Once stabilized from the emergency condition with which patients often present, the insulin injection regimens differ greatly, mainly because it is important to tailor treatment to the individual. Programmes range from one to four injections daily, using short- and long-acting

Table 17.3. Some recommended insulin regimens (from Rang *et al.*, 1999)

Insulin type	Preparation	Peak action (hours)	Duration (hours)	Comments
Fast acting	Neutral insulin	2–4	6–8	Two or more injections required if used alone
Intermediate insulins	Isophane	6–12	12–24	Twice daily injections
	Insulin zinc suspensions (amorphous; semilente)	5–10	12–16	
	Biphasic insulins	3–8	16–22	Mixtures of neutral insulin with either crystalline or isophane insulin
Long-acting	Insulin zinc suspension (crystalline; ultralente)	10–24	24–36	
	Insulin zinc suspension (mixed; lente)	6–14	18–30	30% amorphous + 70% crystalline
	Protamine zinc insulin	10–20	20–36	Given once daily

insulins alone or in combination, as shown in *Table 17.3*. Most, but not all, patients now use genetically engineered human insulin rather than animal derived types.

At one time, the treatment of diabetes involved a rigid diet and dose regimen (Unger, 1982). Most diabetics are now, however, taught to measure their blood glucose at least daily and to adjust their dose and calorie intake according to both the results they find and the guidance provided by their physicians. The aim is to keep glucose levels at or below the normal maximum but at the same time avoiding hypoglycaemia. Even if this is achieved as an average, there are certain to be oscillations which take the glucose concentration outside the tolerances (see also *Figure 17.6* and accompanying text). However, there is clear evidence that in many patients the periods of hyperglycaemia amount to a serious deviation from the intention (Orchard *et al.*, 1997; Saudek, 1993). Many diabetics ignore this because they prefer the risk of hyperglycaemia to that of the more immediately threatening hypoglycaemia, but others simply fail to realize that they are experiencing hyperglycaemia. This is because the condition itself is largely symptomless and blood tests may miss those times of the day when hyperglycaemia occurs. Since the effects of hyperglycaemia are cumulative and the incidence of complications directly related to them, it is clearly important that patients comply with treatment programmes and to encourage them to test often enough to provide the necessary evidence upon which to act appropriately. Careful control has been made simpler by the advent of blood testing kits, the results from which can be interpreted easily even by the partially sighted and which are not painful to use. Injections are also now much easier and less embarrassing to use in public (before a meal, for example), since penfill designs and ready-mixed formulations have become widespread.

Careful control can be taken to fairly extreme levels, such that so-called intensive or tight control is an approach favoured by some authorities (Anonymous, 1996; Azar and Kanaan, 1999; Leslie, 1999; Schiel and Muller, 1999). Intensive treatment involves a programme of extra blood glucose testing and smaller, more frequent insulin injections. There is a practical limit to this in terms of the acceptable frequency of daily invasive events, although some reports mention up to eight injections of soluble insulin daily (Home, 1997). It must also be important to recognize the psychological effects of such introspection. However, it is the statistical analysis of long-term trials with intensive treatment that show that patients with lower glucose levels produce years longer without kidney disease, blindness or amputation for example, compared to those on standard treatment. The main hazard of tight control is hypoglycaemia and another is the obesity sometimes induced because hyperinsulinaemia favours triglyceride deposition in fat (Montague, 1983; Pickup and Williams, 1997). However, most problems can be managed with care. One of the barriers to the use of tight control is the sheer trauma of the process. Clearly, both injection and blood testing are invasive and this is a difficult regimen to impose, particularly on children. Yet it is for the very young diabetic that tight control has the most to offer, since complications normally begin to become obvious when they are still in young adulthood. As a consequence, several experimental routes of insulin delivery have been considered as alternatives to injection. Pulmonary, nasal and oral dosage forms have therefore been proposed, such that treatment compliance and control over glucose levels can be achieved with less inconvenience and trauma. Reviews of these exist elsewhere and they will not be

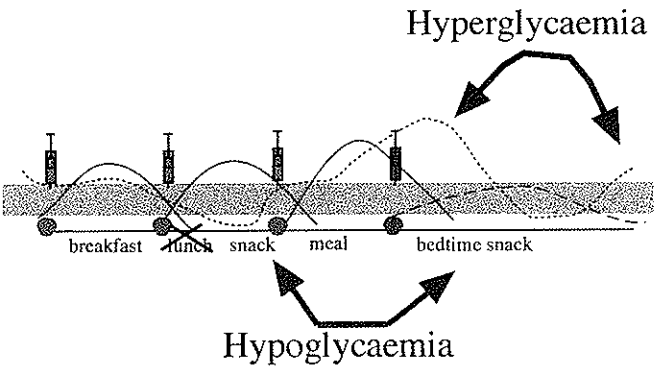

Figure 17.6. Even carefully controlled insulin therapy results in deviations from normal glucose levels. Hyperglycaemia is symptomless and cumulative in its effects.

considered here. (Chetty and Chien, 1998; Hoffman and Ziv, 1997; Pillion *et al.*, 1998; Trehan and Ali, 1998).

Continuous insulin delivery

Delivery of a bolus dose of drug to the body by any route including subcutaneous injection causes its concentration to rise soon after administration with the subsequent distribution of the drug from blood to the tissues at rates and to extents depending on the perfusion and permeability of the drug. This peak rise in concentration level of the drug is followed by a reduction as a consequence of its metabolism and eventual excretion (Rang *et al.*, 1999). Elimination processes can be quantified in terms of a plasma half-life, among other relevant pharmacokinetic terms and the frequency of dosing required to maintain adequate blood levels is an inverse function of the plasma half-life. Clearly, this method of repeated dosing leads to peaks and troughs in the blood level.

Some of the failure to control glucose levels is a result of the periods of time between bolus doses, whether by injection or by one of the proposed alternative routes mentioned. The dose has to contain enough insulin to provide control for several hours but this sometimes depresses the blood level so much that even a small delay in eating can produce hypoglycaemia. However, as the hours pass after an injection, the insulin release falls off in a first order manner as the subcutaneous depot depletes and calorie intake may well lead to high but symptomless circulating levels of glucose. The continuous subcutaneous infusion (CSII) pump was developed to overcome this problem and also to reduce the actual number of injections (Bremer *et al.*, 1997; Steindel *et al.*, 1995). This method of delivery is not a new concept, Metcalf having described it as a means of managing diabetic ketoacidosis as far back as 1934. In the most sophisticated designs, appropriate dose rates can be programmed using algorithms for both the basal dose between meals and for pre-meal boli to control post-prandial glycaemic changes. The patient only has to remember to eat on time. Pumps do not obviate the need for testing frequently and to adjust the programme appropriately, and in this respect CSII can be viewed as a logical progression from conventional intensive treatments as described above. The method has, however,

been dogged by difficulties with both ketoacidosis (Helve *et al.*, 1987) and hypoglycaemia. Other disadvantages of CSII delivery systems include higher cost, bulkiness of pumps, risk of mechanical failure (which induces the ketoacidosis), limitation in activities, and skin infection at the infusion site (Mecklenburg *et al.*, 1984). Despite several statements to the contrary (Koivisto and Tronier, 1983), another theoretical risk of CSII is the development of secondary amyloidosis due to chronic administration of insulin aggregates (Brownlee and Cerami, 1983). CSII has fallen into disfavour with clinicians in the U.K. for these reasons and because of a perceived negligible advantage over more conventional regimens. However, CSII remains popular in other parts of Europe and there are anecdotal claims that in the United States medical staff treat their own diabetes with pumps while their patients tend not to.

There are alternatives to CSII, however. The totally implantable pump, exemplified by the Minimed type, has been in clinical trial for a number of years in Europe and the United States. The device pumps insulin from a reservoir placed surgically in the abdominal subcutaneous tissue and delivers it via a catheter to the peritoneal fluid, where the dose is absorbed via mesenteric vessels into the portal supply. It is possible to programme this system by external telemetry and to refill it through a septum which can be accessed through the skin. Unlike the CSII system, it is not associated with hypoglycaemia because no depot effect is possible, insulin having a short plasma half-life, compared to its fate before absorption from subcutaneous sites. However, there is a tendency for insulin to precipitate in the pump and tubing because of the shear to which it is exposed and there have been many incidences of ketoacidosis where insulin delivery has failed for this reason (Saudek, 1993; Saudek *et al.*, 1996; Selam, 1997). However, its careful use is associated with encouraging clinical outcomes which may justify cost and risk (Anonymous, 1995; Haardt *et al.*, 1994; Lassman-Vague *et al.*, 1995; Lassman-Vague *et al.*, 1997).

Other possibilities exist for the continuous delivery of insulin with basal and boost dosing included. For example, polymer-containing formulations have long been proposed for subcutaneous implant to release the drug by combinations of erosion and diffusion. Such a system can be adapted to provide the drug on demand. This could be achieved by the incorporation of magnetic particles in the polymer so that when an oscillating magnetic field is applied, the beads vibrate, perturbing the polymer matrix and causing increased egress of the drug (Edelman *et al.*, 1984; Edelman and Langer, 1993). If such a device was loaded with a depot of insulin, this mechanism could be used to supplement diabetics' insulin delivery around meal times.

An iontophoretic technique, first described in the 1950s, is widely used in pharmacological research because it allows both the location and duration of drug release to be controlled (Tyle, 1986). It is a facilitative process for the transdermal delivery of peptides, which would otherwise be impossible to deliver across the skin (Chien *et al.*, 1987; Kanikkannan *et al.*, 1999; Pillai *et al.*, 1999). The technique involves the creation of a potential gradient between a micropipette tip, containing a concentrated solution of drug, and the surrounding tissues. This process induces the migration of ionic substances, such as monomeric insulin, through the skin with the flow of electrical current. Successful studies have been conducted in animal models (Meyer *et al.*, 1989; Siddiqui *et al.*, 1987; Stephen *et al.*, 1984) which have shown biologically effective levels of insulin being delivered. Application of iontophoresis to human insulin dosage is dependent on further development of the appropriate forms of

insulin, as well as of the equipment to achieve it comfortably and safely (Kennedy, 1991).

Closed-looped devices for glycaemic control

It is, perhaps obvious that any drug should be administered in a manner that precisely matches physiological requirement (Heller, 1997). If the therapeutic target is effectively a moving one, as with the control of blood sugar, then it follows that delivery might also need to have a flexibility that certainly could not be achieved by using the delivery systems described. Glycaemic control is difficult for two main reasons. First, the fluctuating insulin levels in the blood that result from the repeated nature of subcutaneous doses as discussed above and which can be solved by continuous administration. However, the second reason is more pressing. It is the fact that the blood glucose levels are a function of calorie intake and expenditure, neither of which are constants. In the healthy individual, the islet cells sense this fluctuation, as has been described, and insulin is synthesized and secreted to match the current need. In the diabetic, this system has broken down and the imposed insulin replacement

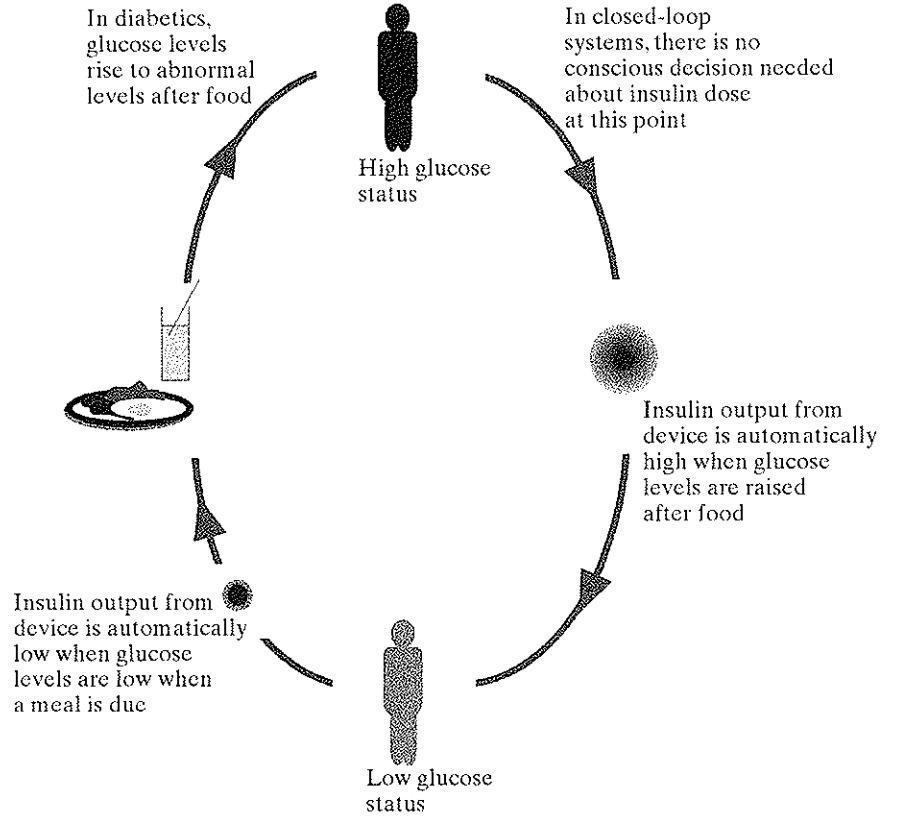

Figure 17.7. Closed-loop design.

mimics the physiological system to an extent that is only partly under the control of the patient. Unfortunately, experience shows that this approximation to glucose control does not normalize the complex metabolic disturbances that characterize the diabetic syndrome or avert many of the long-term complications of diabetes (Orchard *et al.*, 1997). There is, therefore, a need for the development of an insulin delivery system providing a continuous supply of insulin but which, unlike even the programmable pump, is able to meet the fluctuating demands of blood glucose concentrations in a self-regulating manner. A device which is able to sense blood glucose levels and then infuse the appropriate amount of insulin in a continuous feedback fashion ('artificial pancreas') was described in the early 1970s (Albisser *et al.*, 1974). Such a system is referred to as 'closed-loop'. Mechanical devices used for the delivery of insulin are therefore classified as open- or closed-looped systems, depending on whether or not they have a glucose sensor (Kennedy, 1991).

The requirements for an effective closed-loop system are as follows. First, the system should respond to glucose in a highly specific way. Second, the response should be within a time-scale of minutes, like the physiological system. It should respond with accurate shadowing of glucose levels and not with an averaged or exaggerated output. Glucose control within the hepatic region is probably more important than peripheral levels, and so the device should monitor the visceral region if possible. Fifth, the dose should, for perfection, have a pulsatile output since this feature in the healthy pancreas may avoid an abnormal level of down-regulation of insulin receptors. The system should not require the chemical modification of the insulin that it delivers in order to work, since this would be abnormal and expensive. Finally, the device should have no harmful effects. For example, it must not leak insulin or any other component, neither should it be vulnerable to influences which might induce such a leak. Related to this, it should not provoke the immune system.

The most obvious approach is to implant new pancreatic tissue so that the normal physiological control can be re-imposed. Pancreatic transplantations were first attempted in the 1960s (Kelly *et al.*, 1967) and are now being performed in increasing numbers (Bilous *et al.*, 1989; Gill and Ballesteros, 2000; Humar *et al.*, 2000; Paty *et al.*, 2000a; Paty *et al.*, 2000b; Soon-Shiong *et al.*, 1993). The goal of the procedure is not only to make a diabetic person independent of insulin treatment but also to reduce the incidence and severity of diabetic complications. In the latter respect, the evidence is somewhat conflicting but, for example, transplantation does appear to reduce mesangial thickening (Bilous *et al.*, 1989). The main problem associated with pancreatic transplantation is tissue rejection which requires the patient to be on continual immunosuppressive therapy (Halle *et al.*, 1993; Pickup and Williams, 1997), to prevent both graft rejection and the recurrence of insulitis. At present, this requirement has largely restricted transplant to patients who also need renal transplant, but this may change as better immunosuppressants become available. Further evolution of the transplant concept has involved the injection of islet cells into the portal circulation so that insulin-producing cells lodge and grow within liver tissue. Such cells have been encapsulated within beads composed of ceramic, acrylic or alginate which are permeable to glucose and insulin but not to immunoglobulins or cells, the aim of this being to reduce their immunogenicity (Pope *et al.*, 1997).

Electronic sensor systems

Closed-loop insulin infusion systems which work using electronic sensors are the second most obvious possibility and have certainly been used. They are typified by a machine known as the Biostator. This is a piece of hospital equipment through which the blood is circulated extracorporeally. It comprises a glucose sensor with a means to transduce and amplify the signal, a microprocessor and a pump. It was developed in the 1970s to maintain the glucose levels within normal levels for patients in crisis and is still used in some centres (Gin *et al.*, 1998; Meyerhoff *et al.*, 1996; Selam, 1997). It is, in effect, the clinical formalization of the so-called 'glucose clamp', a procedure in which insulin levels are administered intravenously with continual manual adjustments to the infusion rates to maintain a given glucose level. Glucose sensors, as required for this kind of equipment, have been available for some time. They operate on an electron transfer chain which incorporates several linked redox reactions. This results in a current, the size of which is a function of the extent of the original oxidation reaction, which is commonly based on glucose oxidase. This strategy of re-circulating electrons in a chain makes the continuous operation of the system resistant to oxygen depletion and it detects the redox equilibrium which prevails at any given time. However, despite the development of these sensors and the obvious potential for miniaturization, none reliably controls glycaemic levels *in vivo* over extended periods of time. First, the specificity is compromised because a number of drugs interfere with the oxidation cycles, having preferential oxidation potentials. These include ascorbic acid and paracetamol. Second, the electrodes are inactivated frequently by surface reactions that result in loss of sensitivity during continuous use *in vivo*. Third, there are biocompatibility problems resulting in fibrin and platelet deposition at the interface between the sensor and body fluids (Gerritsen *et al.*, 1998). Moreover, therapeutic application of this closed-loop feedback system to daily diabetes care has been limited by size and cost.

Competitive displacement

There have been several proposed methods for a chemically controlled, closed-loop delivery of insulin. Some have their roots in conventional controlled delivery designs, involving rate-determining membrane control and will be discussed later. One system that does not fall into this category, however, relies on the competitive displacement of glucose-modified insulin from glucose receptor sites. A synthetic glucose receptor has been built in a method which involves imprinting.

A material is described by Mayes *et al.* in which a glucose ligand is temporarily bonded to functional monomers and thus imposes a glucose complementarity on the resulting polymer (Mayes *et al.*, 1994). The authors recognize the drug delivery possibilities in this polyvalent artificial lectin-like material but do not elaborate. However, the exploitation of other systems with glucose binding sites gives some clue to this. A similar imprinted polymer has been developed in which di-hydroxyl moieties in sugars bind to phenylboronic acid (PBA) moieties in cellulose and acrylic polymers (Hisamitsu *et al.*, 1997; Kataoka *et al.*, 1998; Kataoka *et al.*, 1999; Kataoka *et al.*, 1994; Kitano *et al.*, 1992; Shiino *et al.*, 1996; Shiino *et al.*, 1995). The insulin delivery relies on its displacement by glucose from the polymer and thus requires an

appropriate derivatization of insulin which, in the example quoted, involves the covalent addition of gluconic acid. The latter was chosen because only the open chain presentation of the diol had the ability to bind to the PBA site. Glucose itself, presumably also as an open chain, is able to displace the modified insulin from the same site, thus allowing it to diffuse from the beaded polymer matrix. The method gives a rapid, sensitive and reversible response that appears to have a correlation with the glucose concentration. It cannot be claimed to be glucose specific, however, since other diols could elicit a response.

Specificity is realized with the natural lectin concanavalin A (con A) which has binding sites that can distinguish ring form glucose from most other substances, as is further discussed later in this article. A system which included the use of this lectin was first pioneered as early as 1979 and modified by others (Baudys *et al.*, 1995; Brownlee and Cerami, 1979; Brownlee and Cerami, 1983; Jeong and Kim, 1984; Jeong *et al.*, 1985; Kim *et al.*, 1994; Kim and Fassihi, 1997a; Kim and Fassihi, 1997b; Kim and Jacobs, 1994; Kim *et al.*, 1984). As with the PBA system, it involves the preparation of insulin derivatives, with covalently attached oligosaccharides which are complementary to the binding sites of the lectin. Influx of glucose through a semipermeable poly-hydroxyethylmethacrylate (poly-HEMA) membrane displaces glycosylated insulin from the con A substrate, making it bioavailable in concentrations directly related to those of the ambient glucose concentration (Saudek, 1993; Saudek *et al.*, 1996; Saudek, 1997).

Unfortunately, con A is mitogenic and therefore, must not make contact with tissue. Several methods of containing con A had to be designed involving the synthesis of conjugates larger than the retaining pore size (Pai *et al.*, 1993; Pai *et al.*, 1992). However, doubts have been raised about the mechanical strength of the poly-HEMA pouch among other aspects of this design. An added major disadvantage is the need

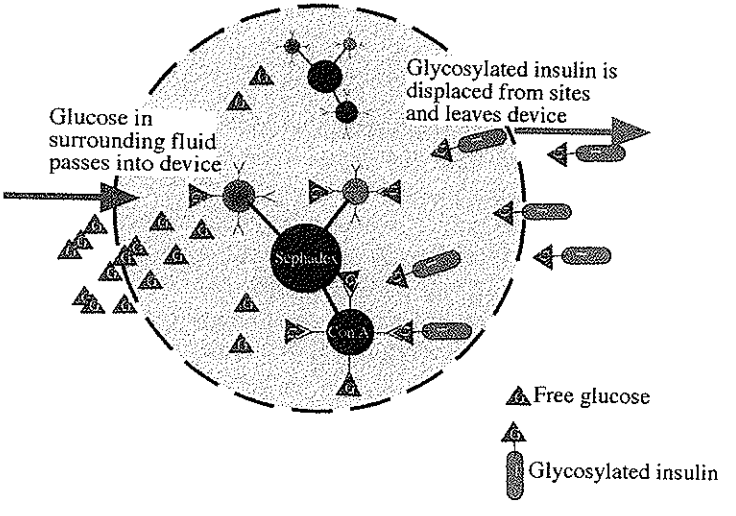

Figure 17.8. In this competitive displacement design, a semi-permeable membrane encloses Sephadex beads bearing covalently attached con A. The system is first loaded with glycosylated insulin which can then be displaced by incoming glucose.

to modify insulin covalently by glycosylation, so that it can participate in the displacement.

Alternative displacement systems involve the lectin being bonded to the inner surface of hollow fibres. These contain insulin and dextran and the precipitation induced by the binding of dextran with con A promotes the blocking of the porous walls of the fibres preventing efflux of insulin (Tomioka *et al.*, 1994). The mechanism for this complexation and its subsequent reversal by glucose displacement is discussed below in the context of a modified reservoir device.

Closed-loop designs based on conventional controlled release systems

In the past two decades, much energy has been expended on designing systems which control the continuous release of a drug such that, despite a short plasma half-life, its frequency of dosing can be reduced. This involves a unit that can control the medication output as a continuous stream, thus delivering several conventional doses over an appropriate time interval. Instead of involving a mechanical pump as in CSII, most designs utilize polymer excipients or membranes to delay the dissolution or diffusion of the drug in some way and have been proposed as oral or implanted dose forms. A variety of systems can be used and many have been aimed at producing a zero-order output (Hennink *et al.*, 1997; van Dijk-Wolthhuis *et al.*, 1997), ie a release rate that is independent of time, despite extended periods of delivery. The aim of zero order delivery is to produce blood levels that not only lie between the minimum effective concentration (MEC) and a minimum toxic concentration (MTC) for an extended time (Siegel, 1997) but which are constant. Zero-order delivery is a worthy aim for many therapeutic purposes. Thus, for example, the control of epilepsy, asthma and pain may well benefit from constant blood levels of the appropriate drug. The following discussion focuses on the matrix and reservoir designs for accomplishing zero-order release because they both lend themselves to modifications that can be glucose sensitive and therefore the basis of closed-loop designs.

Polymeric matrix drug delivery systems

A matrix or monolithic device is a block of material comprising polymeric material throughout which the drug is distributed, possibly above its solubility limit (Chien, 1992; Dunn, 1991; Himmelstein, 1991; Martin, 1993). One example of a matrix has already been discussed above in relation to a magnetically vibrated source of insulin (Edelman and Langer, 1993). The polymers used as matrices for drug delivery can be classified into types that dissolve, others that erode as a result of enzymatic or other degradation processes, and those which remain unchanged in body fluids. The drug may therefore be released as the polymer disperses or it might itself dissolve and diffuse through and away from the intact matrix. It is the latter system which is of interest in the present context of modification for glucose sensitivity. The factors affecting drug release are therefore (i) the diffusion coefficient of the drug in the polymer; (ii) the solubility of the drug in the polymer and (iii) the length of the diffusion pathway of the drug (this simplified discussion assumes no partitioning complications). The overall rate of release may be determined by the diffusion of the drug in solution through the microporous polymer, or it could be dissolution controlled

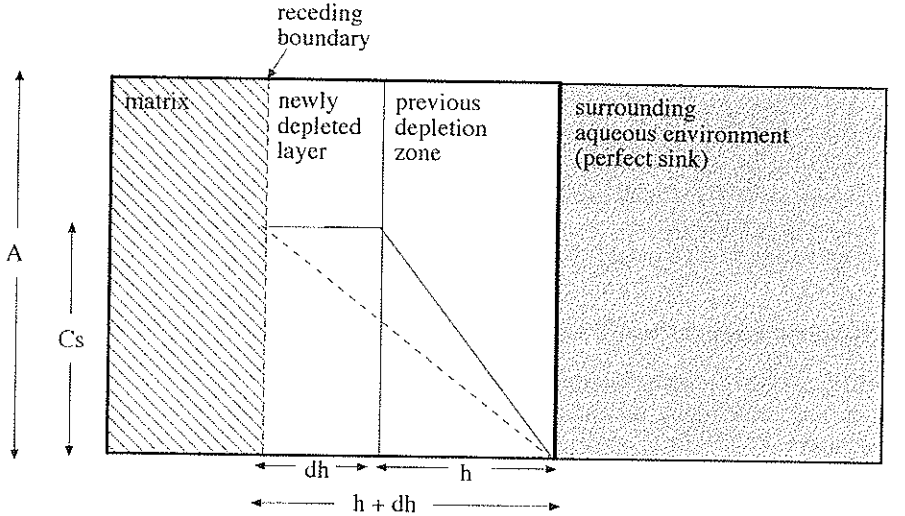

Figure 17.9. Drug release from a polymeric matrix, showing the parameters from which the matrix equation describes the diminishing release rate, which is a function of the increasing depletion zone, h (from Martin, 1993).

(Martin, 1993). Commonly used, non-biodegradable polymers include silicones, poly(ethylene vinyl acetate) and the polyurethanes (Allcock, 1990; Pitt, 1990). As the drug is released from a non-eroding matrix, the boundary between the full and depleted sections recedes, thus increasing the distance for diffusion through the depleted matrix before the drug reaches the surrounding environment.

Figure 17.9 illustrates the matrix two dimensionally, with the total drug content represented by the distance A on the y axis, whereas its solubility is Cs. The drug diffusion coefficient is D and *t* is the time. The value of D is affected by the physical attributes of the matrix material such as hydrogen bonding, pore size, tortuosity and porosity (Martin, 1993).

The increase in the depleted zone h is shown as dh and the concentration gradient across the zone can be seen to fall, as represented by the diagonal as h changes to (h+ dh). A simple geometric proof shows that the total drug delivered dQ as the zone of depletion increases in this way, is equal to:

$$dQ = A\,dh - (1/2)Cs\,dh \tag{17.1}$$

integration gives the Higuchi equation

$$Q = \{D\,Cs\,(2A - Cs)\,t\}^{1/2} \tag{17.2}$$

which simplifies to

$$Q = (D\,Cs\,2A\,t)^{1/2} \tag{17.3}$$

when the total content A is greatly in excess of the solubility Cs.

The rate of delivery at a given instant in time is

$$dQ/dt = 1/2\,\{D\,Cs\,(2A - Cs)/t\}^{1/2} \tag{17.4}$$

simplifying to

$$dQ/dt = (D\ Cs\ A/2t)^{1/2} \quad (17.5)$$

For non-erodible, monolithic devices that are diffusion controlled, therefore, release is linear with the square root of time (Park, 1997). In order to obtain zero-order release from a matrix, it is necessary to compensate for the increasing zone of depletion. This has been attempted by, for example, layering the matrix so that the new zone boundaries contain drug in greater concentration or smaller particle size.

The matrix material itself can be made interactive with its environment. All matrix systems will have some temperature dependence, for example, and reaching critical temperatures can trigger the activity of some. Similarly, some matrices can be made glucose sensitive by methods that can also be applied to reservoir membranes, as discussed below.

Polymeric reservoir drug delivery system

The reservoir system entails the release of the drug through a polymeric membrane. The diffusion of the drug through the membrane is rate determining and, provided certain characteristics of the system remain constant, the release of the drug through such a gateway membrane will have zero-order characteristics (Fessenden and Fessenden, 1994). Reservoir release is described with the familiar Fick's first law which describes: (i) the diffusivity of the individual particles; (ii) the magnitude of the concentration gradient; and (iii) the path length through which diffusion occurs. This is usually expressed by the following equation:

$$(dQ/dt) = (D.K.C)/x \quad (17.6)$$

The amount of drug released per unit time from such devices is a function of the drug diffusion coefficient D, the partition coefficient K (unity in totally aqueous systems), the concentration C, and x, the thickness of the membrane.

This simplified equation makes no allowance for any significant resistance

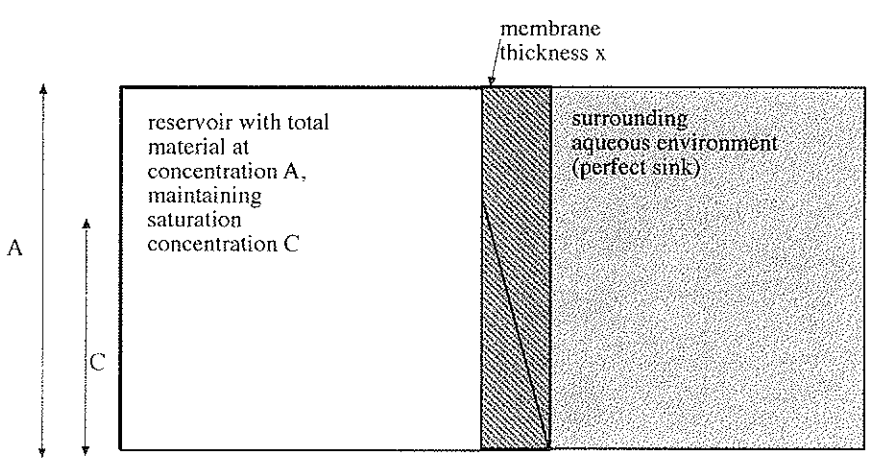

Figure 17.10. Drug release from a reservoir governed by a polymeric membrane. This gives zero order delivery if certain conditions are met.

due to the boundary diffusion layers close to the membrane, and therefore assumes that the membrane is rate determining. In order to achieve zero-order delivery, the thermodynamic activity of the drug in the reservoir must remain constant, using a saturated solution in contact with excess solid (Maple, 1996) and in addition, the membrane itself must not swell, erode or change in any way that affects its permeability. After an initial period, a steady state is reached (Narasimhan and Peppas, 1997) and this may follow a burst of surface held material or a lag period during which the concentration gradient develops.

Matrix and reservoir designs are by no means the only ones which are capable of delivery at a constant rate, but they are common by comparison with others. Of the two, the matrix type is considered preferable because of the intrinsic safety if the unit should fracture. However, a matrix requires careful manipulation to make it even approximate to a zero-order device, whereas the reservoir is inherently capable of producing constant delivery rate. As a consequence, the reservoir type offers a more obvious basis for modification in that if variable rates of delivery are to be deliberately induced by glucose, for example, all other factors should preferably remain constant.

Glucose sensitive modifications of the membranes and matrices

If the polymeric material which comprises the rate-determining matrix or membrane does not have constant geometry (ie swells or erodes) and permeability characteristics, the above descriptions of solute transport kinetics break down. However, if these characteristics change under the influence of glucose and if the effect creates increased drug transport, then such modifications can form the basis of glucose sensitive insulin delivery.

One such system which has been well documented is based on a pH sensitive design in which a drug solute diffuses through a crosslinked tertiary amine polymer prepared from a hydrophilic acrylate which includes N,N-dimethylaminoethyl pendants. The latter become protonated if the pH drops, causing the material to swell as the charged

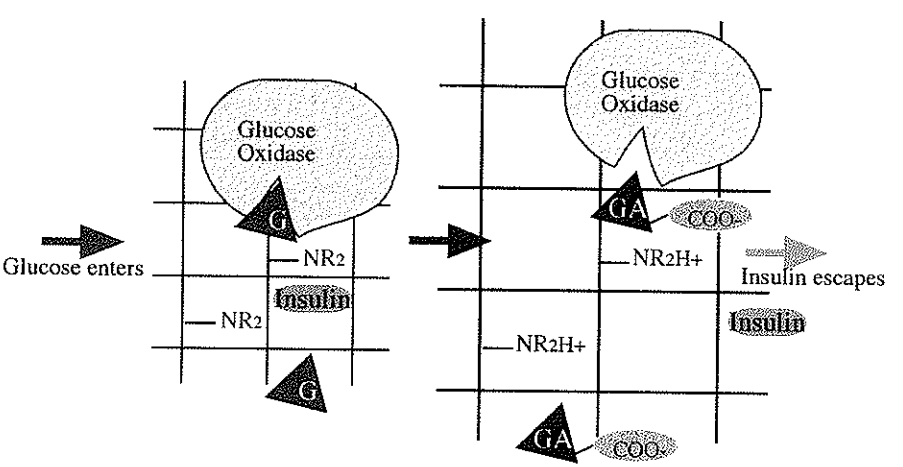

Figure 17.11. An ionizable polymeric construction, formed round glucose oxidase, swells when amine groups are protonated, after enzyme action. This creates increased permeability to insulin.

groups repel, and thus increasing the transport of any solute within the structure. This mechanism can be converted to a glucose-sensitive one by incorporating glucose oxidase as the material is crosslinked. If glucose then diffuses into the acrylic, the enzyme converts it to gluconic acid, generating a proton which interacts with the amine group as before (Albin *et al.*, 1987; Albin *et al.*, 1985; Horbett *et al.*, 1984a; Horbett *et al.*, 1984b; Kost *et al.*, 1985).

Clearly, such a system could operate as a matrix or membrane to control the release of insulin with transport a function of the glucose content, the whole mechanism being reversible. One obvious objection to this system is that although the interaction between glucose and glucose oxidase is highly specific, the overall mechanism is not, because of its sensitivity to protons. A decade later, a variation on this theme was produced in which crosslinked co-polymers of polyethylene glycol and polyelectrolyte were shown to have a similar pH sensitivity. These also can be coupled with glucose oxidase (Lowman *et al.*, 1999; Lowman and Peppas, 1999; Lowman and Peppas, 2000; Peppas *et al.*, 1999; Podual *et al.*, 2000a; Podual *et al.*, 2000b; Podual *et al.*, 2000c; Schwarte and Peppas, 1998; Scott and Peppas, 1999).

Sol-gel controlled devices

A mechanism is still required which can deliver insulin in a specific and direct response to glucose and which is not complicated by issues such as covalent

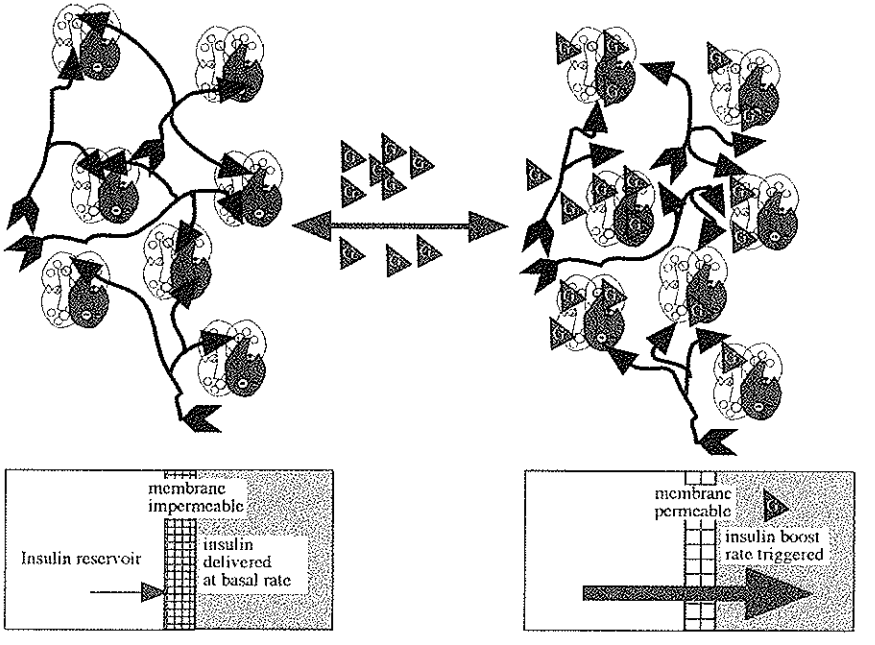

Figure 17.12. A gelatinous complex formed from con A and dextran (among other polysaccharide-like materials), undergoes a reversible gel to sol transformation in response to glucose levels, and can therefore deliver insulin at an increased rate in the glucose-induced sol state. The diagram depicts a reservoir design, where the gel-sol material forms the rate-determining membrane.

modification of insulin or the need for immunosuppression. In the system discussed below, an insulin reservoir is controlled by a membrane composed of a branched glucose polysaccharide and a multivalent glucose receptor molecule, as typified by con A, the lectin discussed earlier in this article (Taylor, 1992; Taylor, 1993; Taylor *et al.*, 1994; Tanna and Taylor, 1994). Lectins have long been known to form precipitated complexes with glucose-containing polysaccharides such as starch, glycogen and dextran (Goldstein *et al.*, 1965; Sumner and Howell, 1936). Indeed, this is a reaction that has been used as the basis for an optical glucose sensor (Nakamae *et al.*, 1994). It was also used in the drug delivery mechanism described above (Tomioka *et al.*, 1994) in which an immobilized dextran-con A complex forms in hollow fibre pores governing the passage of insulin. The complex occurs because terminal glucose moieties lock into the lectin receptors and since both components are multivalent in terms of the interactive moieties, a three-dimensional network forms. The interaction is reversible because the receptors appear to have a similar affinity for free or terminal glucose and therefore the polysaccharide can be displaced, reversibly, from the complex by incoming free glucose.

In the proposed design, however, the complex is a continuous gelatinous phase, rather than a particulate one. The gel forms for the same reasons as the precipitate, but in a limited region of the polysaccharide-water-lectin phase diagram. The mechanism by which the complex could control insulin transport is different from that in which pores are blocked by a precipitated complex. The gelatinous material, when exposed to free glucose, loses its viscosity reversibly as a result of the dismantling of the polysaccharide bonds with the receptors.

It is therefore the equilibrium state of a sol-gel transition which is of interest in this case. In the sol state, it becomes very permeable to insulin until the ambient glucose level drops and dialyses out of the gel. *In vivo*, the glucose level would drop when the released insulin has exerted its pharmacological effect, and thus this mechanism forms the basis of a closed-loop system for diabetes therapy. Gels can be formed using both natural and synthetic polysaccharides such as glycogen or polysucrose, for example, but most studies have concentrated on the use of dextran (Taylor *et al.*, 1995a; Taylor *et al.*, 1994; Taylor *et al.*, 1995b; Tanna and Taylor, 1997; Tanna and Taylor, 1998a; Tanna and Taylor, 1998b; Tanna *et al.*, 1999). An identical gelatinous material formed from dextran and con A has also been proposed purely as the basis for a glucose sensor by others (Ballerstadt and Ehwald, 1994; Ballerstadt and Schultz, 1998; Ehwald and Ballerstadt, 1992). The choice of constituents is important. Dextran is the generic name applied to a large class of soluble α-D-glucans (Pearce *et al.*, 1990). The *Leuconostoc mesenteroides* B-512 strain produces dextran which is characterized by 95% α-1-6 glucopyranosidic linkages and 5% α-1-3 linkages (Larsen, 1989). The α-1-3 linkages the sources of side chains of which 85% are 1–2 glucose residues in length. The remaining 15% of the side chains may have an average length of 33 glucose residues (Jeanes, 1977) and may not be uniformly distributed (Larsen, 1989).

The combination of length and extensive branching incidence are important for the production of a gel and experience shows that polysaccharides like glycogen which are compact, allow much less formulation flexibility because of their tendency to produce precipitates instead. A closed-loop design based on a con A sol-gel transition has since been described by others who have reported success with alternatives to

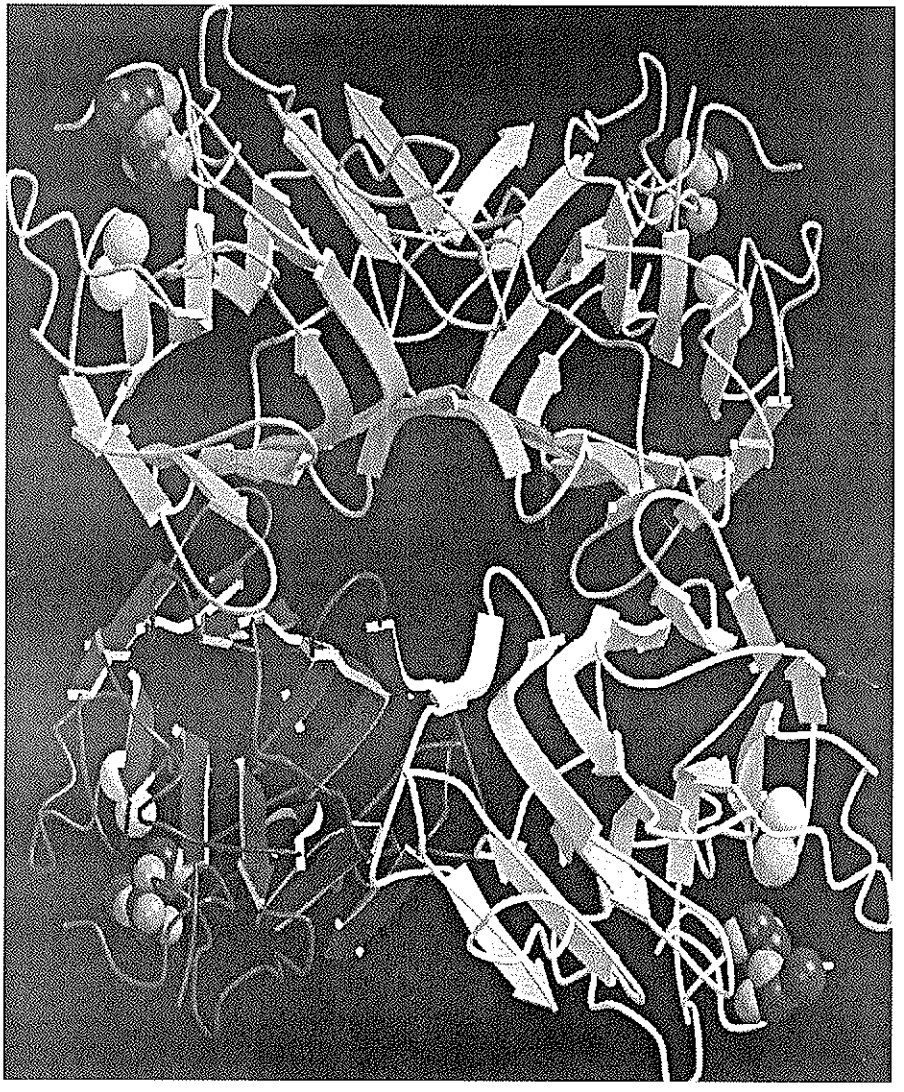

Figure 17.13. The structure of concanavalin A (with bound metal ions and a glucose molecule in each monomer of the tetramer, determined by protein crystallography (with permission of Professor John R. Helliwell, University of Manchester).

dextran in the form of acrylic structures substituted with pendant saccharide groups (Lee and Park, 1994; Obaidat and Park, 1995; Obaidat and Park, 1996; Obaidat and Park, 1997; Valuev *et al.*, 1997; Valuev *et al.*, 1998). These materials closely resemble those used in the optical sensor described above (Nakamae *et al.*, 1994).

The feasibility of using such a system clinically depends on the characteristics of con A. Structurally, this protein (Becker *et al.*, 1976; Becker *et al.*, 1971; Becker *et al.*, 1975) is tetrameric at pH values between 5.6 and 7.0, with each monomer consisting of 237 amino acid residues (Wang *et al.*, 1992). Below about pH 5.6, it separates into

two dimers but aggregation occurs above pH 7.0. In each monomeric unit, the polypeptide chain is folded into a compact ellipsoidal dome, about 40 × 39 Å in cross-section and 42 Å high with a base of approximately 40 × 25 Å. Individual monomers are paired base-to-base across an axis of two-fold symmetry to form ellipsoidal dimers approximately 84 × 40 × 40 Å in size. Each monomeric unit binds calcium and manganese in two different sites which are approximately 4.2 Å apart. This metallic binding is a prerequisite for carbohydrate interaction. It is reported (Shoham *et al.*, 1973) that con A is capable of binding other divalent metal such as cobalt, nickel and cadmium. Con A shows remarkable chemical aptness for use in glucose sensitive designs. It can distinguish α-D glucose from the β-D and L isomers and from galactose. Although this lectin accommodates both mannose and fructose with even greater affinity, there seem to be no physiologically important sugars present in concentrations sufficient to compete seriously with its interaction with glucose. It appears to provide receptor attachments for at least three consecutive mannose units such as occur in some of the derivatized insulins (discussed above in relation to competitive displacement mechanisms) and there may be analogy for the interaction with glucans, upon which the sol-gel system depends. Using molecular modelling for the interaction of methyl-α-D-mannopyranoside, it has been shown that arginine, asparagine, aspartic acid, leucine and tyrosine residues would form hydrogen bonds with mannose hydroxyl oxygens at carbons 3, 4 and 6. The oxygen at the 1 position forms no such bonds and that at position 2 forms bonds with water molecules. An additional stabilization may be formed by van der Waals interactions with mannose

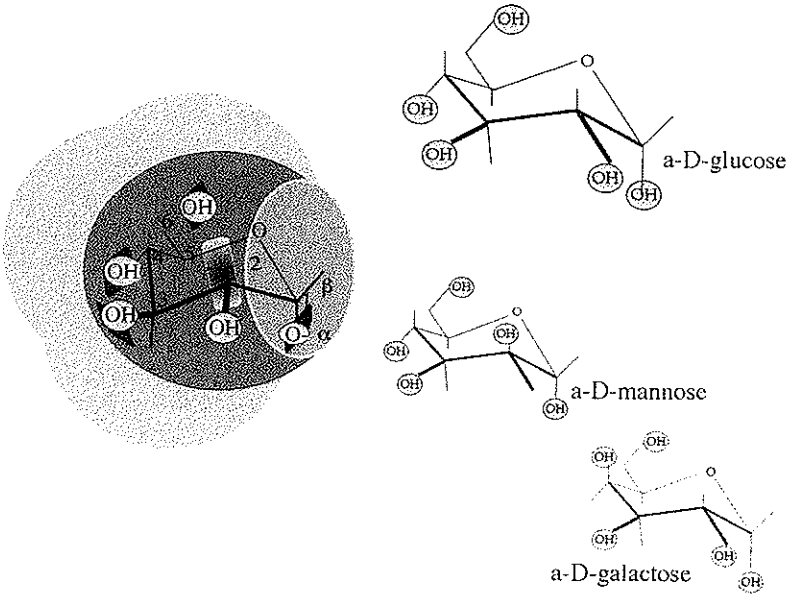

Figure 17.14. Within each con A monomer, there is a receptor which can accommodate glucose, mannose or fructose, but not galactose. This relates to specific reactions around hydroxyl oxygens at positions 3, 4 and 6, which deny access if incorrect, whereas stabilizing van der Waals interactions at position 2 are less critical. The receptor appears to have similar sites further to the interior which may allow interaction with suitable moieties in polysaccharides.

oxygen 2, but is not sufficient to foster galactose fit (which is inappropriately configured at oxygen 4), while hydrophobic interactions with other tyrosine rings also reinforce fit for mannose and glucose (Li *et al.*, 1998).

While chemically well suited for the purpose, however, there are potential biological complications. Con A is mitogenic and has a variety of widely reported effects on cell growth. Clearly, used in an insulin delivery device, the main concern is for containment within the gel. This is also important mechanistically because loss of this component would disable the responsive nature of the gel. Containment was a problem also faced for the competitive displacement design, and the approach in that case was to bond the protein to larger structures within the formulation. Thus, Sephadex-bound con A was used in one variant and a con A aggregate in another (Pai *et al.*, 1993; Pai *et al.*, 1992). Others studying the gel-sol design option (Li *et al.*, 1998) have looked at the possibility of designing more specific artificial lectins than the PBA types described earlier. In the present case (Tanna and Taylor, 1998a; Tanna and Taylor, 1998b; Tanna *et al.*, 1999), the approach has been two-fold. First, the gel membrane assembly has to be confined using thin porous barriers because it is water

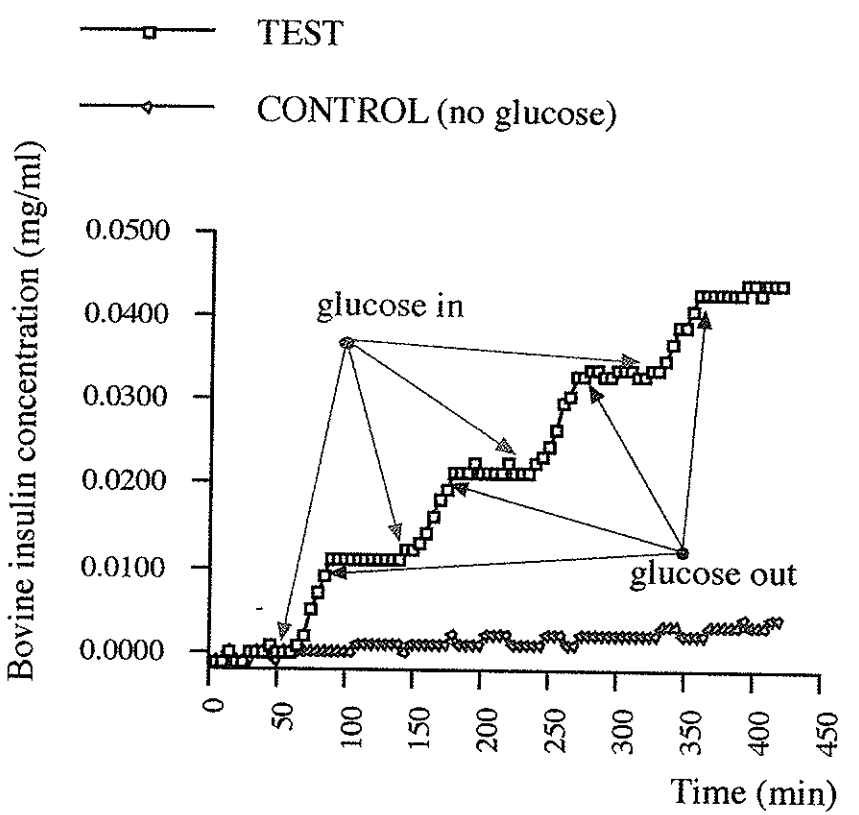

Figure 17.15. *In vitro* release of insulin using a covalent conjugate of con A and dextran. Several glucose triggers are used, showing fast response time for both provocation and recovery. The glucose concentration was 2%w/v, which is considerably higher than diabetic levels, but gels can be made which respond within clinical ranges.

dispersible. The need is to transport insulin which has a molecular weight of between 6 and 36 kDa (depending on the association state) but not the lectin (100 kDa, assuming the tetrameric state at close to neutral pH) or the dextran (mw 2000 kDa). This should allow the use of pores of nominal molecular weight limit of perhaps 80 kDa. Unfortunately, this pore size restricts insulin transport in practice, and larger pores are needed to avoid this and ensure that the gel confined within the assembly is the rate-determining layer. Thus, a second strategy securing the lectin to carriers seems likely to be necessary. Attachment to sections of the dextran, using simple Schiff's base production is one approach that has worked well. In this, the dextran is oxidized using sodium periodate and the product dialysed to remove excess periodate and iodine. Addition of the lectin allows binding via exposed lysines, which does not seem to interfere with the working mechanism. Carbodiimide attachment of lectin and amine substituted dextran to polyacrylic acid carrier particles is also under investigation. The advantage with the latter system may be that the glucose sensitive region may be confined to the interstices between polyacrylic acid crosslinked particles, and this may allow lower lectin concentrations to be used in the formulation.

The proposed system clearly fulfils some of the criteria outlined earlier, in that it operates by a specific and direct interaction with glucose and also that it needs no chemical modification of insulin. Results *in vitro* show that it responds within minutes, although whether it can shadow blood glucose *in vivo* has yet to be discovered.

A device using this system could be placed in the peritoneum similar to the implantable programmable pump and thus could respond to hepatic rather than peripheral levels. However, its delivery pattern is otherwise dissimilar to the physiological output in that it cannot be pulsatile. The longevity of the proteinaceous formulation in proximity to peritoneal fluid will be critical, depending on the size of the pores in the separating membrane and the ability of such pores to exclude enzymes from the gel. Perhaps the most important factors will relate to whether a device based on this gel can operate safely from the viewpoint of glucose control and containment of toxic agents, including insulin and con A. However, this is a design which is simple in operation, has no moving parts and which requires no power source for its fundamental mechanism.

Conclusion

Under normal physiological conditions, euglycaemia is maintained principally by the homeostatic balance of insulin and glucagon which are secreted from the pancreas. In both type 1 and type 2 diabetes mellitus there is a substantial and chronic increase in the circulating glucose concentration. This elevation in glucose levels is accompanied by a plethora of other biochemical disturbances, including disruption of carbohydrate, fat and protein metabolism. Clinical manifestations of diabetes which arise from the metabolic disturbances vary between individuals but are often a serious threat to quality and length of life. Since the discovery of insulin eighty years ago, glycaemic maintenance has been attempted by the use of insulin. Although this improves the short-term consequences, especially in type 1 diabetes, even the strict treatment regimens fall short of the accuracy in glucose control imposed by the healthy pancreas. An effective closed-loop delivery system is required which may

not, however, need to have mechanistic similarity to the physiological one. For example, although it has been shown that the normal pancreas responds to secretagogues other than glucose, it is the high circulating levels of glucose that are harmful to diabetics. Therefore if a simple feedback mechanism could be imposed in which glucose was the driver, this could well lead to a treatment that would be an improvement on the conventional. Clearly, a system in which an implanted reservoir of insulin is needed is itself a health risk, but until pancreas transplantation is routinely applicable to all diabetics, other closed-loop systems, of which several have been discussed in this article, remain of interest.

References

ALBERTI, K.G. AND ZIMMET, P.Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation [see comments]. *Diabetic Medicine* **15**, 539–553.

ALBIN, G., HORBETT, T. AND RATNER, B. (1985). Glucose Sensitive Membranes for Controlled Delivery of Insulin: Insulin Transport Studies. *Journal of Controlled Release* **2**, 153–164.

ALBIN, G., HORBETT, T., MILLER, S. AND RICKER, N. (1987). Theoretical and Experimental Studies of Glucose Sensitive Membranes. *Journal of Controlled Release* **6**, 267–291.

ALBISSER, A.M., LEIBEL, B.S., EWART, T.G., DAVIDOVAC, Z. AND BOTZ, C.K. (1974). An Artificial Endocrine Pancreas. *Diabetes* **23**, 389–396.

ALLCOCK, H.R. (1990). Development and evaluation of the biodegradeable polyphosphazenes. In: *Biodegradable Polymers as Drug Delivery Systems*. Eds. M. Chasin and R. Langer, pp 163–193. New York: Marcel Dekker, Inc.

AMOS, A.F., MCCARTY, D.J. AND ZIMMET, P. (1997). The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabetic Medicine*, S1–85.

ANONYMOUS. (1995). Multicentre trial of a programmable implantable insulin pump in type 1 diabetes. The Point Study II Group. *International Journal of Artificial Organs* **18**, 322–325.

ANONYMOUS. (1996). Lifetime benefits and costs of intensive therapy as practiced in the diabetes control and complications trial. The Diabetes Control and Complications Trial Research Group. *Journal of the American Medical Association* **276**, 1409–1415.

ANONYMOUS. (1999). Blood pressure control in type 2 diabetes – what does the United Kingdom Prospective Diabetes Study (UKPDS) tell us? [editorial]. *Nephrology, Dialysis and Transplantation* **14**, 2562–2564.

AZAR, S.T. AND KANAAN, N. (1999). Intensive insulin therapy compared with conventional insulin therapy does not reduce depressive symptoms in parents of children with type 1 diabetes. *Diabetes Care* **22**, 1372–1373.

BAKAYSA, D.L., RADZIUK, J., HAVEL, H.A., BRADER, M.L., LI, S., DODD, S.W., BEALS, J.M., PEKAR, A.H. AND BREMS, D.N. (1996). Physicochemical basis for the rapid time-action of LysB28ProB29-insulin: dissociation of a protein-ligand complex. *Protein Science* **5**, 2521–2531.

BALDEWEG, S.E. AND YUDKIN, J.S. (1998). Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). U.K. Prospective Diabetes Study (UKPDS) Group [published erratum appears in *Lancet* 1998 Nov 7;352(9139):1557]. *Lancet* **352**, 854–865.

BALLERSTADT, R. AND EHWALD, R. (1994). Suitability of aqueous dispersions of dextran and concanavalin A for glucose sensing in different variants of the affinity sensor. *Biosensors and Bioelectronics* **9**, 557–567.

BALLERSTADT, R. AND SCHULTZ, J.S. (1998). Kinetics of dissolution of Concanavalin A dextran sols in response to glucose measured by surface plasmon resonance. *Sensors and Actuators: B Chemical* **46**, 50–55.

BANTING, F.G. AND BEST, C.H. (1922). The internal secretion of the pancreas. *Journal of Laboratory and Clinical Medicine* **7**, 256–271.

BAUDYS, M., UCHIO, T., HOVGAARD, L., ZHU, E., AVRAMOGLOU, T., JOZEFOWICZ, M., RIHOVA, B., PARK, J., LEE, H. AND KIM, S. (1995). Glycosylated insulins. *Journal of Controlled Release* **36**, 151–157.

BECKER, J.W., CUNNINGHAM, B.A., REEKEJR, G.N., WANG, J.L. AND EDELMAN, G.M. (1971). The Molecular Structure of Concanavalin A. *Journal of Biological Chemistry* **246**, 6123–6125.

BECKER, J.W., REEKE, G.N., WANG, J.L., CUNNINGHAM, B.A. AND EDELMAN, G.M. (1975). The covalent and three-dimensional structure of Concanavalin A. III. Structure of the monomer and its interaction with metals and saccharides. *Journal of Biological Chemistry* **250**, 1513–1524.

BECKER, J.W., CUNNINGHAM, B.A., REEKE, G.N., WANG, J.L. AND EDELMAN, G.M. (1976). The molecular structure of concanavalin A. In: *Concanavalin A as a Tool*. Eds. H. Bittiger and H.P. Schnebli, pp 33–54. London and New York: John Wiley & Sons.

BILOUS, R. (1999). Complications of type 2 diabetes mellitus: a brief overview. *American Osteopathic Association. Journal* **99**, S10–14.

BILOUS, R., MAUER, S., SUTHERLAND, D., NAJARIAN, J., GOETZ, F. AND STEFFES, M. (1989). The effects of pancreas transplantation on the glomerular structure of renal-allografts in patients with insulin-dependent diabetes. *New England Journal of Medicine* **321**, 80–85.

BLOOM, A. AND IRELAND, J. (1992). *Diabetes: A Colour Atlas*. 2nd Edition. Wolfe Publishing Ltd.

BRANGE, J., SKELBAEK-PEDERSEN, B., LANGKJAER, L., DAMGAARD, U., EGE, H., HAVELUND, S., HEDING, L.G., JOERGENSEN, K.H., LYKKEBERG, J., MARKUSSEN, J., PINGEL, M. AND RASMUSSEN, E. (1987). *Galenics of Insulin. The Physico-chemical and Pharmaceutical Aspects of Insulin and Insulin Preparations*. Berlin: Springer-Verlag.

BRANGE, J., ANDERSEN, L., LAURSEN, E., MEYN, G. AND RASMUSSEN, E. (1997). Towards understanding fibrillation. *Journal of Pharmaceutical Sciences* **86**, 517–525.

BRANKIN, E. AND FISHER, M. (1998). The U.K. Prospective Diabetes Study. A review. *Diabetes Care* **21**, C35–38.

BREMER, U., HORRES, C.R. AND FRANCOEUR, M.L. (1997). Protein delivery with infusion pumps. *Pharmaceutical Biotechnology* **10**, 239–254.

BROOK, C. AND MARSHALL, N. (1996). *Essential Endocrinology*. 3rd Edition. Oxford: Blackwell Science.

BROSNAN, J.T. (1999). Comments on metabolic needs for glucose and the role of gluconeogenesis. *European Journal of Clinical Nutrition* **53**, AS107–S111.

BROWNLEE, M. AND CERAMI, A. (1979). A glucose controlled insulin delivery system: semisynthetic insulin bound to lectin. *Science* **206**, 1190.

BROWNLEE, M. AND CERAMI, A. (1983). Glycosylated insulin complexed to Concanavalin A. Biochemical basis for a closed-loop insulin delivery system. *Diabetes* **32**, 499–504.

BURGE, M.R. AND SCHADE, D.S. (1997). Insulins. *Endocrinology and Metabolism Clinics of North America Py*. 575.

BURGE, M.R., WATERS, D.L., HOLCOMBE, J.H. AND SCHADE, D.S. (1997). Prolonged efficacy of short acting insulin Lispro in combination with human ultralente in insulin-dependent diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* **82**, 920–924.

CHANG, X., JORGENSEN, A.M., BARDRUM, P. AND LED, J.J. (1997). Solution structures of the R6 human insulin hexamer. *Biochemistry* **36**, 9409–9422.

CHETTY, D.J. AND CHIEN, Y.W. (1998). Novel methods of insulin delivery: an update. *Critical Reviews in Therapeutic Drug Carrier Systems* **15**, 629–670.

CHIEN, Y.W. (1992). *Novel Drug Delivery Systems*. 2nd Edition. New York: Marcel Dekker, Inc.

CHIEN, Y.W., SIDDIQUI, O., SUN, Y., SHI, W.M. AND LIU, J.C. (1987). Transdermal Iontophoretic Delivery of Therapeutic Peptides, Proteins, Insulin. *Annals of the New York Academy of Sciences* **507**, 32–51.

CLEMENTS, R.S., ROBISON, W.G. AND COHEN, M. (1998). Anti-glycated albumin therapy ameliorates early retinal microvascular pathology in db/db mice. *Journal of Diabetes and its Complications* **12**, 28–33.

COTRAN, R., KUMAR, V. AND ROBBINS, S. (1994). *Pathologic Basis of Disease*. 5th Edition. W.B. Saunders.

DEFRONZO, R.A. (1999). Implications of the United Kingdom prospective diabetes study. *Primary Care* **26**, 809–827.

DUNN, R.L. (1991). Polymeric matrices. In: *Polymeric Drugs and Drug Delivery Systems*. Eds. R.L. Dunn and R.M. Ottenbrite, pp 11–23. American Chemical Society Symposium Series.

EDELMAN, E. AND LANGER, R. (1993). Optimization of Release from Magnetically Controlled Polymeric Drug Release Devices. *Biomaterials* **14**, 621–626.

EDELMAN, E., LINHARDT, R., BOBECK, H., KOST, J., ROSEN, H. AND LANGER, R. (1984). Polymer based drug delivery: magnetically modulated and bioerodible systems. In: *Polymers as Biomaterials*. Eds. S.W. Shalaby, A.S. Hoffman, T.A. Horbett and B.D. Ratner, pp 279–292. New York: Plenum.

EGE, H. (1986). Semantics of insulin. *Diabetic Medicine* **3**, 212–215.

EHWALD, R. AND BALLERSTADT, R. (1992). *Affinitäts-Sensor Patent DE 42 03 466 A1*.

FESSENDEN, R.J. AND FESSENDEN, J.S. (1994). *Organic Chemistry*. 5th Edition. California: Brooks/Cole Publishing Company.

FRAYN, K.N. (1997). *Metabolic Regulation: A Human Perspective*. London: Portland Press.

GAVIN, J.R. (1998). New classification and diagnostic criteria for diabetes mellitus. *Clin Cornerstone* **1**, 1–12.

GERRITSEN, M., JANSEN, J.A., KROS, A., NOLTE, R.J. AND LUTTERMAN, J.A. (1998). Performance of subcutaneously implanted glucose sensors: a review. *Journal of Investigative Surgery* **11**, 163–174.

GIANNOUKAKIS, N., RUDERT, W.A., GHIVIZZANI, S.C., GAMBOTTO, A., RICORDI, C., TRUCCO, M. AND ROBBINS, P.D. (1999). Adenoviral gene transfer of the interleukin-1 receptor antagonist protein to human islets prevents IL-1beta-induced beta-cell impairment and activation of islet cell apoptosis in vitro. *Diabetes* **48**, 1730–1736.

GILL, I. AND BALLESTEROS, A. (2000). Bioencapsulation within synthetic polymers (Part 1): sol-gel encapsulated biologicals. *Trends in Biotechnology* **18**, 282–296.

GIN, H., CATARGI, B., RIGALLEAU, V., RULLIER, E., ROGER, P. AND TABARIN, A. (1998). Experience with the Biostator for diagnosis and assisted surgery of 21 insulinomas. *European Journal of Endocrinology* **139**, 371–377.

GOFF, D.C., D'AGOSTINO, R.B., HAFFNER, S.M., SAAD, M.F. AND WAGENKNECHT, L. (2000). Lipoprotein concentrations and carotid atherosclerosis by diabetes status – Results from the Insulin Resistance Atherosclerosis Study. *Diabetes Care* **23**, 1006–1011.

GOLDSTEIN, I.J., HOLLERMAN, C.E. AND MERRICK, J.M. (1965). Protein-carbohydrate interaction Part (I) the interaction of polysaccharides with concanavalin A. *Biochimica et Biophysica Acta* **97**, 68–76.

GREENSTEIN, B. (1994). *Endocrinology at a Glance*. 1st Edition. Oxford: Blackwell Science.

HAARDT, M.J., SELAM, J.L., SLAMA, G., BETHOUX, J.P., DORANGE, C., MACE, B., RAMANICHE, M.L. AND BRUZZO, F. (1994). A cost-benefit comparison of intensive diabetes management with implantable pumps versus multiple subcutaneous injections in patients with type I diabetes. *Diabetes Care* **17**, 847–851.

HALLE, J., LANDRY, D., FOURNIER, A., BEAUDRY, M. AND LEBLOND, F. (1993). Method for the Quantification of Alginate in Microcapsules. *Cell Transplantation* **2**, 429–436.

HELLER, J. (1997). Feedback controlled drug delivery. In: *Controlled Drug Delivery: Challenges and Strategies*. Ed. K. Park, pp 127–146. American Chemical Society.

HELVE, E., KOIVISTO, V., LEHTONEN, A., LAATIKAINEN, L., MERENMIES, L., PELKONEN, R. AND HUTTUNEN, J. (1987). A crossover comparison of continuous insulin infusion (csii) and conventional injection (cit) therapy of type-i diabetes. *Acta Endocrinologica* **115**, 22.

HENNINK, W.E., FRANSSEN, O., VAN DIJK WOLTHUIS, W.N.E. AND TALSMA, H. (1997). Dextran hydrogels for the controlled release of proteins. *Journal of Controlled Release* **48**, 107–114.

HIMMELSTEIN, K.J. (1991). Interaction between polymeric drug delivery systems and biological systems: Barriers and opportunities. In: *Polymeric Drugs and Drug Delivery Systems*. Eds. R.L. Dunn and R.M. Ottenbrite, pp 34–39. American Chemical Society Symposium Series.

HISAMITSU, I., KATAOKA, K., OKANO, T. AND SAKURAI, Y. (1997). Glucose-responsive gel

from phenylborate polymer and poly(vinyl alcohol): Prompt response at physiological pH through the interaction of borate with amine group in the gel. *Pharmaceutical Research* **14**, 289–293.

HOFFMAN, A. AND ZIV, E. (1997). Pharmacokinetic considerations of new insulin formulations and routes of administration. *Clinical Pharmacokinetics* **33**, 285–301.

HOLLEMAN, F. AND HOEKSTRA, J.B.L. (1997). Insulin lispro. *New England Journal of Medicine* **337**, 176–183.

HOME, P.D. (1997). Intensive insulin therapy in clinical practice. *Diabetologia* **40**, 83–87.

HORBETT, T.A., RATNER, B.D. AND KOST, J. (1984a). Swelling behaviour of glucose sensitive membranes. In: *Polymers as Biomaterials*. Eds. S.W. Shalaby, A.S. Hoffman, T.A. Horbett and B.D. Ratner, pp 279–292. New York: Plenum.

HORBETT, T.A., RATNER, B.D., KOST, J. AND SINGH, M. (1984b). A bioresponsive membrane for Insulin Delivery. In: *Recent Advances in Drug Delivery*. Eds. J. Anderson and S. Kim, pp 209–220. New York: Plenum Press.

HUMAR, A., KANDASWAMY, R., GRUESSNER, R., GRUESSNER, A. AND SUTHERLAND, D. (2000). Chronic rejection in pancreas transplant recipients: A multivariate analysis of risk factors. *Transplantation* **69**, S410.

IMAGAWA, A., HANAFUSA, T., ITOH, N., WAGURI, M., YAMAMOTO, K., MIYAGAWA, J., MORIWAKI, M., YAMAGATA, K., IWAHASHI, H., SADA, M., TSUJI, T., TAMURA, S., KAWATA, S., KUWAJIMA, M., NAKAJIMA, H., NAMBA, M. AND MATSUZAWA, Y. (1999). Immunological abnormalities in islets at diagnosis paralleled further deterioration of glycaemic control in patients with recent-onset Type I (insulin-dependent) diabetes mellitus. *Diabetologia* **42**, 574–578.

JACOBY, E., KRUGER, P., SCHLITTER, J., ROPER, D. AND WOLLMER, A. (1996). Simulation of a complex protein structural-change. *Protein Engineering* **9**, 113–125.

JAEGER, C., HERING, B.J., HATZIAGELAKI, E., FEDERLIN, K. AND BRETZEL, R.G. (1999). Glutamic acid decarboxylase antibodies are more frequent than islet cell antibodies in islet transplanted IDDM patients and persist or occur despite immunosuppression. *Journal of Molecular Medicine* **77**, 45–48.

JEANES, A. (1977). Dextrans and pullulans: industrially significant alpha-D-glucans. In: *Extracellular Microbial Polysaccharides*. Eds. P.A. Sandford and A. Laskin, pp 284–298. Washington, D.C.: American Chemical Society.

JEONG, S.Y. AND KIM, S.W. (1984). Self regulating insulin delivery systems i – synthesis and characterisation of glycosylated insulin. *Journal of Controlled Release* **1**, 57–66.

JEONG, S.Y., KIM, S.W., HOLMBERG, D.L. AND MCREA, J.C. (1985). Self-regulating insulin delivery systems iii – in-vivo studies. *Journal of Controlled Release* **2**, 143–152.

KANIKKANNAN, N., SINGH, J. AND RAMARAO, P. (1999). Transdermal iontophoretic delivery of bovine insulin and monomeric human insulin analogue. *Journal of Controlled Release* **59**, 99–105.

KATAOKA, K., MIYAZAKI, H., MIYAJI, K., HISAMITSU, I., TANAKA, K., OKANO, T. AND SAKURAI, Y. (1994). Novel glucose-sensitive polymers and hydrogels based on phenylborate-containing polymers. *Abstracts of Papers of The American Chemical Society* **208**, 161-POLY.

KATAOKA, K., MIYAZAKI, H., BUNYA, M., OKANO, T. AND SAKURAI, Y. (1998). Totally synthetic polymer gels responding to external glucose concentration: Their preparation and application to on-off regulation of insulin release. *Journal of The American Chemical Society* **120**, 12694–12695.

KATAOKA, K., MIYAZAKI, H., BUNYA, M., OKANO, T. AND SAKURAI, Y. (1999). On-off regulation of insulin-release by totally synthetic polymer gels responding to external glucose concentration. *Abstracts of Papers of The American Chemical Society* **217**, 165-POLY.

KEELE, C.A., NEIL, E. AND JOELS, N. (1982). *Samson Wright's Applied Physiology*. 13th Edition. Oxford: Oxford Medical Publications.

KELLY, W.D., LILLEHE, R.C., MERKEL, F.K., IDEZUKI, Y. AND GOETZ, F.C. (1967). Allotransplantation of the Pancreas and Duodenum Along with the Kidney in Diabetic Neuropathy. *Surgery* **61**, 827–837.

KENNEDY, F.P. (1991). Recent developments in insulin delivery techniques – current status and future potential. *Drugs* **42**, 213–227.

KIM, H. AND FASSIHI, R. (1997a). Application of a binary polymer system in drug release rate modulation.1. Characterization of release mechanism. *Journal of Pharmaceutical Sciences* **86**, 316–322.

KIM, H. AND FASSIHI, R. (1997b). Application of binary polymer system in drug release rate modulation.2. Influence of formulation variables and hydrodynamic conditions on release kinetics. *Journal of Pharmaceutical Sciences* **86**, 323–328.

KIM, S. AND JACOBS, H. (1994). Self-Regulated Insulin Delivery – Artificial Pancreas. *Drug Development and Industrial Pharmacy* **20**, 575–580.

KIM, S., JEONG, S., SATO, S., MCRAE, J. AND FEIJAN, J. (1984). Self-regulating insulin delivery system – a chemical approach. In: *Recent Advances in Drug Delivery Systems*. Eds. J. Anderson and S. Kim, pp 123–136. New York: Plenum Press.

KIM, C.K., IM, E.B., LIM, S.J., OH, Y.K. AND HAN, S.K. (1994). Development of glucose-triggered ph-sensitive liposomes for a potential insulin delivery. *International Journal of Pharmaceutics* **101**, 191–197.

KITANO, S., KOYAMA, Y., KATAOKA, K., OKANO, T. AND SAKURAI, Y. (1992). A novel drug delivery system utilising a glucose responsive complex formation between poly(vinyl alcohol) and poly(N-vinyl-2-pyrrolidone) with a pendent phenylboronic acid moiety. *Journal of Controlled Release* **19**, 162–170.

KNIP, M. (1998). Prediction and prevention of type I diabetes. *Acta Paediatrica. Supplement* **425**, 54–62.

KOIVISTO, V. AND TRONIER, B. (1983). Postprandial blood-glucose response to exercise in type – i diabetes – comparison between pump and injection therapy. *Diabetes Care* **6**, 436–440.

KOST, J., HORBETT, T., RATNER, B. AND SINGH, M. (1985). Glucose Sensitive Membranes containing Glucose Oxidase. Activity, swelling and Permeability Studies. *Journal of Biomedical Materials Research* **19**, 1117–1133.

KRANS, H.M. (1998). [Diabetes mellitus: current classification based on cause and sharpened blood glucose limits for diagnosis]. *Nederlands Tijdschrift voor Geneeskunde* **142**, 225–229.

KUCERA, M.L. AND GRAHAM, J.P. (1998). Insulin lispro, a new insulin analog. *Pharmacotherapy* **18**, 526–538.

LAGUESSE, G.E. (1893). Sur la formation desilots de Langerhans dans le pancreas. *Comptes Rendus des Seances de la Societe de Biologie (Paris)* **45**, 819–820.

LARSEN, C. (1989). Dextran prodrugs-structure and stability in relation to therapeutic activity. *Advanced Drug Delivery Reviews* **3**, 103–154.

LASSMAN-VAGUE, V., BELICAR, P., RACCAH, D., VIALETTES, B., SODOYEZ, J. AND VAGUE, P. (1995). Immunogenicity of long term intraperitoneal insulin administration with implantable programmable pump. Metabolic consequences. *Diabetes Care* **18**, 498–503.

LASSMAN-VAGUE, V., GUERCI, B., HANAIRE-BROUTIN, H., PINGET, M., RENARD, E., SELAM, J.-L. AND VAGUE, P. (1997). Use of implantable insulin pumps: the evadiac position. *Diabetes and Metabolism* **23**, 234–250.

LEE, S. AND PARK, K. (1994). Synthesis of Sol-Gel Phase Reversible Hydrogels Sensitive to Glucose. *Proceedings of the International Symposium of Controlled Release of Bioactive Materials* **21**, 93–94.

LESLIE, R.D. (1999). United Kingdom prospective diabetes study (UKPDS): what now or so what? *Diabetes Metabolism Research Reviews* **15**, 65–71.

LI, T., LEE, H. AND PARK, K. (1998). Comparative stereochemical analysis of glucose-binding proteins for rational design of glucose-specific agents. *Journal of Biomaterials Science. Polymer Edition* **9**, 327–344.

LOWMAN, A.M. AND PEPPAS, N. (1999). Solute transport analysis in pH-responsive, complexing hydrogels of poly(methacrylic acid-g-ethylene glycol). *Journal of Biomaterials Science. Polymer Edition* **10**, 999–1009.

LOWMAN, A.M. AND PEPPAS, N. (2000). Molecular analysis of interpolymer complexation in graft copolymer networks. *Polymer* **41**, 73–80.

LOWMAN, A.M., MORISHITA, M., KAJITA, M., NAGAI, T. AND PEPPAS, N. (1999). Oral delivery

of insulin using pH-responsive complexation gels. *Journal of Pharmaceutical Sciences* **88**, 933–937.

MAPLE, J. (1996). *Advanced Chemistry: An enquiry-based approach.* 1st Edition. London: John Murray Publishers.

MARSHALL, W.J. (1992). *Clinical Chemistry.* 2nd Edition. New York: Gower Medical Publishing.

MARTIN, A. (1993). *Physical Pharmacy: Principles in the Pharmaceutical Sciences.* 4th Edition. London: Lea and Febiger.

MAYES, A., ANDERSSON, L. AND MOSBACH, K. (1994). Sugar Binding Polymers Showing High Anomeric and Epimeric Discrimination Obtained by Noncovalent Molecular Imprinting. *Analytical Biochemistry* **222**, 483–488.

MCGEE, J.O., ISAACSON, P.G. AND WRIGHT, N.A. (1992). *Oxford Textbook of Pathology.* 1st Edition. Oxford: Oxford Medical Publications.

MECKLENBURG, R., BENSON, E., BENSON, J., FREDLUND, P., GUINN, T., METZ, R., NIELSEN, R. AND SANNAR, C. (1984). Acute complications associated with insulin infusion pump therapy – report of experience with 161 patients. *Journal of the American Medical Association* **252**, 3265–3269.

MEYER, B., KATZEFF, H., ESCHBACH, J., TRIMMER, J., ZACHARIAS, S., ROSEN, S. AND SIBALIS, D. (1989). Transdermal delivery of human insulin to albino rabbits using electrical-current. *American Journal of the Medical Sciences* **297**, 321–325.

MEYERHOFF, C., MENNEL, F.J., STERNBERG, F. AND PFEIFFER, E. (1996). Current status of the glucose sensor. *Endocrinologist* **6**, 332–339.

MONTAGUE, W. (1983). *Diabetes and the Endocrine Pancreas: A Biochemical Approach.* 1st Edition. London: Croom Helm.

MORIWAKI, M., ITOH, N., MIYAGAWA, J., YAMAMOTO, K., IMAGAWA, A., YAMAGATA, K., IWAHASHI, H., NAKAJIMA, H., NAMBA, M., NAGATA, S., HANAFUSA, T. AND MATSUZAWA, Y. (1999). Fas and Fas ligand expression in inflamed islets in pancreas sections of patients with recent-onset Type I diabetes mellitus. *Diabetologia* **42**, 1332–1340.

MYSLIWIEC, J., KRETOWSKI, A., KINALSKI, M. AND KINALSKA, I. (1999). CD11a expression and soluble ICAM-1 levels in peripheral blood in high-risk and overt type 1 diabetes subjects. *Immunology Letters* **70**, 69–72.

NAKAMAE, K., MIYATA, T., JIKIHARA, A. AND HOFFMAN, A.S. (1994). Formation of poly(glucosyloxyethyl methacrylate): Concanavalin A complex and its glucose-sensitivity. *Journal of Biomaterials Science. Polymer Edition* **6**, 79–90.

NARASIMHAN, B. AND PEPPAS, N.A. (1997). The role of modelling studies in the development of future controlled release devices. In: *Controlled Drug Delivery: Challenges and Strategies.* Ed. K. Park, pp 529–557. American Chemical Society.

NASR, C.E., HOOGWERF, B.J., FAIMAN, C. AND REDDY, S.S. (1999). Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). U.K. Prospective Diabetes Study (UKPDS) Group. *Journal of the American Medical Association* **281**, 2005–2012.

NOVAK, T.J. AND HANDFORD, A.G. (1998). *Essentials of Pathophysiology.* Melbourne: William C. Brown.

OBAIDAT, A.A. AND PARK, K. (1995). Gel-sol phase-reversible hydrogels sensitive to glucose. *Pharmaceutical Research* **12**, 9 (supplement).

OBAIDAT, A.A. AND PARK, K. (1996). Characterization of glucose dependent gel-sol phase transition of the polymeric glucose-concanavalin A hydrogel system. *Pharmaceutical Research* **13**, 989–995.

OBAIDAT, A. AND PARK, K. (1997). Characterization of protein release through glucose-sensitive hydrogel membranes. *Biomaterials* **18**, 801–806.

OBSIL, T. AND PAVLICEK, Z. (1997). Glycation of proteins and phospholipids: Maillard reaction in vivo. *Chemicke Listy* **91**, 558–569.

ORCHARD, T.J., FORREST, K.Y., ELLIS, D. AND BECKER, D.J. (1997). Cumulative glycemic exposure and microvascular complications in insulin-dependent diabetes mellitus. The glycemic threshold revisited. *Archives of Internal Medicine* **157**, 1851–1856.

PAI, C.M., BAE, Y.H., MACK, E.J., WILSON, D.E. AND KIM, S.W. (1992). Concanavalin a

microspheres for a self-regulating insulin delivery system. *Journal of Pharmaceutical Sciences* **81**, 532–536.

PAI, C., JACOBS, H., BAE, Y. AND KIM, S. (1993). Synthesis and characterization of soluble concanavalin-a oligomer. *Biotechnology and Bioengineering* **41**, 957–963.

PARK, K. (1997). Controlled drug delivery. In: *Controlled Drug Delivery: Challenges and Strategies.* Ed. K. Park, pp 1–5. American Chemical Society.

PATY, B., LANZ, K., SUTHERLAND, D. AND ROBERTSON, R. (2000a). Hypoglycemia counterregulation is intact in successful, long-term pancreas transplant recipients. *Diabetes* **49**, A30.

PATY, B.W., LANZ, K.H., SUTHERLAND, D. AND ROBERTSON, R. (2000b). Hypoglycemia counterregulatory responses in successful pancreas transplant recipients of 11–19 years duration. *Journal of Investigative Medicine* **48**, A75.

PEARCE, B., WALKER, G., SLODKI, M. AND SCHUERCH, C. (1990). Enzymatic and methylation analysis of dextrans and (1-]3)-alpha-D-glucans. *Carbohydrate Research* **203**, 229–246.

PEPPAS, N.A., KEYS, K., TORRES LUGO, M. AND LOWMAN, A. (1999). Poly(ethylene glycol)-containing hydrogels in drug delivery. *Journal of Controlled Release* **62**, 81–87.

PICKUP, J.C. AND WILLIAMS, G. (1997). *Textbook of Diabetes.* Oxford: Blackwell Science.

PILLAI, O., NAIR, V., PODURI, R. AND PANCHAGNULA, R. (1999). Transdermal iontophoresis. Part II: Peptide and protein delivery. *Methods and Findings in Experimental and Clinical Pharmacology* **21**, 229–240.

PILLION, D.J., HOSMER, S. AND MEEZAN, E. (1998). Dodecylmaltoside-mediated nasal and ocular absorption of lyspro-insulin: independence of surfactant action from multimer dissociation. *Pharmaceutical Research* **15**, 1637–1639.

PITT, C.G. (1990). The biodegradeability of polyester blends. *Biomaterials* **11**, 108–112.

PODUAL, K., DOYLE, F.J. AND PEPPAS, N. (2000a). Dynamic behavior of glucose oxidase-containing microparticles of poly(ethylene glycol)-grafted cationic hydrogels in an environment of changing pH. *Biomaterials* **21**, 1439–1450.

PODUAL, K., DOYLE, F.J. AND PEPPAS, N. (2000b). Glucose-sensitivity of glucose oxidase-containing cationic copolymer hydrogels having poly(ethylene glycol) grafts. *Journal of Controlled Release* **67**, 9–17.

PODUAL, K., DOYLE, F.J. AND PEPPAS, N. (2000c). Preparation and dynamic response of cationic copolymer hydrogels containing glucose oxidase. *Polymer* **41**, 3975–3983.

POPE, E.J.A., BRAUN, K. AND PETERSON, C. (1997). Bioartificial organs. 1. Silica gel encapsulated pancreatic islets for the treatment of diabetes mellitus. *Journal of Sol Gel Science and Technology* **8**, 635–639.

PORTH, C. (1998). *Pathophysiology: Concepts of Altered Health States.* 3rd Edition. New York: J.B. Lippincott.

QUINN, R. AND ANDRADE, J.D. (1983). Minimizing the aggregation of neutral insulin solutions. *Journal of Pharmaceutical Sciences* **72**, 1472–1473.

RAHUEL-CLERMONT, S., FRENCH, C.A., KAARSHOLM, N.C. AND DUNN, M.F. (1997). Mechanisms of stabilization of the insulin hexamer through allosteric. *Biochemistry* **36**, 5837–5845.

RANG, H.P., DALE, M.M. AND RITTER, J. (1999). *Pharmacology.* 4th Edition, Edinburgh: Churchill Livingstone.

ROYSARKAR, T., GUPTA, A., DASH, R. AND DOGRA, M. (1993). Effect of Insulin Therapy on Progression of Retinopathy in Noninsulin-Dependent Diabetes-Mellitus. *American Journal of Ophthalmology* **115**, 569–574.

RUGGIERO, D., LECOMTE, M., MICHOUD, E., LAGARDE, M. AND WIERNSPERGER, N. (1997). Involvement of cell-cell interactions in the pathogenesis of diabetic retinopathy. *Diabetes and Metabolism* **23**, 30–43.

SAKAI, H., JINDE, K., SUZUKI, D., YAGAME, M. AND NOMOTO, Y. (1996). Localization of glycated proteins in the glomeruli of patients with diabetic nephropathy. *Nephrology, Dialysis and Transplantation* **11**, 66–71.

SANGER, F. (1959). Chemistry of insulin. *Science* **129**, 1340–1344.

SAUDEK, C. (1993). Future Developments in Insulin Delivery Systems. *Diabetes Care* **16**, 122–132.

SAUDEK, C., DUCKWORTH, W., GIOBBI-HURDER, A., HENDERSON, W., HENRY, R., KELLY, D.,

EDELMAN, S., ZIEVE, F., ADLER, R., ANDERSON, R., HAMILTON, B., DONNER, T., KIRKMAN, M. AND MORGAN, N. (1996). Implantable insulin pump vs multiple dose insulin for non-insulin-dependent diabetes mellitus: a randomised clinical trial. Department of Veterans Affairs Implantable Insulin Pump Study Group. *Journal of the American Medical Association* **276**, 1322–1327.

SAUDEK, C.D. (1997). Novel forms of insulin delivery. *Endocrinology and Metabolism Clinics of North America,* 599.

SCHIEL, R. AND MULLER, U. (1999). Intensive or conventional insulin therapy in type 2 diabetic patients? A population-based study on metabolic control and quality of life (The JEVIN-trial). *Experimental and Clinical Endocrinology and Diabetes* **107**, 506–511.

SCHIFFERDECKER, E., SCHMIDT, K., BOEHM, B. AND SCHATZ, H. (1994). Long-term compliance of intensified insulin therapy. *Diabetes Research and Clinical Practice* **23**, 17–23.

SCHORR, A.B. (1999). Implications of the UKPDS Study for general practice care of type 2 diabetes [letter]. *British Journal of General Practice* **49**, 1009.

SCHWARTE, L.M. AND PEPPAS, N. (1998). Novel poly(ethylene glycol)-grafted, cationic hydrogels: preparation, characterization and diffusive properties. *Polymer* **39**, 6057–6066.

SCOTT, R.A. AND PEPPAS, N. (1999). Compositional effects on network structure of highly crosslinked copolymers of PEG-containing multiacrylates with-acrylic acid. *Macromolecules* **32**, 6139–6148.

SELAM, J.L. (1997). Management of diabetes with glucose sensors and implantable insulin pumps – From the dream of the 60s to the realities of the 90s. *Asaio Journal* **43**, 137–142.

SHIINO, D., MURATA, Y., KUBO, A., KIM, Y.J., KATAOKA, K., KOYAMA, Y., KIKUCHI, A., YOKOYAMA, M., SAKURAI, Y. AND OKANO, T. (1995). Amine containing phenylboronic acid gel for glucose-responsive insulin release under physiological pH. *Journal of Controlled Release* **37**, 269–276.

SHIINO, D., KUBO, A., MURATA, Y., KOYAMA, Y., KATAOKA, K., KIKUCHI, A., SAKURAI, Y. AND OKANO, T. (1996). Amine effect on phenylboronic acid compiler with glucose under physiological pH in aqueous solution. *Journal of Biomaterials Science. Polymer Edition* **7**, 697–705.

SHIMADA, A., IMAZU, Y., MORINAGA, S., FUNAE, O., KASUGA, A., ATSUMI, Y. AND MATSUOKA, K. (1999). T-cell insulitis found in anti-GAD65+ diabetes with residual beta-cell function. A case report [see comments]. *Diabetes Care* **22**, 615–617.

SHOHAM, M., KALB, A.J. AND PECHT, I. (1973). Specificity of Metal Ion Interaction with concanavalin A. *Biochemistry* **12**, 1914–1917.

SIDDIQUI, O., SUN, Y., LIU, J. AND CHIEN, Y. (1987). Facilitated transdermal transport of insulin. *Journal of Pharmaceutical Sciences* **76**, 341–345.

SIEGEL, R.A. (1997). Modelling of self-regulating oscillatory drug delivery. In: *Controlled Drug Delivery: Challenges and Strategies.* Ed. K. Park, pp 501–526. American Chemical Society.

SIGNORE, A., CHIANELLI, M., PARISELLA, M.G., CAPRIOTTI, G., GIACALONE, P., DI LEVE, G. AND BARONE, R. (1999). In vivo imaging of insulitis in autoimmune diabetes. *Journal of Endocrinological Investigation* **22**, 151–158.

SMITH, G.D., SWENSON, D.C., DODSON, E.J., DODSON, G.G. AND REYNOLDS, C.D. (1984). Structural stability in the 4-zinc human insulin hexamer. *Proceedings of the National Academy of Sciences of the United States of America* **81**, 7093–7097.

SOON-SHIONG, P., FELDMAN, E., NELSON, R., HEINTZ, R., YAO, Q., YAO, Z., ZHENG, T., MERIDETH, N., SKJAKBRAEK, G., ESPEVIK, T., SMIDSROD, O. AND SANDFORD, P. (1993). Long-Term Reversal of Diabetes by the Injection of Immunoprotected Islets. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 5843–5847.

STEINDEL, B.S., ROE, T.R., COSTIN, G., CARLSON, M. AND KAUFMAN, F.R. (1995). Continuous subcutaneous insulin infusion (CSII) in children and adolescents with chronic poorly controlled type 1 diabetes mellitus. *Diabetes Research and Clinical Practice* **27**, 199–204.

STEPHEN, R., KIM, S. AND JACOBSEN, S. (1984). Potential novel methods for insulin administration .2. self-regulating internal-drug delivery systems. *Biomedica Biochimica Acta* **43**, 559–560.

STRYER, L. (1988). *Biochemistry*. 3rd Edition. New York: W.H. Freeman.

SUMNER, J.B. AND HOWELL, S.F. (1936). The identification of the hemagglutinin of the Jack Bean with Concanavalin A. *Journal of Bacteriology* **32**, 227–237.

SWIDAN, S.Z. AND MONTGOMERY, P. (1998). Effect of blood glucose concentrations on the development of chronic complications of diabetes mellitus. *Pharmacotherapy* **18**, 961–972.

TANNA, S. AND TAYLOR, M.J. (1994). A self regulating delivery system using high molecular weight solutes in glucose sensitive gel membranes. *Journal of Pharmacy and Pharmacology* **46**, 1051b.

TANNA, S. AND TAYLOR, M.J. (1997). Glucose-responsive gels based on dextran covalently coupled with lectin to control insulin delivery. *Journal of Pharmacy and Pharmacology* **49**, 76.

TANNA, S. AND TAYLOR, M.J. (1998a). Characterization of model solute and insulin delivery across covalently modified lectin-polysaccharide gels sensitive to glucose. *Pharmacy and Pharmacology Communications* **4**, 117–122.

TANNA, S. AND TAYLOR, M. J. (1998b). *Insulin delivery governed by lectin-glycogen gels sensitive to glucose.* Proceedings of the International Symposium of Controlled Release of Bioactive Materials, Las Vegas.

TANNA, S., TAYLOR, M.J. AND ADAMS, G. (1999). Insulin delivery governed by covalently modified lectin-glycogen gels sensitive to glucose. *Journal of Pharmacy and Pharmacology* **51**, 1093–1098.

TAYLOR, M.J. (1992). *Patent WO 93/13803 Drug System.* January 10, 1992.

TAYLOR, M.J. (1993). *Patent WO 95/01186 Drug System II.* June 30, 1993.

TAYLOR, M.J. AND TANNA, S. (1994). A self regulated delivery system using unmodified solutes in glucose sensitive gel membranes. *Journal of Pharmacy and Pharmacology* **46**, 1051a.

TAYLOR, M.J., TANNA, S., COCKSHOTT, S. AND VAITHA, R. (1994). *A novel self regulated delivery system using unmodified solutes in glucose sensitive gel membranes.* Proceedings of the Third European Symposium on Controlled Drug Delivery, The Netherlands.

TAYLOR, M.J., TANNA, S. AND ADAMS, G. (1995a). *Insulin delivery using a novel glucose-sensitive formulation.* Proceedings of the International Symposium of Controlled Release of Bioactive Materials, Seattle.

TAYLOR, M.J., TANNA, S., TAYLOR, P.M. AND ADAMS, G. (1995b). The delivery of insulin from aqueous and non-aqueous reservoirs governed by a glucose sensitive gel membrane. *Journal of Drug Targeting* **3**, 209–216.

TOMIOKA, K., FUKUDA, H. AND TANIGUCHI, H. (1994). Insulin delivery system based on an affinity chemical valve. *Journal of Fermentation and Bioengineering* **77**, 442–444.

TOMLINSON, D.R. (1998). Future prevention and treatment of diabetic neuropathy. *Diabetes and Metabolism* **24**, 79–83.

TREHAN, A. AND ALI, A. (1998). Recent approaches in insulin delivery. *Drug Development and Industrial Pharmacy* **24**, 589–597.

TSUKUSHI, S., KATSUZAKI, T., AOYAMA, I., TAKAYAMA, F., MIYAZAKI, T., SHIMOKATA, K. AND NIWA, T. (1999). Increased erythrocyte 3-DG and AGEs in diabetic hemodialysis patients: Role of the polyol pathway. *Kidney International* **55**, 1970–1976.

TURNER, R.C. (1999). United Kingdom Prospective Diabetes Study (UKPDS). Effects of glucose and blood pressure control on complications of type 2 diabetes mellitus. *Cleveland Clinical Journal of Medicine* **66**, 247–253.

TURNER, R.C., CULL, C.A., FRIGHI, V. AND HOLMAN, R.R. (1999). Pharmacologic therapy for type 2 diabetes mellitus. *Annals of Internal Medicine* **131**, 281–303.

TYLE, P. (1986). Iontophoretic devices for drug delivery. *Pharmaceutical Research* **3**, 318–326.

UNGER, R.H. (1982). Benefits and risks of meticulous control of diabetes. *Medical Clinics of North America* **66**, 1317–1324.

VALUEV, I.L., CHUPOV, V.V., SYTOV, G.A., VALUEV, L.I. AND PLATE, N.A. (1997). Phase-reversible hydrogels based on acrylamide-N-(2-D- glucose)acrylamide copolymers. *Vysokomolekulyarnye Soedineniya Seriya A and Seriya B*, 751–754.

VALUEV, I.L., CHUPOV, V.V. AND VALUEV, L.I. (1998). Chemical modification of polymers with physiologically active species using water-soluble carbodiimides. *Biomaterials* **19**, 41–43.

VAN DIJK-WOLTHHUIS, W.N.E., HOOGEBOOM, J.A.M., VAN STEENBERGEN, M.J., TSANG, S.K.Y. AND HENNINK, W.E. (1997). Degradation and release behaviour of dextran based hydrogels. *Macromolecules* **30**, 4639–4645.

VARMA, S.D., DEVAMANOHARAN, P.S. AND ALI, A. (1997). Formation of advanced glycation end (AGE) products in diabetes: Prevention by pyruvate and alpha-keto glutarate. *Molecular and Cellular Biochemistry* **171**, 23–28.

VON MERING, J. AND MINKOWSKI, O. (1890). Diabetes mellitus nach pankreasextipation. *Archives Experimental Pathology and Pharmacology* **26**, 371–387.

WANG, J., TAKEDA, A., YANG, J. AND WU, C. (1992). Conformation of concanavalin-a and its fragments in aqueous-solution and organic solvent-water mixtures. *Journal of Protein Chemistry* **11**, 157–164.

WHO. (1985). *Diabetes Mellitus: Report of a WHO Study Group*. Geneva: WHO.

WILDE, M.I. AND MCTAVISH, D. (1997). Insulin lispro: a review of its pharmacological properties and therapeutic use in the management of diabetes mellitus. *Drugs* **54**, 597–614.

WILLATTS, S. (1992). *Lecture Notes on Fluid and Electrolyte Balance*. Oxford: Blackwell Scientific Publications.

WILLIAMS, G. AND PICKUP, J.C. (1998). *Handbook of Diabetes*. Oxford: Blackwell Science.

WOOD, M.E. (1998). New criteria for the detection, diagnosis, and classification of diabetes mellitus. *Nurse Practitioner Forum* **9**, 38–41.

YIP, C.M., DEFELIPPIS, M.R., FRANK, B.H., BRADER, M.L. AND WARD, M.D. (1998). Structural and morphological characterization of ultralente insulin crystals by atomic force microscopy: evidence of hydrophobically driven assembly. *Biophysical Journal* **75**, 1172–1179.