INTRODUCTION
DIABETES AN OVERVIEW

The disorder, mainly in carbohydrate metabolism, known as ‘Diabetes’, appears in ancient Sanskrit literature. The Egyptian papyrus of Ebers, dating from 1550 BC references to dietary remedies for those abundant urine (polysuria). The first clinical account of diabetes is given in the writings of Aretaies the Cappodocian (AD 170) who described ‘this mysterious affection... as ‘a melting down of flesh and limbs into urine... life is short, disgusting and painful, thirst unquenchable, death inevitable...’ To this day we are unable to challenge his observation that diabetes is a mysterious disease. (Bloom and Ireland, 1992).

Thomas Cawley was the first to suggest the pancreatic involvement in diabetes. Writing in the London Medical Journal of (1788) he described a case of diabetes which at autopsy showed marked pancreatic damage and led Cawley to postulate that pancreatic disease may be casually related to diabetes. The importance of the pancreas was established by Oskar Minkowski in 1889, who described with Von Mering, how pancreatectomy made a laboratory dog urinate excessively and how the urine attracted flies, he tested the urine for sugar and found large amount of it present (Bloom and Ireland, 1992).

Earlier, Paul Langerhans, while still a medical student, wrote a treatise on the pancreas which included the first clear description of the islets tissues which bear his name, although he did not ascertain the significance of these islets. Gustave Laguesse (1893) suggested that they might produce an endocrine secretion. After the development of the concept of hormones by Ernst Starling in 1904, scholars in Germany, Romania and elsewhere concentrated their attention
on extracting insulin from the pancreas. Finally a Canadian surgeon Fredrik Banting, working with his student Charles Best was successful in discovering and isolating insulin in 1921.

The discovery of insulin was promptly translated into a lifesaving remedy. This was made possible by the efforts of James Bertram Collip and the pharmaceutical industry, within two years of the discovery of insulin by Banting and Best. Thus a new era dawned for the treatment of diabetes by insulin. (Bloom and Ireland, 1992)

**Diabetes can be defined as a chronic disorder characterized by a raised level of glucose in the blood.** Since there are many factors leading to this elevation of blood glucose, diabetes can be an outcome of any of these factors. Which can be hereditary, environmental or hormonal.

From the clinical point of view, the etiology of the commonest forms of diabetes is unknown and is sometimes referred to as primary diabetes. The forms of diabetes include; **Type I or insulin dependent diabetes mellitus (IDDM); Type II or non-insulin dependent diabetes mellitus (NIDDM); and much rarer type of diabetes usually associated with other hereditary disorders.** Apart from these there are reports, mostly from the developing countries of the occurrence of malnutrition dependent diabetes mellitus (MRDM).

With epidemiological evolution and demographic transitions it has been estimated that 50 million people with diabetes currently live in developing countries and this number may grow to 78 million over the current decade. Nearly two third of these will be in Asia and West-Asia, of which one third will be in South East Asia. India will account for half of the total diabetes patients in
South East Asia. Fortunately, the Type I diabetes (IDDM) is not so prevalent in South East Asia including India as it is in the West. Instead, young people in India have Malnutrition Related Diabetes Mellitus (MRDM), which can be efficiently controlled or reversed by proper and balanced diet (Bajaj and Madan, 1993).

Though in diabetes there is two to three fold increase in blood glucose concentration from normal levels, the tissues themselves are starved of glucose. Apart from high glucose in blood, there is also an increase of fat and protein in blood, which could accumulate. The body may break these down to obtain energy. This condition can arise as a result of:

i) inadequate production of insulin by the pancreas

ii) the problems preventing insulin from reaching body tissues

iii) the inadequate response of tissues to insulin, insulin resistance

(Morrison, 1993)

The normal fasting blood glucose of humans is 75-100 mg/dl, which may increase upto 350 mg/dl in severe cases of diabetes.

PATHOLOGICAL CONDITIONS

Type I diabetes (IDDM)

Type I diabetes or IDDM most commonly effects children or young adults, but there could be a late onset as well. The victims of this type of disorder usually emaciate (occasionally there are cases of overweight as well) and suffer
from excessive thirst, polyuria and lassitude. The urine, at diagnosis, contains sugar and significant amount of acetone, while blood examination reveals evidence of dehydration, a tendency of ketoacidosis and a blood glucose exceeding 11 mmol/l (200mg/dl). Level of plasma insulin shows a decrease and also poor response to the elevated glucose. Excess of glucose and pancreatic polypeptide can also be demonstrated and these may be additional factors in the pathogenesis of diabetes of this type.

Type I diabetics need insulin to survive, since without it the symptoms worsen, drowsiness gives place to increased ketoacidosis and even to fatal coma. Hence, these patients are referred to as insulin dependent.

One of the causes of IDDM has been reported to be T cell mediated auto-immunity towards glutamic acid decarboxylase (GAD) (Harrison et al 1993). The authors had studied the first degree relatives of IDDM patients, with risk of this disease. Those with cell mediated response towards GAD progressed to the disease rapidly, whereas those with only anti GAD antibodies progressed slowly and were found not to acquire the disease at all. The scholars postulated that if GAD is a pathogenic autoantigen, sensitization to B cells by GAD is more likely to lead to IDDM when the immune response deviates towards the expansion of auto-reactive T cells rather than towards the generation of auto-antibodies. There are also some reports regarding the involvement of a 30 kDa, pancreatic autoantigen in the sera of IDDM patients (Kim et al 1993).

In large part, NIDDM is due to insulin resistance, a state in which target cells no longer respond to ordinary levels of circulating insulin (White and Kahn, 1994). Circumstantial evidence has accumulated pointing to viral infection as the
vector most likely to precipitate diabetes. The role of coxsackievirus in this regard is now quite evident (Ramsingh et al 1991). Diabetes in children is much less frequently in the summer than in the winter months, when viral infections are epidemic. Animals inoculated experimentally with certain viruses have developed diabetes. These evidences are very suggestive but so far no definite proof is available.

The explanation of etiology of IDDM may lie in the fact of matter that the disease occurs because of the combinations of several factors namely hereditary, immunological, environmental and upto certain extent nutritional.

**Type II diabetes (NIDDM)**

Type II diabetes is certainly the commonest type of diabetes, it is late onset i.e. occurring mostly after thirty five years of age. The diagnosis is made by routine examination of the urine for the presence of sugar and the symptoms shown or elicited on direct examination and questioning. Thirst, polyuria and loss of weight may be present but are seldom severe. The diagnosis can be established by finding an elevated blood sugar or by performing a glucose tolerance test, if there is any doubt. The urine contains sugar but acetone is not found. The study on plasma show that insulin is present but lower than the normal level.

Occasionally, Type II diabetes occurs in children or growing adults in some families of this category the inheritance may be dominant. When diabetes occurs in identical twins, it is always concordant (both twins have diabetes) in the maturity onset type, again suggesting strong genetic inheritance. Evidences suggest that the receptors on the islet cells do not respond to the elevated blood
Classification of diabetes and other categories of glucose tolerance (Bloom and Ireland, 1992)

Class

1. Insulin dependent diabetes mellitus, IDDM (Type I)

2. Non-insulin dependent diabetes mellitus, NIDDM (Type II)
   a) Obese
   b) Non obese

3. Malnutrition related diabetes mellitus, (MRDM)
   a) Fibrocalculous pancreatic diabetes
   b) Protein deficient pancreatic diabetes

4. Gestational diabetes mellitus (GDM)

5. Other types of diabetes including diabetes associated with certain conditions and syndromes
   a) Pancreatic disease
   b) Hormonal
   c) Drug or chemical induced
   d) Insulin or its receptor abnormalities
   e) Certain genetic syndromes
   f) Miscellaneous

6. Impaired glucose tolerance (IGT)
   a) Non obese IGT
   b) Obese IGT
   c) IGT associated with pancreatic diseases, hormonal conditions, drug or chemical induced, insulin receptor abnormalities, certain genetic syndromes
      e.g. Bloom syndrome

7. Previous abnormalities of glucose tolerance (PreIGT)

8. Potential abnormalities of glucose tolerance (PotIGT)

Former Terminology

juvenile diabetes, juvenile onset diabetes, JOD, ketosis prone diabetes, brittle diabetes

adult/maturity onset diabetes, MOD, ketosis-resistant diabetes, brittle diabetes

tropical diabetes, pancreatic diabetes, ketosis resistant diabetes of the young

gestational diabetes

secondary diabetes

asymptomatic diabetes, chemical diabetes, subclinical diabetes, borderline diabetes, latent diabetes

latent diabetes, prediabetes

prediabetes, potential diabetes
glucose well, may be because of the dwindling number of these receptors on the islet cells (Bloom and Ireland, 1992).

The factor most likely to enhance the problem of diabetes is obesity. Carbohydrate rich diet increases the demand for insulin and obesity causes resistance in the peripheral tissues to the action of insulin. The treatment of Type II diabetes is thus self-evident. Obesity of the patient must be reduced, preferably by restricting the intake of refined carbohydrate and saturated fats in the diet. This leads to an increased sensitivity to circulating plasma insulin and return of blood sugar to normal level in most cases. Still, in some patients despite dieting, the blood sugar level remains elevated and in these cases, oral hypoglycaemic agents are prescribed like some sulphonylureas (Bloom and Ireland, 1992).

Though there are quite a few dissimilarities between Type I and Type II diabetes, there are some similarities as well. Both types may develop the same degenerative changes in the blood vessels, nerves, kidneys and eyes, suggesting that the degeneration arise due to hyperglycaemia rather than any inherited etiological factor which may be responsible for degenerative changes leading to long-term complications (Bloom and Ireland, 1992).

METABOLIC AND RELATED CHANGES

NEUROLOGICAL CHANGES

Regional ischemia appears to be a central process in the development of diabetic retinopathy exacerbated by an early, sustained increase in retinal capillary permeability, changes in endothelial and neural cell morphology, and perhaps in basement membrane thickening. Abnormal growth hormone secretion
has been observed in some diabetics and this may contribute, in various ways, to the development of microangiopathy (Brownlee and Cerami, 1981).

Diabetic neuropathy is characterised by a variety of morphological changes associated with decreased sensory and motor conduction velocities including decreased number of intra-membranous particles on the myelin surface; endoneural edema with resultant shrinkage of axon and Schwann cells; increased permeability of nodal gap substances; basement membrane thickening in the intra- and peri-neural vessel; axonal degeneration; and segmental demyelination. Following biochemical changes, some changes are observed also in nerves; changes in the composition and synthetic rate of various myelin lipid and protein components; increased activity of polyol pathway; decreased concentration and synthetic rate of myoinositol and phosphatidyl inositol and decreased exoplasmic transport of choline acetylase, acetylcholine esterase, norepinephrine and several glycoproteins (Keen and Jarret, 1982).

Changes in enzymes in the Central Nervous System

There is an increase in the activity of Na⁺K⁺-ATPase at the early onset of diabetes, which slowly recovers; whereas the increase in the activity of Mg²⁺-ATPase in all the region of brain of diabetic rats, recovers by the administration of insulin (Mayanil et al 1982b). There is a decrease in the activity of malic enzyme in the brain of diabetic rats which is brought to normal level by the administration of insulin (Murthy and Baquer, 1983). In hypoglycaemia, type I hexokinase isoenzyme showed a small decrease in both soluble and particulate fractions from the cerebral hemisphere (Kaur et al 1983). A small increase in the
activity of glucose 6 phosphatase (G6Pase) and fructose 1,6 diphosphatase (FDPase) was found in various regions of brain in diabetic condition (Kaur et al 1981). There was a decrease in the activity of monoamine oxidase (MAO) in different regions of the brain, early in diabetes which reversed by insulin administration to diabetic rats (Mayanil et al 1982a). In diabetes, phosphofructokinase (PFK) and pyruvate kinase (PK) activity decreases in cerebral hemisphere and cerebellum whereas it remains unaffected in brain stem. These effects are reversed by insulin administration (Srivastava and Baquer, 1984a; Srivastava and Baquer, 1984b).

**VASCULAR AND METABOLIC CHANGES**

Accelerated large vessel disease in diabetes may be due to synergistic pathological mechanism involving hyperlipidaemia, altered platelet behaviour and abnormalities in arterial wall function.

Elevated levels of VLDL, triglyceride and LDL cholesterol have been observed in all major classes of diabetes patients. HDL cholesterol may or may not change. Hypercholesterolaemia may be due to increased intestinal VLDL as well as LDL production and, perhaps also to an LDL removal effect. Hyperglycaemia induced endothelial cell dysfunction, increased platelet interaction with subendothelial structures, increased serum growth factor mediated smooth muscle cell proliferation, increased secretion of altered collagen molecules alterations in proteoglycans, and impaired intracellular degradation of LDL may all contribute to the development of microangiopathy. (Brownlee and Cerami, 1981; Orchard, 1990).
Figure 1: Schematic representation of changes in glucose metabolism and potential damage of tissues in diabetes.
The effect of diabetes on the metabolic pathways and their interrelationships is very complex, as virtually all the metabolic pathways of macromolecules in most tissues are affected in uncontrolled diabetes (Taylor and Agius, 1988).

Normally, fasting blood glucose is maintained constant by control of hepatic glucose output. After an overnight fast, about 75% of hepatic glucose is accounted for by glycogenolysis and rest by gluconeogenesis from lactate, alanine, glycerol and pyruvate in decreasing order (Hers and Hue, 1983; Taylor and Agius, 1988). Hepatic glucose output is controlled by basal levels of insulin and glucagon. Basal hepatic glucose extraction of insulin and glucagon is approximately 50% (Waldhausl et al 1982); and hence insulin and glucagon concentration, in the peripheral circulation, are lower than in portal vein. At least 70% of extrahepatic glucose utilization occurs in insulin-insensitive tissues namely brain, RBC and renal medulla (Taylor and Agius, 1988). In NIDDM, fasting blood glucose is raised in direct proportion to hepatic glucose output (Bogardus et al 1984; Revers et al 1984; DeFronzo et al 1985), and appears unlikely to be a result of decreased insulin action at the periphery as it has not been shown to correlate closely with insulin stimulated glucose disposal (DeFronzo et al 1982; Bogardus et al 1984).

As fasting plasma insulin and C-peptide concentrations are normal in NIDDM, the elevated hepatic glucose output is likely to reflect a degree of hepatic insulin insensitivity. Study of the insulin dose-response of suppression of hepatic glucose output supports this concept (Revers et al 1984). In NIDDM subjects, study of plasma insulin levels throughout the day has demonstrated that,
although the incremental responses to meal are decreased and delayed, the prolonged postprandial peaks ensure that mean diurnal plasma insulin levels are elevated (Liu et al 1983). Postprandial rates of gluconeogenesis are not suppressed (Taylor and Agius, 1988) and total hepatic glucose release as assessed isotopically is excessive (Firth et al 1986). Gluconeogenesis, from meal-derived 3-carbon precursors is somewhat greater in NIDDM than in normal subjects (Taylor and Agius, 1988).

Hexokinase and phosphofructokinase activities were found to be decreased in human muscle (Falholt et al 1988), suggesting a potential decrease in glycolysis in NIDDM subjects; whereas glucose-6-phosphate dehydrogenase and malic enzyme activity was elevated, and muscle triglycerol level was elevated too. In IDDM subjects, postprandial hyperglycaemia was due to the lack of sharp increase in circulating insulin levels and reflects inadequacies in the dynamics of insulin delivery. Hepatic glucose is released uninhibited, along with this there is relative insulin resistance in the peripheral tissues (Proitto et al 1983; Yki Jarvinen et al 1984), which results in postprandial hyperglycaemia (Taylor and Agius, 1988).

Among the circulating metabolites, lactate level was found to be most elevated compared to others. Even 24 hours after the administration of insulin the lactate level was found to be elevated, and Cori's cycle too is suppressed (Nossadini et al 1982). Restoration of portal-peripheral insulin gradient returns lactate towards normal (Stevenson et al 1983a; Stevenson et al 1983b; Jiminez et al 1985).
One of the consequences of hyperglycaemia in human diabetes mellitus is increased metabolism of glucose by sorbitol pathway. This reaction is catalysed by aldose reductase. Sorbitol is converted to fructose by sorbitol dehydrogenase. Aldose reductase is present in human brain, nerves, aorta, muscles, erythrocytes and ocular lens (Srivastava et al. 1984; Das and Srivastava, 1985a). Aldose reductase has a low affinity for glucose (Km = 100 mM), it can be activated by glucose-6-phosphate, NADPH and glucose (Das and Srivastava, 1985a; Das and Srivastava, 1985b). Sorbitol is impermeable to cell membrane and tends to accumulate in the cell (Taylor and Agius, 1988; Saxena et al. 1992a). At high glucose concentration the sorbitol pathway in rat rabbit lens may account for one-third of glucose metabolism (Gonzalez et al. 1983; Gonzalez et al. 1986). This has important implications in terms of redox changes of NADP and NAD⁺ couples and metabolism of glucose by alternative pathways (Jeffery and Jornvall, 1983; Askar and Baquer, 1994).

**CHANGES IN LIPID METABOLISM**

There is rapid mobilisation of fatty acids from adipose tissues in IDDM. Excessive lipolysis during insulin deficiency is the combined result of insulin absence and insulin resistance (Singh et al. 1987). One of the consequences of excessive mobilisation of fatty acids in IDDM is the production of ketone bodies in liver. The rate of transfer of fatty acyl units to the mitochondria is regulated by the activity of carnitine palmitoyl transferase I, which faces the intermitochondrial membrane space and catalyses the first step specific to mitochondrial fatty acid oxidation (Taylor and Agius, 1988). Carnitine palmitoyl
transferase I is regulated by malonyl CoA, an intermediate in fatty acid synthesis, and it is also regulated by a phosphorylation mechanism. In insulin deficiency, the rate of fatty acid synthesis in liver declines and consequently the concentration of malonyl CoA also decreases, and the affinity of palmitoyl transferase I for malonyl CoA also decreases (Gamble and Cook, 1985). Thus, relieving the inhibition of carnitine palmitoyl transferase by malonyl CoA (Taylor and Agius, 1988). In muscle, at ketone body concentrations attained in prolonged starvation or diabetic ketosis, the rate of uptake reaches saturation. Consequently, with increasing ketone body production and thereby plasma concentration, there is progressive decrease in total fractional clearance (Fery and Balasse, 1985).

In humans, plasma ketone concentration correlates with, and is generally higher than, acetoacetate concentration in fasting and diabetic ketosis (Owen et al 1982). Conversion of acetoacetate to acetone can occur either non-enzymatically or is catalysed by acetoacetate decarboxylase (Argiles, 1986; Taylor and Agius, 1988). Acetone is converted to pyruvate either directly or indirectly (Cassaza et al 1984), whereas propane-1,2-diol is either oxidized to L-lactate or converted to acetate and formate (Kosugi et al 1986a; Kosugi et al 1986b). Studies on the incorporation of 2-14C acetone into glucose in the rat have shown that at low plasma acetone concentration, it is metabolized primarily to lactate and pyruvate but when the concentration of acetone is high in blood plasma, acetate formation predominates (Kosugi et al 1986a).

Diabetes leads to the absence of insulin or in those circumstances in which available insulin is ineffective. Insulin deficiency is accompanied by the
underutilization of glucose by skeletal muscle, adipocytes and liver, the overproduction of glucose by liver via glycogenolysis and gluconeogenesis is also present. Therefore effect of glucose is evident in both the insulin dependent (where transport of glucose is dependent on insulin) and independent (where the transport is not dependent on insulin and is transported through a concentration gradient across the cell membrane) tissues. In the case of insulin dependent tissues namely adipocytes, liver, muscle, there is relative deficiency of glucose in the cell. Where as in the case of insulin independent tissues there is an increased uptake of glucose as it is dependent on the glucose gradient.

In liver, cells do not retain glucose because the enzyme glucokinase, which is essential for the first step in glucose metabolism, is deficient when insulin is absent like in diabetes. Without phosphorylation, the glucose that has freely entered the hepatocyte flow out immediately. Therefore the glucose which is produced in these insulin dependent cells is ejected out leading to diminished glucose utilization, while some of it is converted to glucose-6-phosphate with the low Km type I hexokinase which is not affected by insulin deficiency (Kaur et al 1983).

HAEMATOLOGICAL AND EXCRETORY FUNCTION

Relative tissue hypoxia may play some part in the development of several diabetic complications. Haematological abnormalities in diabetes which could lead to decreased tissue oxygenation, include, increased erythrocyte aggregation with increased micro-viscosity and decreased deformability; increased level of non-enzymatically glycosylated haemoglobins; which alters oxygen affinity;
decreased effective levels of 2,3 diphosphoglycerol (2,3 DPG); abnormalities in platelet function, including increased adhesiveness; increased sensitivity to several aggregating agents and accelerated thrombogenic prostaglandin derivatives; plasma protein abnormalities, which lead to increased blood viscosity; accelerated fibrinogen consumption; decreased level of anti-thrombin III; increased levels of Von Willebrand Factor (vWF); and decreased fibrinolysis (Brownlee and Cerami, 1981)

Since, the description and characterisation of glycosylated haemoglobin and glucose have been found also to modify a variety of other proteins in-vivo. Increased production of such adducts, in insulin dependent tissues, namely liver and adipocytes could play a role in pathogenesis of some diabetic complications.

Increased filtration rate is also observed in the patients of diabetes leading to increased excretion of albumin and other proteins in urine. Increased mesengial actomyosin-like material and persistence of certain extravasated serum proteins with glomerular basement membrane adversely affects the normal functions in the kidney. Diminished localised mesengial macro-molecular clearing mechanisms and increased glomerular size, glomerular tuft volume, capillary luminal volume and capillary filtration surface area, results in increase in the volume of total output of urine. Continual basement membrane thickening, over many years, ultimately leading to progressive glomerular capillary occlusions and chronic renal failures. It has been demonstrated that increased synthesis is a major factor in excessive accumulation of glomerular basement membrane in diabetic kidney (Brownlee and Cerami, 1981)
VANADIUM AS A TRACE ELEMENT

A Swedish scientist Nils Sefstrom observed rainbow of colours possessed by compounds of the new element he discovered in 1830. He was so struck by the sight that he named the newly discovered element after the Norse goddess of beauty, Vanadis. Vanadium abundance in the earth crust is 0.02%, and while there are over 65 vanadium containing minerals, it is extracted as a by-product from the processing of other metals. Vanadium compounds are also found in the biosphere. Vanadium is found in the specialized blood cells of sea squirts in concentration as high as 0.15 M in the form of tunichrome which is thought to play an important role in cell metabolism. Vanadoenzymes (haloperoxidases) are found in marine algae and certain nitrogen-fixing bacteria (Azotobacter) contain vanadium. In mammals it is an ultra trace element which is widely distributed in tissues. The total body pool is estimated to be about 100 μg based on a daily intake of 10-60 μg (Bevan et al 1995)

ROLE OF VANADIUM IN BIOLOGICAL SYSTEM

The most remarkable effect of vanadium salts is their insulin-mimetic ability to normalize blood glucose in Type I diabetes and Type II diabetes animal models. Vanadium salts also improved the metabolic abnormalities associated with both Type I and Type II models of diabetes mellitus. It is well established that biological actions of insulin are initiated by binding of insulin to a specific receptor located on the membrane of target cells (Kahn, 1976; Pang and Shafer, 1984; Azam et al 1991). The insulin receptor is a hetero-tetrameric glycoprotein composed of two extracellular α-subunits and two transmembrane β-subunits.
List of insulin like action mediated by vanadate in various responsive tissues modified from (Shechter, 1990)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Direction of effect</th>
<th>Target tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexose transport</td>
<td>Stimulated</td>
<td>Skeletal muscle, rat adipocytes, brain, liver</td>
</tr>
<tr>
<td>Glucose oxidation</td>
<td>Stimulated</td>
<td>Rat adipocytes, rat diaphragm, liver</td>
</tr>
<tr>
<td>Gluconeogenesis</td>
<td>Inhibited</td>
<td>Rat liver</td>
</tr>
<tr>
<td>Glycogenesis</td>
<td>Stimulated</td>
<td>Skeletal muscle, rat adipocytes, rat hepatocyte</td>
</tr>
<tr>
<td>Glycogenolysis</td>
<td>Inhibited</td>
<td>Rat liver, skeletal muscle</td>
</tr>
<tr>
<td>Lipogenesis</td>
<td>Stimulated</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>Lipolysis</td>
<td>Inhibited</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>Ketogenesis</td>
<td>Inhibited</td>
<td>Rat liver</td>
</tr>
<tr>
<td>Urea cycle enzymes</td>
<td>Inhibited</td>
<td>Rat liver</td>
</tr>
<tr>
<td>Mitogenic activity</td>
<td>Augmented</td>
<td>Various cultured cells</td>
</tr>
<tr>
<td>Translocation of IGF-II</td>
<td>Stimulated or augmented</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>K⁺ uptake</td>
<td>Stimulated</td>
<td>Cardiac muscle cells</td>
</tr>
<tr>
<td>Ca²⁺-Mg²⁺ ATPase</td>
<td>Inhibited</td>
<td>Plasma membrane from rat adipocytes</td>
</tr>
<tr>
<td>Ca²⁺ influx</td>
<td>Stimulated</td>
<td>Adipose tissue</td>
</tr>
<tr>
<td>Intracellular pH</td>
<td>Elevated</td>
<td>A-431 cells</td>
</tr>
<tr>
<td>PTPase</td>
<td>Inhibited</td>
<td>Rat liver</td>
</tr>
<tr>
<td>Cytoplasmic protein tyrosine kinase</td>
<td>Stimulated</td>
<td>Rat adipocyte</td>
</tr>
</tbody>
</table>
The α-subunit possesses the insulin binding activity and the β-subunit has an intrinsic protein tyrosine kinase (PTK) activity. The binding of insulin to the subunit of its receptor activates the PTK activity of the β-subunit and results in autophosphorylation of the β-subunit in tyrosine residues as well as the tyrosyl phosphorylation of endogenous substrate. Insulin receptor β-subunit autophosphorylation and activation of its PTK activity is believed to be a major pathway mediating the insulin action since cells with PTK deficient receptors are unable to elicit several of the biological effects of insulin. However, the mechanism by which vanadium compounds exert their insulin like effects remains to be still established (Pandey et al 1995).

Vanadium salts are potent inhibitors of protein tyrosine phosphatases (PTP) and thus the initial thought was that they activate the insulin receptor PTK activity by preventing the dephosphorylation of insulin receptor β-subunit. However, recent work has suggested that the site of action of vanadium might not involve insulin receptor PTK (Pandey et al 1995).

An important step in insulin signalling cascade appears to be activation of a group of protein serine/threonine kinase which include mitogen activated protein (MAP) kinases, 90 kDa ribosomal S6 kinase, (p90\textsuperscript{rb}) and 70kDa ribosomal S6 kinase, (p70\textsuperscript{6k}). It has been already shown earlier that sodium orthovanadate stimulates the tyrosyl phosphorylation and activation of MAP kinase in an insulin receptor PTK independent manner (Pandey et al 1995).
Figure 1: Schematic model showing possible target sites of vanadium actions in relation to insulin signalling cascade. Abbreviations: elf-4- eukaryotic protein synthesis initiation factor-4, GRB-2- growth factor receptor bound protein-2, GSK-3- glycogen synthase kinase-3, IRS-1- insulin receptor substrate-1, ISPK- insulin stimulated protein kinase, MAPK- mitogen activated protein kinase, MAPKK- mitogen activated protein kinase kinase, PHAS-1- phosphorylated heat and acid stable protein regulated by insulin, PI3K- phosphatidylinositol 3-kinase, PP1-G- protein phosphatase-glycogen bound form, PTK- protein tyrosine kinase, PTPase- protein tyrosine phosphatase, rsk- ribosomal s6 kinases, shc- src homology containing protein, SOS- son of sevenless (Adapted from Pandey et al 1995)
Glucose Transport

Vanadium Compounds

Other signalling system

Protein Synthesis

Glycogen Synthesis

Nuclear Activity

MAPK

PHAS-I

elf-4

MAPKK

PTPase

Vanadium Compounds
ROLE OF VANADIUM IN REVERSAL OF DIABETES

Insulin has a drawback in being effective only as an injectable hormone. Slow-acting and slow releasing gadgets have been developed as an alternative to insulin. A large number of diabetic cases are of the insulin resistant, Type II diabetes. An alternative to insulin that acts possibly at the intracellular level and will be effective in both types of diabetes, needs to be developed. For this understanding of the regulatory mechanisms of glucose homeostasis is vital.

Insulin mimetic action of vanadium compounds

Vanadium, a group V element (atomic weight 50.94), occurs in traces in biosphere. Its salts form complexes with organic compounds with ease. Its absorption, accumulation and excretion in animals appear to be under homeostatic control. Some characteristic deficiency symptoms are known in some birds and plants but not in animals, the insulin mimetic role of vanadium has been reviewed recently (Ramasarma, 1996). Participation of vanadium compounds in biological activities are now being recognized like bromoperoxidation (Macara, 1980; Cammack, 1986; Vilter, 1984).

A report in 1971 by Meek and coworkers on the decrease in the expired $^{14}$CO$_2$ on oxidation of glucose-1-$^{14}$C in rats given vanadate in drinking water, was the first to show a possible relationship of vanadium and glucose metabolism. This was supported by experiments of Tolman and coworkers (1979) who found that addition of metavanadate or vanadyl sulphate to preparations of adipocytes, hepatocytes and diaphragm from the rat showed the following effects: stimulation of glycogen synthesis in the liver and the diaphragm, stimulation of glucose
oxidation and transport in adipocytes and inhibition of hepatic gluconeogenesis, similar to that obtained with insulin. These investigators opined that the action of vanadate was ‘at a step beyond or in addition to that effected by insulin’. These effects were later confirmed (Dubyak and Klienzeller, 1980; Shechter and Karlish, 1980) where investigations indicated that the effects depended on the ability of vanadate to inhibit the activity of some phosphatases, but not Na⁺-K⁺ ATPase. Triggered by these reports a spate of investigations followed and these confirmed that vanadium salts mimicked a number of insulin effects in its signalling cascade with some exceptions, e.g. lack of effect on muscle protein metabolism (Clark et al 1985). Innumerable effects on related systems were reported and these may have yet undeciphered physiological significance (Ramasarma, 1996).

**VANADIUM AS A POTENTIAL ANTIDIABETIC AGENT**

The pharmacological value of metavanadate was recognized a century ago and it was claimed as ‘panacea universelle’ for treatment of a variety of disease including malnutrition, anaemia, tuberculosis, syphilis and diabetes. Amazingly little evidence was on record. Lyonnet et al (1899) found that urinary glucose output decreased in two out of three diabetic patients treated with metavanadate (4-5 mg doses). This was followed by caution on the accompanying diarrhoeal response (Martin, 1899). Decrease in plasma cholesterol by vanadate was reported in 1959 (Curran et al 1959) and the site of its action was shown to be the rate-limiting enzyme in the pathway, HMG-CoA reductase (Menon et al 1980) also inhibited by H₂O₂ (Omkumar and Ramasarma, 1993).
The first evidence on the effectiveness of metavanadate given in drinking water (0.8 mg/ml) in decreasing glucose and triglycerides to normal levels was found in STZ-treated diabetic rats in 1985 by McNeill and coworkers (Heylinger et al 1985b) in Canada. The response was obtained within a week of starting the treatment and was maintained for several months. It was independent of changes in food consumption or weight gain. Similar decreases in blood glucose were obtained by Ramanadham (1989a; 1989b) with vanadyl sulphate substituting metavanadate in drinking water (0.25-1.25 mg/ml) in a three week period. This effect sustained for 13 weeks after withdrawal in their experiments possibly because vanadium administered as vanadyl was retained in the cells. This is in contrast to the reversal of the effect of discontinuing the treatment reported by Shechter and coworkers (Meyerovitch et al 1987).

One year long treatment with vanadyl also improved secondary effects in diabetic condition such as kidney damage and cataract formation (Dai et al 1994). The effects of these vanadium compounds mostly in STZ-diabetic rats were studied in many laboratories (Brichard et al 1988; Valera et al 1993; Saxena et al 1993; Malibu et al 1994). Some differences observed in these studies seem to depend on mild and severe states of the diabetic condition of the animals. The three forms of vanadium seem to show similar effects although some investigators claimed better response with the vanadyl form.

The following Type II models of NIDDM also respond to oral vanadate treatment and showed reduction of blood glucose: Zucker (fa/fa) genetically obese rat, db/db mice, ob/ob mice, and rats on high sucrose diet (Brichard et al 1989; Pugazhenti et al 1993) (108-112) on withdrawal of vanadate treatment the blood
glucose returned to initial high levels. Lower food consumption and gain in body weight in vanadate-treated animals were observed, and therefore raised doubts (Malibu et al 1994) whether these effects indirectly cause reduction of blood glucose. But careful studies on pair-feeding dispelled these doubts.

There are also indications that vanadium treatment of these models of diabetic animals increased in Type I and decreased in Type II of concentration of insulin in blood plasma when compared to the controls without vanadate treatment (Ramasarma, 1996). The effects of treatment of normal animals with STZ (Type I) and high sucrose diet (Type II) without and with vanadate are also encouraging (Ramasarma, 1996). Compared with the normal animals as 100%, insulin levels decrease on treatments with STZ (27 and 17%) as well as vanadate (71 and 32%) in both the experiments. In the combined treatment vanadate seemed to diminish the effect of STZ and therefore gives an apparent increase. The change in actual value (28 pM) is hardly 10% of the normal. On the other hand insulin levels increased to 266% in animals on high sucrose diet and the increase was restricted in superimposed vanadate treatment (Ramasarma, 1996). The apparent increase in Type I and decrease in Type II are minor changes due to multiple treatments the animals were subjected to.

It appears that vanadate treatment decreases the circulating insulin in all these animals compared to normal animals. What is interesting to note in these data is that vanadate produced a large decrease in the concentration of insulin but not glucose in the blood plasma in normal animals. Even in these animals the changes in the enzymes consequent to vanadate treatment are produced. The basis of maintaining glucose homeostasis needs to be explained (Ramasarma,
Attempts are now being made to find less toxic, more tolerated organic vanadate derivatives obviating poor absorption and gastrointestinal disturbances of inorganic vanadium compounds. Of these bis(maltolalto)oxovanadium (BMOV), a water soluble compound given at a dose of 0.75 mg/ml drinking water was found to be effective in reducing blood sugar and triglyceride levels in STZ-diabetic rats (McNeill et al 1992). BMOV was effective at much smaller doses than metavananadate or vanadyl with minimal side-effects on chronic treatment (Yuen et al 1993). BMOV was also effective in Type II diabetes in fa/fa Zucker rats (McNeill et al 1995). BMOV was found to be 2-fold more effective than vanadyl sulphate and a well tolerated insulin-mimic. The $V^+$-analogue of BMOV was less effective (McNeill et al 1995).

Peroxo derivatives of vanadate were also found to possess hypoglycaemic activity in animals at much lower concentrations compared to vanadate or vanadyl. Pyridine-2-carboxylate-bis-peroxovanadate (bp-V-pic) produced a 30% decrease in plasma glucose in 30 min at a dose of 0.075 mmole/100g body weight in fasted rats whereas 8-fold higher dose of vanadate was ineffective (Posner et al 1994). Similar effect was observed in STZ- and BB-diabetic rats (Shisheva et al 1994), and also with other derivatives. The basis of their action appears to be the same type of vanadium interaction with intracellular proteins and the different derivatives may be acting as vehicle to carry vanadium to the appropriate tissues.

Initially, the target of action of vanadate was considered to be the
membrane-based insulin receptor (Tamura et al 1984), but later findings pointed to post receptor functions in the cytosol in addition insulin receptor substrate 1 (IRS-1). Under conditions wherein good antilipolytic action occurred, vanadate showed marginal activation of phosphorylation of insulin receptor (Mooney et al 1989). Insulin-like effects were obtained with vanadate even in adipocytes depleted of insulin receptors (Green, 1986). Also cytosolic protein kinases are known to influence glucose metabolism-mitogen activated protein kinase (MAPK) (D’Onofrio et al 1994) and a nonreceptor protein kinase (Elberg et al 1994) were found to be activated in vanadate-treated cells. the most significant end-result of vanadate treatment of the cells is to improve and retain the phosphorylation status of proteins (Swarup et al 1982; Pandey et al 1995) which also include enzymes, such as glycogen-bound protein phosphatases (PP1-G), in the downstream signal cascade of insulin. The insulin mimic action of vanadate is implicit.

The activity of PP1-G is regulated by an isoform of p<sub>90</sub><sup>Mr</sup> (ribosomal s6 kinase, 90 kDa protein) which responds to insulin (Donella-Dean et al 1993). Srivastava and coworkers established a role for ser/thr kinases (Pandey et al 1995), such as MAPK, p<sub>90</sub><sup>Mr</sup> and p<sub>90</sub><sup>Mr</sup> in insulin mimic action of vanadate found that the activation of these enzymes is achieved by H<sub>2</sub>O<sub>2</sub> (D’Onofrio et al 1994) and also by diperoxovanadate. It is becoming apparent that the regulation of these enzymes important in insulin signal cascade may use both ‘phos-dephos’ and redox reactions (Ramasarma, 1996).
Studies on hypoglycaemic action of vanadium compounds

Essentiality of vanadium is not established in humans although all the tissues possess it in the concentration range of 1-40 ng/g. The dietary requirement is unclear. The reported values of daily intake vary widely from 10 μg to 2 mg per day and probably depends on food habits. Most plant and animal tissues tested contain vanadium in the range of 1-100 ng/g dry weight (Nachay, 1984). High concentrations up to 1 μg/g or more were found in black pepper, tea leaf, cocoa powder, tobacco and some mushrooms. These are consumed by humans and some are habit forming. Tissue concentrations can be increased by dietary supplements, particularly chicken (Nachay, 1984). It should not be forgotten that the importance of vanadium as a powerful inhibitor of Na/K-ATPase was recognized after it was found as a contaminant in crystalline ATP derived from horse muscle. Vanadium concentration in human blood normally at 5 μM increased five folds in cancer patients (Agrawal and Sant, 1978). No correlation between vanadium content as a condition of health or disease is found with certainty.

Absorption of dietary vanadium is poor and nearly 80% of it is excreted in faeces (about 2 μg/g dry weight). During passage through blood, vanadium is bound to transferrin and a small amount is taken up by erythrocytes through the anion channel. It is transferred to tissue, primarily kidney, liver, testes and spleen, but is largely excreted in the urine. Only small amounts are retained in the body pool, estimated to be no more than 0.2 mg (Nachay, 1984), understandable in view of its toxicity.
A preliminary investigation on clinical evaluation vanadate treatment (125 mg/day, oral) of five each of IDDM and NIDDM patients gave encouraging results (Agrawal and Sant, 1978). The treatment was well tolerated with no obvious side-effects. The rate of glucose utilization remained unchanged in IDDM patients at low and high doses of insulin whereas the insulin sensitivity in NIDDM patients improved on vanadate treatment. The activities of MAPK and s6K in leucocytes increased significantly on vanadate treatment in both IDDM and NIDDM patients (Pandey et al 1995). The results led to the conclusion that the animal models of diabetes are valid and the responses in patients were similar. More extensive clinical trials with derivatives of vanadium are likely to follow. Several organic complexes are likely to be formed with vanadium, especially the phenolics abundant in plants. It will be of interest to see whether any of the plant extract possess such organic vanadates. The desirable features of the appropriate vanadium compound are good absorption, retention in tissues and availability of active vanadium in cell for an extended period (Ramasarma, 1996).

Forms of vanadium and their reactivity

Vanadium can form compounds mainly in three valence states of 3, 4 and 5, both anionic and cationic species. Trivalent vanadium is unstable at physiological pH and undergoes rapid oxidation. It is known to accumulate in acidic vacuoles in marine tunicates reaching a concentration of about 0.15 M (Ciereszko et al 1963). Tetravalent vanadyl cation is considered to be the less toxic storage form (Macara, 1980). Vanadyl is not easily oxidized (Shi and Dalal, 1993) and is not capable of generating superoxide on auto-oxidation as often
Figure : Different coordination compounds of vanadium
presumed (Liochev and Fridovich, 1990). Vanadyl can be oxidized by H$_2$O$_2$ and cytochrome c yielding vanadate (Brooks and Sicilio, 1971; Liochev and Fridovich, 1990). Pentavalent vanadium compounds and other vanadium compounds (V$_2$ and V$_4$) exist in oligomeric forms at high concentration (Jaswal and Tracey, 1991). The chemical reactions of vanadium are similar to those of phosphorus in many species. The V-O bonds are longer than the P-O bonds and this is the basis for the high competitiveness of vanadate in phosphate ester hydrolysis (Macara, 1980). Vanadium compounds have a remarkable property of forming stable coordination complexes with O- and N- ligands of a variety of organic compounds. A typical example is the vanadate-EDTA complex (Amos and Sawyer, 1972). This complex being stable explains why EDTA interferes with many reactions dependent on vanadate and diperoxovanadate such as NADH oxidation (Ravishankar and Ramasarma, 1995) and oxygen release by catalase (Ravishankar et al 1995).

**Redox reactions of vanadium compounds**

Vanadate (V$^+$) can be reduced to vanadyl (V$^+$) by cellular reductants such as ascorbate, glutathione and catechol compounds (e.g. noradrenaline) but at high concentrations. A microsomal NADH-dependent enzyme system reduces decavanadate more readily than metavanadate (Patole et al 1986). Vanadyl is also oxidized by diperoxovanadate and produces a free radical species of the type $^*$OV(O$_2$), less powerful than the related $^*$OH radicals in reacting with quenching agents (Ravishankar and Ramasarma, 1995). This radical species degrades and releases half equivalent of oxygen (Ravishankar et al 1994). But it can also serve
as an oxidant and withdraw electrons from some compounds, such as NADH (Ravishankar and Ramasarma, 1995). Peroxovanadate and the derived \( \text{OV(O)}_2 \) radical species, may function as oxidants of cysteine-SH at the active site of proteins such as MAP kinase (D'Onofrio et al 1994) and protein tyrosine phosphatase (Barford et al 1994). In the insulin-response cascade, inactivation of this significant reaction by vanadate derived active intermediates will explain insulin mimic action of vanadate. Modifications of proteins by \( \text{H}_2\text{O}_2 \) are implicated in regulation of several enzymes (Ramasarma, 1990) including pyruvate dehydrogenase, another insulin responding enzyme. Like phosphorylation-dephosphorylation, redox reactions of protein can take part in intracellular responses of insulin. It will not be surprising if some of the plant extracts described above possess this property. Vanadate compounds exemplify this potential.

The activities of key gluconeogenic and shunt enzymes in hepatic and red blood cells (RBC) respectively were found to return to normal levels when diabetic rats were fed with a 60% ethanol extracted and solvent evaporated residue of Momordica charantia (Shibib et al 1993).

**CHANGES IN ENZYMES OF GLYCOLYTIC AND RELATED PATHWAYS IN INSULIN DEPENDENT AND INDEPENDENT TISSUES DURING DIABETES**

**Hexokinase** (D-hexose: ATP 6-phosphotransferase, EC 2.7.1.1)

Hexokinase is the first enzyme of glycolysis and it catalyses the
phosphorylation of D-glucose to glucose-6-phosphate.

Hexokinase is a regulatory enzyme and controls the flux of glucose inside the cell. According to its electrophoretic mobility, the enzyme has been shown to occur in four isoforms in tissues: Hexokinase I, II, III, IV (Katzen and Schimke, 1965). Type I, II and III isozymes have low Km for glucose (0.01 - 0.1 mM), while Type IV which is also known as glucokinase, has a relatively high Km of about 10 mM, this enzyme is localized in liver only (Katzen and Schimke, 1965; Katzen, 1967; Singh et al. 1987). An incubation of extracts at 45 °C for 1 hour completely destroys the activity of Type II isozyme (Gumma and McLean, 1972). In most tissue with an exception of liver, hexokinase in present both in cytosol and particulate fractions of the cells, the particulate fraction contributing upto 30-75% percent of the total activity of the enzyme (Baquer et al. 1975; Wilson, 1980; Sochor et al. 1985).

The total activity of hexokinase in liver is high owing to the contribution of glucokinase compared to the total hexokinase activity in kidney, though in kidney it is present in both cytosolic as well as mitochondrial forms (Sochor et al. 1985). The ratio soluble/particulate hexokinase in kidney is close to unity in control rats. Hexokinase is inhibited by its reaction products glucose-6-phosphate and ATP. The high Km enzyme, glucokinase, however, is insensitive to glucose-6-phosphate inhibition (Weinhouse, 1976). Based on the distribution of hexokinase in subcellular fractions and the different properties of the enzymes in the fraction, a mechanism was suggested by which the equilibrium of hexokinase between soluble and the particulate fraction may be an important factor in the regulation of glucose phosphorylation (Wilson, 1968; Baquer and McLean, 1972).
Interrelationships of routes of glucose metabolism

- Sorbitol
- Glucose
- Fructose
- Glucose-6-P
- F6P
- PEP
- Pyruvate
- TCA
- Lactate

Enzymes:
- AR
- SDH
- HK
- G6PDH
- PK

Co-factors:
- NAD⁺, NADH, NADP⁺, NADPH
- ATP, ADP
- CO₂

Pathways:
- Glycolysis
- Pentose Phosphate Pathway
- TCA Cycle
The soluble mitochondrial hexokinase equilibrium has been shown to be sensitive to metabolic control in vivo particularly to glucose-6-phosphate levels.

The activities of hexokinase type II and glucokinase are greatly diminished in the absence of insulin (Sochor et al. 1985; Taylor and Agius, 1988). The activity of these isoenzymes are restored to almost normal levels after both insulin and vanadium treatment in liver (Sochor et al. 1985; Saxena et al. 1992a).

**Pyruvate Kinase** (ATP: pyruvate phosphotransferase, EC 2.7.1.40)

Pyruvate kinase is also a regulatory enzyme in the glycolytic sequence of reactions. The product of this reaction, pyruvate is crucial in metabolism and it feeds to various pathways. The main pathway of this flux is tricarboxylic acid cycle apart from other routes of its metabolism.

The enzyme exhibits a broad specificity for the nucleotide substrate. It may utilize GDP, IDP and UDP apart from ADP with different affinities. Mg$^{2+}$-ADP is believed to be its true substrate. The enzyme shows a high degree of specificity towards the other substrate, phosphoenol pyruvate (PEP) (Seubert and Schoner, 1971; Harada et al. 1978).

There are at least three isoforms of pyruvate kinase known to exist, in higher animals. These are designated as L-type, $M_1$-type and $M_2$-type of A-type (Imamura and Tanaka, 1972; Harada et al. 1978; Denton et al. 1979). The L-type also forms the major component of the kidney cortex enzyme. In liver, adipose tissue and kidney pyruvate kinase isozymes may occur in interconvertible forms with different catalytic properties (Bailey et al. 1968). The interconversion is dependent on temperature and the concentration of the effector, fructose.
Liver is one of the main sites for glycolysis as well as gluconeogenesis. The control of pyruvate kinase is important because any significant, simultaneous activity of pyruvate kinase during gluconeogenesis would create a 'futile cycle', recycling pyruvate at the expense of ATP. It is therefore necessary to subject this enzyme to regulation by a number of effectors like alanine, PEP, FDP and intracellular pH (Seubert and Schoner, 1971). The intracellular concentration of ATP and alanine are sufficient to cause a complete block in the activity of liver pyruvate kinase. Fructose-1,6-diphosphate effectively counteracts the negative control by ATP and alanine, and therefore, the activity of pyruvate kinase in liver is very sensitive to the changes in the level of this glycolytic intermediate (Seubert and Schoner, 1971; Denton et al 1979).

**Aldose Reductase** (alditol: NADPH oxidoreductase, EC 1.1.1.2)

Aldose reductase is the rate limiting enzyme of sorbitol pathway. Aldose reductase is NADPH specific and exhibits a broad substrate specificity reducing a number of aldoses and aldehydes to their corresponding polyols and alcohols (O’Brein and Schofield, 1980). It was first demonstrated to be present in the extracts of sheep seminal vesicle (Hers, 1956; Hers, 1957). The enzyme is widely distributed and shown to be present in kidney, brain, nerve, aorta, muscle, erythrocytes and ocular lens (Gabbay, 1973; Ludvigson and Sorenson, 1980; Das and Srivastava, 1985a; Das and Srivastava, 1985b). In kidney, it is mainly localized in renal medulla while its activity is very in cortex (Chauncey et al 1988).
In erythrocytes, though its activity is very low, yet it plays an important role in diabetes when the enzyme gets activated in the presence of elevated glucose levels (Srivastava et al 1986). Aldose reductase has been isolated and purified to homogeneity from erythrocytes (Das and Srivastava, 1985b; Hamada et al 1991). Aldose reductase has a low affinity for glucose Km about 100 mM (Moonsammy and Stewart, 1967). It is activated by glucose, glucose-6-phosphate and NADPH (Das and Srivastava, 1985a; Das and Srivastava, 1985b). The relative affinities of aldose reductase with different substrates are as follows: DL-glyceraldehyde> D-xylulose> D-glucuronate> D-galactose> D-glucose (Bagnasco et al 1987). Conversion of glucose to sorbitol by aldose reductase requires NADPH and forms NADP⁺, thereby it competes with other NADPH requiring reactions e.g., the conversion of oxidized form of glutathione to reduced form, and fatty acid and cholesterol biosynthesis (Taylor and Agius, 1988).

The pentose phosphate pathway is the major source of NADPH in most tissues showing a higher lipogenic rate and its flux is generally determined by the NADP⁺/NADPH ratio. Hence, polyol pathway helps in the activation of pentose phosphate pathway by removing the competitive inhibitor NADPH and oxidising it to its activator NADP (Gonzalez et al 1986; Sochor et al 1988; Taylor and Agius, 1988).

**Sorbitol Dehydrogenase** (i-iditol dehydrogenase, EC 1.1.1.14)

Sorbitol dehydrogenase is the second enzyme of polyol pathway that converts sorbitol to fructose by a reversible reaction. It also has a broad substrate specificity for many sugar alcohols, additionally converting xylitol to D-xylulose
and ribitol to D-ribulose (Gabbay, 1973). Conversion of sorbitol to fructose is coupled to reduction of NAD' to NADH and this competes with glycolysis at the glyceraldehyde dehydrogenase step for NAD' (Gonzalez et al 1986; Taylor and Agius, 1988). An increase in the NADH/NAD' ratio favours increase conversion of dihydroxy acetone phosphate to glycerol-3-phosphate (Gonzalez et al 1983).

The presence of sorbitol dehydrogenase has been shown in kidney, liver, adrenal gland and brain (Gabbay, 1973; Leissingt and McGuinness, 1982). Schwann cell sheath has the unique ability to synthesize free (non-phosphorylated) fructose with the help of this enzyme for various metabolic purposes (Brownlee and Cerami, 1981). However, the role of this enzyme in insulin dependent tissue is not very clear (Bagnasco et al 1986; Bagnasco et al 1987). In kidney, the enzyme is mainly localized in the cortical region while its activity is very low in the medullary region (Chauncey et al 1988).

**Glucose-6-phosphate dehydrogenase** (D-glucose-6-phosphate: NADP oxidoreductase EC 1.1.1.49)

Glucose-6-phosphate dehydrogenase is the first enzyme of the pentose phosphate shunt which occurs widely in living cells where one of its main functions is to provide the NADPH necessary for the synthesis of fatty acids and other specific reductions. The biosynthesis of fatty acids is related to the oxidative part of the shunt where the enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase produce the necessary NADPH. The above facts along with the strategic position of glucose-6-phosphate dehydrogenase at a metabolic branch point, suggest that the glucose-6-phosphate dehydrogenase
reaction is an important site of metabolic control in most tissues. Its behaviour is also influenced by the various hormonal and nutritional levels, enzyme quaternary structure and various metabolites including ATP, ADP, spermine and palmitoyl-CoA. (Kanji et al 1976).

Phosphate has long been known to inhibit glucose-6-phosphate dehydrogenase, particularly at concentrations above 0.1 M. Magnesium ions show a stimulating effect in glycylglycine buffer which is not evident in dilute phosphate buffer. Glucose-6-phosphate dehydrogenase is very specific for NADP⁺.

The lyophilized preparations are stable indefinitely, provided that they are kept cold and dry. Aqueous solutions lose activity when stored in the refrigerator but may be kept frozen at -16°C for weeks with only slight loss. Care must be taken to avoid shaking during freezing and thawing.