Introduction
I. DIABETES MELLITUS: A THREAT TO MANKIND

(A) GENERAL

Diabetes mellitus is a major source of suffering and morbidity afflicting 20-30 million people of all social conditions throughout the world. Clinically, diabetes is not one but rather a group of related diseases, all of which are characterized by hyperglycemia (Brownlee and Cerami, 1981; Arky, 1983). There are two major forms of diabetes mellitus: (i) Type I diabetes (insulin-dependent diabetes) often has an early life onset and is characterized by insufficient production of insulin by the beta cells of Islet of Langerhans. It can in principle be treated by exogenous administration of insulin. (ii) Type II diabetes (noninsulin-dependent diabetes) has relatively late onset and is characterized by an inability of tissues to respond to insulin (resistance) and it is often accompanied or preceded by obesity. In general, insulin-dependent diabetes is more severe than noninsulin-dependent diabetes mellitus (Bjorntrop, 1988; Reaven, 1988). Both these clinical syndromes are characterized by absolute or relative insulin deficiency which can be ameliorated by regulating diet, monitoring insulin injections or oral hypoglycemic agent therapy. However, these standard treatments does not prevent the development of chronic complications of diabetes that affect almost every organ of body including eyes, kidney, nerves and arteries giving rise to the pathogenic conditions retinopathy, nephropathy.
neuropathy and atherosclerosis respectively (Brownlee and Cerami, 1981; Taylor and Agius, 1988).

(B) DEVELOPMENT OF DIABETES

Despite extensive research, there is still a dearth of precise knowledge about the pathogenesis of diabetes, however, on the basis of current studies the development of type I diabetes mellitus is thought to be dependent on either the autoimmunity or the interaction of environmental agents with the pancreatic beta cells, or both in a genetically susceptible host (Yoon et al, 1987). As environmental factors affecting the induction of type I diabetes, diabetogenic chemicals and viruses are likely candidates as primary injurious agents for pancreatic beta cells in man and animals. A number of structurally related chemicals including alloxan, streptozotocin, chlorozotocin, vacor, and cyproheptadine are diabetogenic mainly in rodents and sometimes in man. However, alloxan and streptozotocin are the much used beta cell cytotoxin to induce insulin dependent diabetes and a single injection to rats or multiple injections to mice may be used to achieve varying severities of diabetes (Junod et al, 1969). For the most part, the diabetes induced by these two chemicals is a reasonable analog to the clinical condition (characterized by hyperglycemia, glucosuria, polydipsia, polyuria, loss in body weight despite polyphagia, hyperlipidemia, ketourea and acidosis) and these metabolic alterations are shown to be reversed by external insulin treatment suggesting that there is a minimum
of nonspecific damage to other organs (Rowland and Bellush, 1989).

The possible mechanisms for the beta cell destruction by these chemicals include (i) generation of oxygen free radicals and alterations in endogenous scavengers of these reactive species (ii) breakage of DNA and consequent increase in the activity of poly ADP ribosynthetase, an enzyme depleting NADP+ in beta cells and (iii) inhibition of active calcium transport and calmodulin activating protein kinase activity (Yoon et al, 1987; Rowland and Bellush, 1989).

In fact far more questions about its pathogenesis and beta cell killing remain, than have been answered to date.

(C) COMPLICATIONS OF DIABETES

The diabetic individual is prone to complications which are a major threat to both the quality and length of life. Until relatively recently, knowledge about diabetic complications was in large part limited to clinical description and morphological characterization. Information from physiological and biochemical investigations of diabetic complications has been accumulating rapidly to elucidate the biochemical and molecular mechanisms involved in the eruption of diabetic complications. These complications are a heterogeneous group of clinical disorders which affect the retina, the kidney, the vascular system, the peripheral nerves, the lens and the skin (Brownlee and Cerami, 1981; Taylor and Agius, 1988).
In the eye, retinal capillary damage leading to oedema, new vessel formation, and hemorrhage makes blindness 25 times more common among diabetics. Opacity of the ocular lens, cataract, occurs earlier and more frequently in patients with diabetes (Brownlee and Cerami, 1981). In a number of experimental diabetic animals, occasionally in severe juvenile onset diabetes, the development of cataract is particularly rapid. Capillary damage in the glomerulus associated with basement membrane thickening makes chronic renal failure with proteinuria 17 times more common in diabetes (Brownlee and Cerami, 1981). After 5 years of clinical diabetes, basement membranes are 25-30% thicker than in normal subjects as shown in Fig. 1 (Brownlee and Cerami, 1981; Keen and Jarret, 1982; Taylor and Agius, 1988). However, tight metabolic control can partially reverse the basement membrane thickening (Raskin et al, 1983; Taylor and Agius, 1988). In the diabetic peripheral nerve, axonal dwindling and segmental demyelination are associated with a very high prevalence of motor, sensory and autonomic dysfunction, including impotence, which affects 40% of diabetic males (Brownlee and Cerami, 1981).

Diabetes is also a risk factor for atherogenesis and arterial disease. Increased atheromata in medium and large arteries make coronary artery disease and stroke twice as common and symptomatic peripheral arterial disease 3-4 times more common among diabetics (Clements, 1979; Graf et al, 1979; Taylor and Agius, 1988). Gangrene leading to amputation is at least 5 times more
Hypothetical diagram of normal and diabetic glomerular capillary basement membrane showing thickening of the basement membrane structure in diabetes (Adapted from Keen and Jarret, 1982).
frequent among diabetics. The average life expectancy of diabetic patients is only two thirds that of the general population (Brownlee and Cerami, 1981; Kesaniemi et al, 1983).

Relative tissue hypoxia may also play some part in the development of several diabetic complications. The decreased tissue oxygenation may result from hematological abnormalities which occur in diabetes such as increased erythrocyte aggregation with increased microviscosity and decreased deformability, increased levels of glycosylated hemoglobin and abnormalities in platelet function (Schmid-Schonbein and Volgar, 1976; Brownlee and Cerami, 1981; Jones and Peterson, 1981). Although, half of those diagnosed as diabetic before age 31 die before they reach 50, largely as a result of cardiovascular or renal complications, often with many years of crippling and debilitating disease beforehand (Brownlee and Cerami, 1981).

The organizing concept that emerges from the previous studies is that insulin deficiency and its metabolic consequences are the primary etiologic events in the pathogenesis of diabetic complications.

(D) HYPERGLYCEMIA: A ROOT CAUSE OF DIABETIC COMPLICATIONS

Increasing number of evidences suggest that persistent hyperglycemia during diabetes is the root cause of all the diabetic complications (Engerman and
Kern, 1986; Wolff and Dean, 1987; Hunt et al, 1990). Thus, it is reasonable to postulate that glucose is toxic and that increased plasma and tissue glucose is a proximal source of the increased oxidative stress apparent in diabetes (Wolff, 1982; Halliwell and Gutteridge, 1984; Wolff et al, 1991).

Protein glycation, a factor possibly contributing to tissue damage in diabetes mellitus, has been extensively investigated modelled by exposure of proteins to glucose in vitro (Hunt et al, 1988; Pigeolet and Remacle, 1991; Wolff et al, 1991). In glycation, glucose reacts with free amino groups (α-amino groups of terminal amino acid residues and ε-amino groups of peptide bound lysine residues), to form a stable practically irreversible ketoamine structure (Adachi et al, 1991). Increased glycation of proteins is thought to be involved in not only structural but also functional changes in proteins such as lower affinities for their receptors (insulin and LDL) [Dolhofer and Wieland, 1979; Schleicher et al, 1981] and modified enzymatic activities for example activation of aldose reductase (Srivastava et al, 1989) and inactivation of copper zinc-superoxide dismutase (Arai et al, 1987).

Enzymic glycosylation is a highly regulated post translational process responsible for conferring specific structural and functional changes on proteins. In contrast, non-enzymatic glycosylation is governed by prevailing glucose concentration. The latter mechanism has gained much interest as recent work from several laboratories suggest the possibility that the modifications of proteins
by non-enzymatic glycosylation in high glucose environment (diabetes) might play a role in the pathogenesis of the sequelae of diabetic complications such as retinopathy, nephropathy, atherosclerosis, myocardial infarction, neuropathy and other lesions (Wieland, 1983; Taylor and Agius, 1988). Schematic representation of the development of various diabetic complications as a result of persistent hyperglycemia during diabetes is shown in Fig 2. Glycation of basal membrane proteins such as collagen and fibronectin, leading to increased membrane permeability, has been implicated in diabetic microangiopathy (Wieland, 1983; Taylor and Agius, 1988). Opacification of eye lens is due to the presence of high molecular weight aggregates, resulting from the oxidative linkage of glycated proteins (eg. disulfide bonds) [Engerman and Kern, 1986]. Advanced glycation products are probably directly toxic for the cells because of the consequent cross linking process (theory of ageing and diabetes) [Wieland, 1983; Monnier, 1989]. Early glycation product, however, could act in several ways by altering the protein three dimensional structure. In persistent hyperglycemia, during diabetes, the glycation of red blood cell membrane proteins participates in altering the membrane fluidity which controls a number of membrane functions including permeability, active transport and ligand affinity; all these are modified in diabetes (Wieland, 1983; Monnier, 1989).

In addition to the above mechanism it has been reported that glucose can reduce molecular oxygen, yielding ketoaldehyde and hydrogen peroxide. This
"glucose autoxidation" and/or related metal-ion-catalysed autoxidation processes may also contribute to protein modification (Wolff and Dean, 1987; Hunt et al, 1990; Wolff et al, 1991).

Evidences from clinical observations and experiments on animals strongly suggest that improved control of the diabetic state (i.e. maintenance of nearly normal levels of blood glucose concentration) will reduce the complication rate (Furth and Harding, 1989; Ceriello et al, 1991). The plausible explanation for this is that the achievement of stable normoglycemia would not allow protein glycation and glucose autoxidation which are deterrent for protein structure and function, thus could effectively control the pathogenic mechanisms leading to all or most of the complications of diabetes. Increasingly, therapeutic approach is being intensified to achieve this objective of attaining stable normoglycemia in diabetics by antidiabetogenic agent/oral hypoglycemic drug therapy.

II. ENZYMES OF CARBOHYDRATE METABOLISM, POLYOL PATHWAY AND RELATED METABOLISM

(A) CARBOHYDRATE METABOLISM

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

Hexokinase catalyzes the phosphorylation of glucose by ATP.

\[ \text{Mg}^{2+} \]

\[ \text{D-glucose} + \text{ATP} \quad \text{----} \quad \text{D-glucose-6-phosphate} + \text{ADP} \]
Fig. 2. Schematic representation of glucose overutilization and induced pathological changes in insulin-independent tissues (like kidney, RBC, lens), resulting from non-insulin requiring pathways, due to persistent hyperglycemia in diabetes (Adapted from Riordan et al, 1988).
It is the first regulatory enzyme that regulates the entry of glucose in the glycolytic route by its phosphorylation, thereby controlling the glucose flux inside the cell. Based on the electrophoretic mobility, the enzyme has been shown to occur in four isozymic forms in tissues: hexokinase I, II, III and IV (Katzen and Schimke, 1965). Type I and II isozymes have low Km for glucose (0.01-0.1 mM) while type IV, also known as glucokinase, has a relatively high Km of about 10 mM (Sols, 1968). Type IV isozyme is confined to liver whereas other isozymes have a varied distribution in tissues. Type-I isozyme is heat stable while type-II isozyme is heat labile. An incubation at 45°C for 1 hour totally destroys the activity of type II isozyme (Gumma and McLean, 1972).

The activity of hexokinase in different tissues of rat is shown in Table 1. In most tissues except liver, hexokinase is present both in cytosol and particulate fractions of the cells, the latter contributing as much as 30-75 % of the total enzyme activity (Baquer et al, 1975; Wilson, 1980; Sochor et al, 1985). In liver, the total hexokinase activity (including glucokinase) is higher than the total hexokinase activity in kidney though the enzyme in kidney is present in both particulate as well as in soluble fractions (Sochor et al, 1985). The quotient soluble/particulate hexokinase in kidney is close to unity in control rats. Hexokinase is inhibited by its reaction products glucose-6-phosphate and ADP. 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 fractions, Wilson (1968) suggested a mechanism by which the equilibrium of hexokinase between the soluble and the particulate fraction may be an important factor in the control of glucose phosphorylation. The soluble mitochondrial hexokinase equilibrium has been shown to be sensitive to metabolic control in vivo particularly to glucose-6-phosphate levels.

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

Pyruvate kinase catalyzes the third rate-limiting reaction of glycolysis as given below:

\[
\text{Mg}^{2+} \\
\text{Phosphoenol pyruvate + ADP} \quad \longrightarrow \quad \text{Pyruvate + ATP}
\]

The product of the reaction, pyruvate feeds into a number of metabolic pathways, thus placing this enzyme at a primary intersection, regulating the entry of glucose flux into tricarboxylic acid cycle and other routes of metabolism.

The pyruvate kinase activity in various tissues of rat is given in Table 1. The enzyme exhibits a broad specificity for the nucleotide substrate. Besides ADP, the enzyme may utilize GDP, IDP and UDP with different affinities. Mg-ADP is, however, believed to be the true substrate. The enzyme shows a high degree of specificity towards the other substrate, phosphoenolpyruvate (Seubert and Schoner, 1971; Harada et al, 1978).

Atleast three isozymic forms of pyruvate kinase are known to exist in
<table>
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\(^a\) Enzyme activity in m unit/gram.  
\(^b\) K/g tissue where K is first order rate constant.  
\(^c\) Enzyme activity in Bergmeyer unit.  
\(^d\) K/g Hb, K is first order rate constant.  
\(^e\) Enzyme activity in mice.
higher animals designated as L-type (Liver type), M₁-type (Muscle type) and M₂ or 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. The pyruvate kinase isozymes from liver, adipose tissue and kidney can also exist in two inter-convertible forms with different catalytic properties (Bailey et al, 1968). The interconversion is dependent on temperature and the concentration of the effector, fructose diphosphate.

Liver is the site of both glycolysis and gluconeogenesis. The control of pyruvate kinase in this tissue is of considerable importance 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, not surprising that the enzyme in liver is subjected to regulation by so many effectors like alanine, phosphoenolpyruvate, fructose diphosphate and intracellular pH (Seubert and Schoner, 1971). The intracellular concentrations of ATP and alanine are sufficient to cause a complete block in the activity of liver pyruvate kinase. Fructose 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).

(iii) **Malic Enzyme** [malate : NADP⁺ oxidoreductase (oxaloacetate-decarboxylating), EC 1.1.1.40]
Malic enzyme or NADP⁺-malate dehydrogenase catalyses the following reaction:

\[
\text{L-Malate} + \text{NADP}^+ \rightleftharpoons \text{Pyruvate} + \text{CO}_2 + \text{NADPH}
\]

The enzyme has been well studied in several mammalian tissues like liver (Hsu, 1970, Saito and Tomita, 1973), kidney (Saito and Tomita, 1973), muscle (Taroni et al, 1987), erythrocytes (Shows et al, 1970) and brain (salganicoff and Koepp, 1967). The malic enzyme activity in different tissues of rat is depicted in Table 1. Malic enzyme is an important source of NADPH for lipogenesis which is a minor pathway in kidney compared to that in liver and therefore there is a difference in the levels of this enzyme in the two tissues. In liver the activity of this enzyme is about 5 fold higher than in kidney (Sochor et al, 1985). A correlation between malic enzyme activity and lipid synthesis is suggested (Luine and Kauffman, 1971) and it was shown that malic enzyme activity paralleled changes in the lipid content.

The distribution of malic enzyme in several animal species appears to be tissue specific (Bradickza and Pette, 1971; Frenkel, 1975). The enzyme appears to be present exclusively in the cytosol of rat liver, adipose tissue, and adrenal medulla (Frenkel, 1975). Whereas a high proportion of malic enzyme activity has been detected in the mitochondrial fraction of brain and heart (Bradickza and Pette, 1971; Frenkel, 1975). Hsu (1970) postulated a general mechanism of liver malic enzyme action according to which an enzyme bound oxaloacetate is
required as an intermediate in the enzyme reaction.

(B) SORBITOL (POLYOL) PATHWAY

One of the consequence of hyperglycemia in diabetes mellitus is increased metabolism of glucose by the sorbitol pathway. This involves the reduction of glucose to sorbitol by aldose reductase and the oxidation of sorbitol to fructose by sorbitol dehydrogenase as shown in Fig. 3.

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

It 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'Brien and Schofield, 1980). It was first demonstrated in extracts of sheep seminal vesicle by Hers (1956, 1957). The enzyme is shown to be present in kidney, brain, nerve, aorta, muscle, erythrocytes and ocular lens (Gabbay, 1973; Ludvigson and Sorenson, 1980; Akagi et al, 1984; Srivastava et al, 1984; Das and Srivastava, 1985a, 1985b). In kidney, it is mainly localized in renal medulla while its activity is very low 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). Das and Srivastava (1985a) and Hamada et al (1991) have isolated and purified the aldose reductase near homogeneity from the erythrocytes.
Although the purified enzyme has a low affinity for glucose (Km about 100 mM; Moonsammy and Stewart, 1967), it can be activated by glucose, glucose-6-phosphate and NADPH (Das and Srivastava, 1985a, 1985b). The relative activities of aldose reductase with different substrates are: 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. NADPH is required for the conversion of oxidized to reduced glutathione (Fig. 3), a powerful antioxidant which protects cellular components from oxidative damage, and for fatty acid and cholesterol biosynthesis (Taylor and Agius, 1988). The pentose phosphate pathway is the major source of NADPH in most tissues and its flux is generally determined by NADP+/NADPH ratio. Thus, an activated polyol pathway by reoxidizing NADPH also activates the pentose phosphate pathway (Gonzales et al, 1986; Sochor et al, 1988; Taylor and Agius, 1988).

(ii) **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 (Fig.3). It has 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...
The interrelationship of polyol pathway, pentose phosphate pathway and glycolysis (Adapted from Taylor and Agius, 1988).
glycolysis at the glyceraldehyde dehydrogenase step for NAD⁺ (Gonzalez et al., 1986; Taylor and Agius, 1988) as shown in Fig. 3. An increase in the NADH/NAD⁺ ratio favours increased conversion of dihydroxyacetone phosphate to glycerol-3-phosphate (Gonzales et al., 1983) [Fig. 3].

The presence of this enzyme has been shown in kidney, liver, adrenals, and brain (Gabbay, 1973; Leissintg and McGuinness, 1982). The 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 Carami, 1981). However, the role of this enzyme in insulin - dependent tissue like liver is not clear (Bagnasco et al., 1986, 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).

(C) ANTIOXIDATIVE SYSTEM

(i) **Superoxide Dismutase** (Superoxide oxidoreductase, EC 1.15.1.1)

Superoxide dismutase dismutases the superoxide radical (O₂⁻) to peroxide (H₂O₂) and oxygen as shown below:

\[ 2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \]

The superoxide anion has been implicated in inflammation, hyperoxic cell damage and reperfusion injury (McCord, 1986) and is involved in a wide range of diseases (Bulkley, 1983). The enzyme superoxide dismutases are the primary
defense against the superoxide radical. Although $O_2^-$, once formed, undergoes spontaneous dismutation to peroxide and oxygen, the presence of superoxide dismutase increases the reaction rate by $10^9$ fold.

The nonenzymatic dismutation of $O_2^-$ has also been reported to result in the production of singlet oxygen, and it has, therefore, been proposed that the function of superoxide dismutase is to protect the aerobic cell from the toxic effects of not only $O_2^-$ but singlet oxygen ($^{1}{O}_2$) as well.

The superoxide dismutase activity in different tissues of rat is given in Table 1. There are at least three isozymes in the mammalian body; CuZn-superoxide dismutase in the cytosol of cells, Mn-superoxide dismutase in the mitochondrial matrix, and extracellular superoxide dismutase (EC-superoxide dismutase) in the extracellular space (Marklund, 1984; Erlansson et al., 1990). In erythrocytes, only CuZn-superoxide dismutase is present (Loven et al., 1986). EC-superoxide dismutase is heterogeneous in vivo and can be divided into at least three forms with regard to heparin-affinity; A which does not bind, B which binds weakly, and C which binds relatively strongly to heparin (Karlson and Marklund, 1988).

(ii) **Catalase** (Hydrogen peroxide: hydrogen peroxide oxidoreductase, EC 1.11.1.6)

Hydrogen peroxide is damaging in living systems as it can give rise to the
formation of toxic reactive hydroxyl radicals ('OH). It is, therefore, advantageous for cells to control the amount of hydrogen peroxide and to prevent its accumulation. Two types of enzymes exist to remove H₂O₂ within the cells; catalase and peroxidase (Halliwell and Gutteridge, 1989).

Most aerobic cells contain catalase which was first isolated and obtained in crystalline form from ox liver and later from blood and other sources (Aebi, 1974). Catalase has a double function, because it catalyzes the following reactions (1) decomposition of H₂O₂ to give H₂O and O₂ (2) oxidation of H⁺ donors, for example methanol, ethanol, formic acid, phenols, with the consumption of 1 mol of peroxide:

\[
\begin{align*}
\text{catalase} \\
(1) \quad 2\text{H}_2\text{O}_2 & \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \\
\text{catalase} \\
(2) \quad \text{ROOH} + \text{AH}_2 & \rightarrow \text{H}_2\text{O} + \text{ROH} + \text{A}
\end{align*}
\]

The predominating reaction depends on the concentration of H⁺ donor and the steady-state concentration or rate of production of H₂O₂ in the system. In both cases the active catalase-H₂O₂, complex I is formed first. The decomposition of H₂O₂, in which a second molecule of H₂O₂ serves as H⁺ donor for complex I, proceeds exceedingly rapidly, whereas peroxidative reactions proceed relatively slowly (Aebi, 1974).

The catalase activity of tissues varies greatly; it is present in high
concentrations in erythrocytes, liver and kidney and low in connective tissue. In tissues, it is mainly particle-bound (in mitochondria and peroxisomes), whereas it exists in a soluble state in erythrocytes (Matkovics et al., 1982; Halliwell and Gutteridge, 1989). The activity of catalase in various tissues of rat is given in Table 1.

(iii) **Glutathione Peroxidase / Glutathione Reductase System** (glutathione: Hydrogenperoxide oxidoreductase, EC 1.11.1.9; NADPH: oxidized glutathione oxido-reductase, EC 1.6.4.2)

Glutathione peroxidase has a key role in the enzymatic defense system against oxygen derived free radicals (Raes et al., 1987). It detoxifies H$_2$O$_2$ or any hydroperoxide utilizing GSH as a reductant and results in the formation of H$_2$O and oxidized glutathione (GSSG), as shown in Fig. 4. NADPH is needed to reduce GSSG back to GSH in a secondary reaction utilizing glutathione reductase. The two enzymes function within the cell in a coupled form and as a result maintain the intracellular GSSG/GSH ratio at an optimum level (Kirkman and Gaetani, 1984) [Fig. 4]. The activities of glutathione peroxidase and glutathione reductase in different tissues of rat are shown in Table 1. Glutathione peroxidase is present as both a selenium-independent form and a selenoenzyme. The selenoenzyme is capable of catalyzing the reduction of both hydrogen peroxide and lipid peroxides (Asayama et al., 1986).

Hydrogen peroxide (H$_2$O$_2$) is produced within the cell as a result of
The coupled action of glutathione peroxidase and glutathione reductase to remove hydrogen peroxide. Catalase also performs the same action but does not require glutathione.
various metabolic processes. Under normal conditions, it is mostly destroyed by catalase and in part by glutathione peroxidase; its accumulation within the cell is thus prevented (Mavelli et al, 1982a). Although human erythrocytes contain large amounts of catalase, the principle means for disposal of hydrogen peroxide in these cells has been considered to be the NADPH, glutathione reductase / peroxidase pathway (Cohen and Hochstein, 1963; Beutler, 1977). However, Gaetani et al (1989) have demonstrated that both catalase and glutathione peroxidase pathway are dependent on NADPH for function and both systems are actively involved in the disposal of H$_2$O$_2$ and that a failure in the generation of NADPH, as with glucose-6-phosphate dehydrogenase deficiency, impairs both mechanisms for detoxification of H$_2$O$_2$.

**Reduced Glutathione (GSH)**

Reduced glutathione (GSH), a tripeptide (γ-glutamyl-cysteine-glycine), is present in all cell types and is important in the regulation of the redox state and the protection of cells from oxidative damage by virtue of its ability to donate hydrogen atoms to form a disulfide compound, oxidized glutathione (GSSG) (Younes and Siegers, 1980; Comporti, 1987). GSH is used as a substrate for the H$_2$O$_2$-removing enzyme glutathione peroxidase. The role of intracellular GSH in the detoxification of electrophilic metabolites of xenobiotics and reactive oxygen intermediates has been well established (Adams et al, 1983). Its importance is exemplified by the findings that fibroblasts and hepatocytes depleted of
glutathione die prematurely, a phenomenon prevented by the presence of an antioxidant (Bannai et al, 1977; Miccadei et al, 1988). Red cells and lens are two tissues that have a very high level of glutathione and 2.5-15% of total glutathione exists in the red cells as oxidized glutathione (GSSG) (Srivastava, 1971).

A deficiency of GSH (either via abnormalities of synthesis or regeneration) results in severe impairment of tissue protective system and have serious consequences such as hemolysis (Beutler, 1957). One of the possible roles of GSH could be to act as the first line of defense against oxidants such as singlet oxygen, hydrogen peroxide, lipid peroxides and hydroperoxides. In the absence of GSH, the oxidizing agents could oxidize protein sulfhydryls to form high molecular weight protein aggregates linked by intra- and inter-molecular disulfide bridges (Srivastava, 1971). In different forms of experimental cataracts in animals and in human senile cataract, the amount of high molecular weight protein aggregate increases drastically that is believed to be the basis for increased light scattering and lens opacification (Srivastava, 1971; Engerman and Kern, 1986).

III. METABOLIC ALTERATIONS IN DIABETES

(A) GENERAL

During diabetes various tissues of the body are severely but differently affected. Although, diabetes has classically been considered a disease of "glucose underutilization", there are now many indications that in diabetes a shunting of
glucose from insulin-dependent pathways to those not requiring this hormone may take place (Spiro, 1976; Brownlee and Cerami, 1981; Alberti and Press, 1982; Sochor et al, 1985). On the basis of this hypothesis tissues can be categorized in two groups:

(a) Insulin-dependent tissues (e.g. liver, adipose tissue, skeletal muscle) which require insulin for glucose uptake, glucose phosphorylation and the entry of glucose-6-phosphate into different metabolic pathways.

(b) Insulin-independent tissues (e.g. kidney, erythrocytes, lens, brain) which do not require insulin for glucose transport and overutilize glucose during persistent hyperglycemia in diabetic state.

Thus, the tissues in the body respond differently to the diabetic conditions and the metabolic pathways are also altered differently giving rise to several pathological conditions in a chronic state (Spiro, 1976; Sochor et al, 1985).

(B) **INSULIN DEFICIENCY STATE**

The consequences of diabetes or insulin deficiency state are cardinally manifested by hyperglycemia, which results from (i) decreased entry of glucose into cells, (ii) its decreased utilization in insulin-dependent tissues and from (iii) the increased production of glucose (gluconeogenesis) by liver (Granner, 1985; Taylor and Agius, 1988).

Polyurea, polydipsia and weight loss in spite of adequate calorie intake are
the major symptoms of insulin deficiency. During diabetes, after a certain plasma glucose level is attained (< 180 mg/dl) the maximum level of renal tubular reabsorption of glucose is exceeded and sugar is excreted in the urine (glucosuria). The urine volume is increased owing to osmotic diuresis and coincident obligatory water loss (polyurea), and this in turn leads to dehydration (hyperosmolarity), increased thirst, and excessive drinking of water (polydipsia). Glucosuria causes a substantial loss of calories (4.1 KCal/g of glucose excreted) which when coupled with the loss of muscle and adipose tissue, results in severe weight loss in spite of increased appetite (polyphagia) [Unger and Dobbs, 1978; Granner, 1985].

In the absence of insulin, protein synthesis decreases due to diminished transport of amino acids which also serve as substrate for gluconeogenesis. In diabetes, the antilipolytic effect of insulin is lost as insulin inhibits gluconeogenesis mainly by decreasing the quantity and activity of liver enzymes required for gluconeogenesis, thus enhancing the plasma free fatty acids which causes accumulation of 2- hydroxybutyric acid and acetoacetic acid (ketosis) and severe metabolic acidosis supervenes (Granner, 1985; Taylor and Agius, 1988).

(C) ALTERATIONS IN CARBOHYDRATE METABOLISM

Insulin influences the intracellular utilization of glucose in a number of ways. In a normal person, about half of the glucose ingested is converted to energy through the glycolytic pathway and about half is stored as fat or glycogen.
As a result in the diabetic state, characterized by insulin deficiency, glycolysis is decreased and the anabolic processes of glycogenesis and lipogenesis are impeded. Indeed, only 5% of an ingested glucose load is converted to fat in an insulin-deficient diabetic (Straus, 1984; Granner, 1985; Taylor and Agius, 1988). In normal subjects, insulin causes enhanced uptake of glucose by increasing the glucokinase activity in liver which causes initial phosphorylation of glucose after it diffuses into the liver cells. Since glucose-6-phosphate is unable to go out of the plasma membrane, this action of insulin results in the retention of glucose within the liver cell. The glycolysis is enhanced by insulin due to the stimulated activities of several key enzymes including hexokinase, phosphofructokinase and pyruvate kinase (Hedekov, 1980; Granner, 1985). Pyruvate kinase isozymes show differing sensitivities towards insulin. The L-type enzyme has been reported to decrease in diabetic rats (Tanaka et al, 1967). It has been shown that a major action of insulin on hepatic gluconeogenesis is exerted at the pyruvate kinase level (Feliu et al, 1976) and that insulin counteracts the glucagon stimulated phosphorylation and inactivation of hepatic pyruvate kinase (Claus et al, 1979).

In addition to short term effects on the activities of the key glycolytic enzymes, insulin also exerts long-term affects on the de novo synthesis of these enzymes. Weber and co-workers have shown that insulin acts as an inducer of hexokinase, phospho-fructokinase and pyruvate kinase in rat liver (Weber et al,
1965; Weber and Singhal, 1965; and Weber et al, 1966). The induction of the enzymes was found to be synchronous with each other. The activities of all the three enzymes (hexokinase, phosphofructokinase and pyruvate kinase) are reported to be severely depressed in muscle and liver under conditions of insulin deficiency (Ali et al, 1980; Chen and Ianuzzo, 1982; Sochor et al, 1985).

Malic enzyme activity in liver is also regulated by insulin. In diabetes, a decrease in the malic enzyme activity is seen which is reversed by the administration of insulin (Belfiore et al, 1974; Nepokroeff et al, 1974). This insulin induced increase has been shown to be due to the enhancement in both the enzyme quantity as well as the specific activity (Thompson and Drake, 1982a, 1982b; Drake et al, 1984).

In liver and muscle, insulin enhances glucose incorporation into glycogen by cAMP mediated stimulation of glycogen synthase. Insulin also activates a phosphatase that dephosphorylates glycogen synthase, thereby resulting in the activation of this enzyme. Thus, the net effect of insulin on glycogen metabolism is also anabolic that increases the amount of glycogen in the liver (Hers, 1976). The glycogen can increase to total amount about 5 to 6 % of the liver mass which is equivalent to almost 100 grams of stored glycogen. Insulin stands alone against an array of hormones like glucagon, glucocorticoids and epinephrine that attempt to counteract the effect of insulin in decreasing the blood glucose level and promoting the deposition of glycogen in liver and muscle (Hers, 1976; Granner,
In contrast, the glucose metabolism in insulin-insensitive tissues like kidney, erythrocytes, lens and nerves is different from that in insulin-sensitive tissues, as glucose uptake and its utilization is not modulated by the presence of insulin. At least 70% of extrahepatic glucose utilization occurs in these tissues (Taylor and Agius, 1988). Thus during diabetes, unlike liver and muscle, the enzyme activities and metabolite levels (glucose-6-phosphate) are found to be enhanced in insulin independent tissues (Spiro, 1976; Alberti and Press, 1982) that further lead to the following metabolic and pathological changes:

(i) activation of polyol pathway and sorbitol accumulation (Gonzalez et al., 1986; Raskin and Rosenstock, 1987)
(ii) activation of glycogen synthase causing accumulation of glycogen as shown in rat kidney (Brownlee and Cerami, 1981; Taylor and Agius, 1988)
(iii) thickening of basement membrane as in kidney and nerves (Brownlee and Cerami, 1981)
(iv) glycosylation of proteins (Hunt et al., 1988; Wolff et al., 1991).

Thus, the carbohydrate metabolism in the insulin-dependent and insulin-independent tissues are differentially altered in the diabetic state.

(D) ALTERATIONS IN POLYOL PATHWAY

The polyol pathway is a minor pathway of glucose metabolism and under
normal conditions there is little flux through the pathway in most tissues as shown in Fig. 5. However, in diabetic hyperglycemic conditions, the flux through this pathway increases dramatically, leading to increased intracellular concentration of sorbitol in tissues like kidney, erythrocyte and lens (Spiro, 1976; Alberti and Press, 1982) [Fig. 5]. Because of the impermeability of most cells to sorbitol, the accumulation of this metabolite establishes an osmotic imbalance that ultimately causes the development of a number of pathological conditions in various tissues (Kinoshita et al, 1979; Crabbe et al, 1980; O’ Brien and Schofield, 1980; Judzewitsch et al, 1983).

In diabetes, the sorbitol content in the kidney is elevated to several folds leading to an increase in osmotic pressure and cell disruption, thereby, causing kidney dysfunction and renal failure at a later stage (Gabbay, 1973; Kinoshita, 1986; Chauncey et al, 1988). In diabetes, there is a marked increase in kidney growth characterized by hypertrophy and hyperplasia in contrast with the general pattern of tissue loss (Gabbay, 1973; Beyer-mears et al, 1984). Persistent hyperglycemia, during diabetes, activates aldose reductase (Gabbay and Cathcart, 1974; Beyer-mears et al, 1983) which in turn stimulates the pentose phosphate pathway by reoxidizing the NADPH produced by it leading to increased formation of nucleotide precursors and activation of other biosynthetic mechanisms finally causing a net increase in kidney growth (Hutton et al 1975; Sochor et al, 1988). Strong links have been reported between polyol pathway and the small blood
The insulin unregulated glucose transport in insulin-independent tissues and its flux into glycolysis and polyol pathway at normal glucose concentration. Increased glucose flux in polyol pathway in hyperglycemic conditions during diabetes (Adapted from Clarke et al, 1984 and slightly modified).
vessel complications of diabetes mellitus (Brownlee and Cerami, 1981). During diabetes, this pathway is responsible for sorbitol accumulation in erythrocytes thus resulting in membrane deformability in red blood cells that may lead to several pathogenic consequences (Gabbay, 1973; Robey et al, 1987). In the lens, the sorbitol accumulation is responsible for the development of diabetic cataractogenesis (Kinoshita et al, 1979, Crabbe et al, 1980) and in the peripheral nerves, it has been implicated in the development of diabetic neuropathy (Judzewitsch et al, 1983). In the brain, it has been suggested to be the causative agent in the development of total cerebral oedema (O'Brien and Schofield, 1980). Thus, as suggested, the inhibition of aldose reductase and control of hyperglycemia may represent a direct pharmacologic approach in the treatment of certain diabetic complications (Sochor et al, 1988; Bhatnagar et al, 1990).

(E) ALTERATIONS IN ANTIOXIDATIVE SYSTEM

Evidence is accumulating which suggest that toxic reactive oxygen derived free radicals (superoxide, peroxide and hydroxyl radicals) play a crucial role in diabetes (Wolff and Dean, 1987; Wohaeib and Godin, 1987; Oberley, 1988; Wolff et al, 1991). The levels of these reactive oxygen species are controlled by antioxidant enzymes namely superoxide dismutase, catalase, glutathione peroxidase/oxidized glutathione reductase systems and nonenzymatic scavengers like reduced glutathione and vitamin E (Halliwell and Gutteridge, 1984; Simmons, 1984).
Wohaeib and Godin (1987) reported marked alterations in antioxidant enzyme activities and tissue GSH concentrations in diabetic rats and all the changes were almost normalized by insulin treatment. Matkovics and coworkers found a decrease in superoxide dismutase activity in the liver, kidney, spleen, heart, testis, pancreas, skeletal muscle and erythrocytes of diabetic rats (Matkovics, 1977; Matkovics et al, 1982). Oral glutathione treatment is shown to restore the superoxide dismutase activity of renal cortex and liver, but not erythrocytes of rats with streptozocin- induced diabetes (Loven et al, 1986). Glutathione peroxidase has been found to be reduced in human polymorphonuclear leukocytes from type I diabetic patients (Chari et al, 1984). Hepatic glutathione levels decrease in rats with streptozocin induced diabetes (Loven et al, 1986; Wohaeib and Godin, 1987).

The chemical agents like alloxan and streptozocin which have selective cytotoxicity for pancreatic beta cells, have been shown to generate reactive oxygen radicals for their diabetogenic properties (Asayama et al, 1984a, 1984b; Oberley et al, 1988). Patients with type II diabetes and angiopathy had 91% more thiobarbituric acid (TBA) reactive material than controls (Oberley, 1988). Serum TBA-reactive material is reported to be increased by 61% in adult subjects with poorly controlled type-I or II diabetes, but remains unchanged in patients with well controlled diabetes (Oberley, 1988).

Diabetic complications like myocardial ischemic/reperfusion injury
(McCord, 1985), retinal damage (Gouch et al, 1978) and renal injury (Paller et al, 1984) have also been suggested to involve free radical related processes. There is also evidence that oxidative transformation of plasma lipoproteins enhance their ability to induce atherosclerosis (Fogelman et al, 1980) which also shows a high prevalence in diabetics.

There are now ample evidence that during diabetes, persistent hyperglycemia causes increased production of free radicals via autoxidation of glucose (Wolff and Dean, 1987; Hunt et al, 1990) and also via nonenzymatic protein glycation (Wolff et al, 1991) that may lead to disruption of cellular functions and oxidative damage to membranes (Oberley, 1988).

IV. VANADIUM : A BIOLOGICALLY RELEVANT ELEMENT

(A) INTRODUCTION

Vanadium has been recognised as an essential nutritional requirement in the higher animals for nearly a decade but its function remains completely obscure (Underwood, 1977; Golden and Golden, 1981). Vanadium was identified in 1831 in Sweden by Sefstrom who named it after vanadis, the Scandinavian goddess of beauty, youth and lustre (Nechay et al, 1986). This is of course, because vanadium containing crystals and salts in solution give so many striking colours. Even persons exposed to excess vanadium may have green tongues caused by deposition of green vanadium compound. During this century several
actions of vanadium have been discovered, examined, or proposed without definitive proof or identification of its function. This had to do with nutrition, prevention of dental caries, treatment of infections, diabetes, atherosclerosis and anemia (Schroeder et al, 1963; Walters, 1977).

In late 1970's interest in vanadium was revived from discoveries that vanadate ion is an extremely potent and novel inhibitor of the Na⁺ K⁺ ATPase system and from that it may be a physiological regulator of cation pump by changing oxidation states with different inhibitory effectiveness. The novelty as an inhibitor is based on the fact that vanadate inhibits the cation pump at the cytoplasmic Na⁺ site in contrast to digitalis which inhibits the pump at the K⁺ step outside the cell (Macara, 1980; Nechay, 1984).

(B) CHEMISTRY

Vanadium is a group V transitional trace element that has a complex chemistry which is attributed to (i) its multiple oxidation states (ii) hydrolysis and (iii) polymerization. The redox chemistry of this metal undoubtedly plays a role in its biochemical action (Macara, 1980). The oxidation states of biological interest of vanadium are +3, +4, and +5. The first, V³⁺ is stable only in acidic solution (<pH 2) and in the absence of oxygen. On a priori grounds, therefore, it might not be expected to occur in vivo. The best known example of the occurrence of V³⁺ is in the vanadocytes of the blood of tunicates where it is the
predominant state of the metal, otherwise vanadium is largely found in the +4 and +5 oxidation states both of which are readily accessible under physiological conditions (Chasteen, 1981; Smith, 1989; Wever and Kustin, 1990).

\[ V^{4+} \] too is stable only in acidic solution, being rapidly oxidized by dissolved oxygen at physiological pH. Below pH 3 it exists as the blue vanadyl cation, VO\(^{2+}\). The vanadyl cation is also present in tunicate blood cells. This ion forms strong complexes with a diversity of ligands and is known to bind to numerous proteins (Chasteen, 1981). Above pH 2-3, solutions of the vanadyl (\(V^{4+}\)) ion tend to undergo air oxidation to form vanadate (\(V^{5+}\)). This susceptibility to oxidation makes biological studies with vanadyl (VO\(^{2+}\)) more difficult than with vanadate (\(V^{5+}\)) and probably accounts for the fact that vanadyl (VO\(^{2+}\)) has not been extensively studied as an inhibitor. Hydrolysed species of VO\(^{2+}\) are very prone toward air oxidation. When the VO\(^{2+}\) is chelated, however, oxidation is considerably retarded. Reduction of vanadate (\(V^{5+}\)) to vanadyl (\(V^{4+}\)) takes place in the presence of a number of reducing agents common to cells. These include L- ascorbic acid (Chasteen, 1981), glutathione (Macara et al, 1980), norepinephrine (Cantley et al, 1978) and possibly NADH (Vykocil et al, 1980; Ramasarma et al, 1981).

In the physiological range pH 6-8, when the total vanadium concentration is less than 10 mM the species present in appreciable amount are vanadates, VO\(_3^-\) (+5 oxidation state) [Chasteen, 1983]. The inhibitory or activating effect of
vanadium on many phosphohydrolases appears to reside largely, but not exclusively in the +5 oxidation state. The similarity between the chemistry of the vanadate and the phosphate is undoubtedly responsible for much of the biological activity of the metal (Chasteen, 1983). To examine the effects of vanadate, a wide range of its concentrations from 1 μM to 10 mM was used in various in vitro systems as reported in the literature (Tolman et al., 1979; Dubyak and Kleinzeller, 1980; Shechter and Karlish, 1980; Smith, 1983).

(C) DISTRIBUTION

Vanadate is found in plants and animals, although its biological role is unclear. The vanadate +5 form of vanadium has been found in body fluids and the vanadyl +4 form of the compound has been located intracellularly in humans (Nechay, 1984). In mammals, vanadium is an ultra trace element. The most sensitive of commonly used methods for measuring vanadium are the techniques of neutron activation analysis and flameless atomic adsorption spectroscopy (Stroop et al., 1982 and Byrne and Kosta, 1978). Most tissues of higher animals contain intracellular vanadium at concentrations varying between 0.1 and 1 μM (Simon, 1979; Macara, 1980; Ramasarma and Crane, 1981). In the reference human the total body pool of vanadium is ~100 μg with a daily intake of 10-60 μg. Approximate wet tissue concentration (in nanograms of vanadium per gram) are: liver 13, kidney 5, bone, spleen and thyroid 3, brain, fat, milk, colostrum, bile and urine <1, lungs and hair 12-140 (Byrne and Kosta, 1978 and Nechay,
The mean human serum concentration of vanadium is 0.035 ng/ml (range 0.014 - 0.939) according to Cornelis et al (1981) and Versieck and Cornelis (1980), and 0.67 ng/ml (range 0.26 - 1.3) according to the more sensitive technique of Simonoff et al (1984). The above values show that vanadium in the body is found mainly inside the cells.

In plasma, vanadium apparently exists as metavanadate ($\text{VO}_3^-$, +5) and inside cells in reduced form as vanadyl ($\text{VO}^{2+}$, +4) (Rubinson, 1981). NMR spectroscopy confirms that vanadium in tissues exists in the +4 oxidation state (Sakurai et al, 1980).

Erythrocytes transport vanadate from the environment by two mechanisms, one sensitive to anion transport inhibitors and a second that remains unidentified (Heinz et al, 1982). Because of the 100-400 fold weaker binding of vanadate to proteins than the binding of vanadyl to phosphates, the equilibrium favours reduction of vanadate ($V$, +5 oxidation state) to vanadyl ($V$, +4 oxidation state) as soon as vanadate enters the cell and the subsequent binding of vanadyl to phosphates (Nechay et al, 1986).

(D) ROLE IN BIOLOGY

(i) Ignition of interest in enzymology of vanadium

Cantley et al (1977) reported an inhibitor of $\text{Na}^+\text{K}^+$-ATPase in the ATP prepared by the Sigma chemical company from equine muscle. The
Na⁺K⁺-insensitive ATPase in their preparation and adenylate cyclase (EC 4.6.1.1) were not inhibited. The inhibitor was not present in Sigma Grade II ATP prepared by phosphorylation of adenosine with enzymes from yeast.

Subsequently, Cantley et al (1978) also noticed that the inhibition depended on the source of the ATP. In fact the ATP, was needed only to provide the inhibitor. Cantley tuned his Na⁺K⁺-ATPase assay system for maximum sensitivity to the inhibitor and separated it from Sigma equine ATP by chromatography with chelex-100 and sephadex. When the isolated inhibitor was submitted to electron probe microanalysis and microwave emission spectroscopy vanadium could be identified (Cantley et al, 1977, 1978).

(ii) Inhibition of phosphoenzymes

Vanadium has been known since 1965 to inhibit Na⁺K⁺-ATPase. This class of membrane enzymes has two principal conformations called E1 and E2. For this reason this class is sometimes called the E1/E2 class of enzymes. In the E1 conformation, the enzyme accepts a phosphate group reversibly from ATP and in the E2 conformation it accepts a phosphate group reversibly from Pi (inorganic phosphate). All members of this class are inhibited by vanadate (Macara, 1980; Chasteen, 1983; Nechay et al, 1986).

Vanadate inhibits Na⁺K⁺-ATPase from the cytoplasmic site as a deadend transition state analog of inorganic phosphate by forming a stable inactive complex in a reversible reaction with the E2 conformation of the enzyme (Huang
and Askari, 1981). Inhibition by vanadate requires a divalent cation such as Mg$^{2+}$, as does phosphorylation from ATP (Cantley et al, 1978).

Almost all the phosphoenzymes including ion transport ATPases, Ca$^{2+}$-ATPase of sarcoplasmic reticulum, gastric H$^+$K$^+$-ATPase and other ATPases are inhibited by vanadate (Frust and Solioz, 1985; Walderhaug et al, 1985). However, it may fail to inhibit these enzymes if the assay system is improperly adjusted (Chasteen, 1983).

Acid and alkaline phosphatases, phosphotyrosyl protein phosphatase can also be inhibited by vanadate (Chasteen, 1983; Swarup et al, 1982).

(iii) Stimulation of enzymes

Interestingly, adenylate cyclase is stimulated six fold by 3 mM vanadate (Combert and Johnson, 1983; Nechay et al, 1986). Glyceraldehyde-3-phosphate dehydrogenase is uncoupled by vanadate as effectively as by arsenate. An NADH-oxidizing activity in plasma membrane is also shown to be stimulated by vanadate. Phosphoglucomutase and glucose-6-phosphate dehydrogenase accept glucose as a poor substrate and there is a large stimulation of this impaired activity by addition of vanadate. Apparently, glucose-6-vanadate is formed sufficiently and rapidly that serves as a good substrate to promote the reaction (Chasteen, 1983).

(E) INSULIN-LIKE EFFECTS OF VANADIUM COMPOUNDS

In recent years, there has been increasing interest in the biological
significance of vanadium since the demonstration that vanadium compounds possess potent insulin-like properties. Its insulin-mimetic effects were early documented in 1980 when it was shown that vanadate could stimulate glucose uptake and oxidation in rat adipocytes (Shechter and Karlish, 1980 and Dubyak and Kleinzeller, 1980). Subsequently, several studies were performed that amply indicate that vanadate is a novel and potent insulin-mimetic agent. Of great interest was the demonstration by Heyliger et al (1985) that vanadate inclusion in the drinking water of diabetic rats normalizes the elevated blood glucose level and corrects the disturbed cardiac performance. This discovery came as a breakthrough and several laboratories focussed extensive research to elucidate and explore other insulin-like or antidiabetic properties of vanadium compounds on various metabolic disorders.

The insulin-mimetic effects of vanadate is shown to be unrelated to the previously known action of vanadate in inhibiting Na⁺K⁺-ATPase activity (Shechter, 1990). In fact, vanadate (VO₃⁻) over a concentration range of 0.1-0.7 mM does not inhibit Na⁺K⁺-ATPase activity in various intact cellular systems, probably due to the fact that internalized vanadate (VO₃⁻, +5 oxidation state) is reduced intracellularly to vanadyl ion (VO²⁺, +4 oxidation state) which is an ineffective inhibitor of the ATPases (Nechay, 1984; Shechter, 1990).

(i) **In vitro studies**

In recent years, vanadate has been found to mimic many of the
documented actions of insulin in various cell types such as adipocytes, skeletal muscle and hepatocytes. The various insulin-like effects of vanadate in \textit{in vitro} systems are documented in Table 2. Vanadate is shown to simulate both the glucose transport-dependent and -independent intracellular actions of insulin in rat adipocytes and the mutagenic action of insulin in quiescent swiss 3T3 and 3T6 cell lines (Smith, 1983). Shechter and Karlish (1980) reported that externally applied vanadate (V$^{+5}$) at low concentrations mimic fully the effect of insulin on glucose oxidation in rat adipocytes. They suggested that this stimulation seemed mainly due to the effects of vanadyl (V$^{+4}$) ions produced within the cells and not primarily to the inhibition of sodium pump. Dubyak and Kleinzeller (1980) also showed that vanadate caused insulin like effects by increasing hexose transport in adipocytes. The degradation of endogenous proteins found at a high rate in isolated rat hepatocytes (when incubated in amino acid free medium) is inhibited by vanadate and insulin (Seglen and Gordon, 1981). Vanadate also augmented the stimulatory effect of insulin on DNA synthesis in the mammary gland (Hori and Oha, 1980) and directly stimulated DNA synthesis in quiescent human fibroblasts (Carpenter, 1981).

Later, Tamura et al (1983, 1984) demonstrated that like insulin, vanadate stimulates the activities of glycogen synthase and glycogen phosphorylase in rat adipocytes in a dose and time dependent manner and no additional effect was observed when insulin and vanadate were added together. Furthermore, both
vanadate and insulin increase the phosphorylation of 95000-dalton subunit of adipocyte insulin receptor and this phosphorylation might be related to the intracellular activation of glycogen synthase (Tamura et al, 1983). In a subsequent study, Green (1986) showed that vanadate, in contrast to insulin, could stimulate glucose transport in adipocytes by acting at a post receptor level even when the insulin receptors were down regulated. Other insulin-like effects of vanadate on adipocytes include the stimulation of glucose uptake, glucose metabolism (Tolman et al, 1979; Clausen et al, 1981, Tamura et al, 1984), glucose incorporation (Sakurai et al, 1990), glucose oxidation via glycolysis as well as HMP shunt (Degani et al, 1981; Brichard et al, 1990).

Like in adipocytes, vanadate produces insulin- like effects in skeletal muscle and hepatocytes and has been shown to increase the glucose transport and its metabolism (Tolman et al, 1979; Clausen et al, 1981; Brichard et al, 1990). Clark et al (1985) demonstrated that vanadate and insulin causes qualitatively similar changes in muscle glucose metabolism and increases glucose uptake (2-fold), glycolysis (2 fold) and glycogen synthesis (3-fold). Vanadate also causes augmentation of Type II insulin-like growth factor (IGF) receptor translocation in the liver (Kadota et al, 1987), a well established rapid insulin effect.

Vanadate is also shown to effectively inhibit the isoproterenol-dependent lipolysis and has been suggested to be a more potent antilipolytic agent than insulin, though it does not affect tyrosine phosphorylation (Mooney et al, 1989).
<table>
<thead>
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<th>Activity</th>
<th>Direction of activation</th>
<th>Target tissue</th>
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<tr>
<td>Hexose transport</td>
<td>Stimulated</td>
<td>Skeletal muscle, rat adipocytes</td>
</tr>
<tr>
<td>Lipogenesis</td>
<td>Stimulated</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>Glucose oxidation</td>
<td>Stimulated</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>Lipolysis</td>
<td>Inhibited</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>Glycogen synthase</td>
<td>Stimulated</td>
<td>Skeletal muscle, rat adipocytes, Rat hepatocytes</td>
</tr>
<tr>
<td>Mitogenic activity</td>
<td>Augmented</td>
<td>Various cultured cells</td>
</tr>
<tr>
<td>Translocation of IGF-II receptors</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 membranes 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>
</tbody>
</table>

IGF-II, insulin-like growth factor II.

(Adapted from Shechter, 1990).
Similarly, Degani et al (1981) demonstrated that vanadate activates glucose oxidation as effectively as insulin and could be more potent than insulin as an antilipolytic agent. Vanadate is reported to regulate glucose transporter (Glut-1) expression in NIH3T3 mouse fibroblasts suggesting that the rapid effects of vanadate on glucose transport may be due to the altered expression of one or more genes encoding the glucose transporters (Mountjoy and Flier, 1990). Thus, there are now ample evidence to firmly establish that vanadate possess potent insulin like properties.

(ii) *In vivo* studies

In an interesting extension of earlier reports regarding the *in vitro* insulin-mimetic activity of vanadate in isolated cells, Heyliger et al (1985) for the first time demonstrated that vanadate could be orally administered to diabetic rats. They found that vanadate inclusion in the drinking water (0.6 to 0.8 mg/ml) of diabetic rats restores the much elevated blood glucose levels to normal values and also eliminates the depressed cardiac performance. Since this demonstration of the *in vivo* insulin like effects of vanadate, interest in its other antidiabetic properties and effects on biochemical parameters have increased. As a result, several laboratories became actively engaged in further characterizing this very important aspect of vanadium in relation with therapeutic cure of diabetes and its complications. Subsequently, the studies of various researchers established that sodium orthovanadate in a dose of 0.6 to 0.8 mg/ml in drinking water has

Oral administration of vanadate to experimentally induced diabetic rats not only effectively causes stable normoglycemia but also improves many metabolic disorders related to diabetes, though the decreased level of insulin is not restored (Heyliger et al, 1985; Shechter, 1990). To reduce the toxicity of vanadate, 80 mM NaCl was also supplemented with vanadate in the drinking water of diabetic rats (Heyliger et al, 1985; Meyerovitch et al, 1991). Vanadate therapy has been demonstrated to exert a prophylactic effect on the progressive cardiodepression and to improve myocardial contractile function in the diabetic rats (Paulson et al, 1987).

Meyerovitch et al (1987) established optimal conditions for sodium metavanadate administration and found that inclusion of metavanadate at a dose of 0.2 mg/ml (in the drinking water) maintains a longlasting stable normoglycemia making the diabetic rats anabolic. Sodium orthovanadate and vanadyl sulfate have also been administered by intraperitoneal injections to diabetic rats in the therapeutic doses and were shown to produce similar insulin-mimetic effects (Strout et al, 1990; Sakurai et al, 1990). However, the much used and convenient vanadate treatment route seems to be through drinking water (Heyliger et al, 1985; Meyerovitch et al, 1987; Gil et al, 1988; Valera et al, 1993).
Diabetes is characterized by a decrease in the levels of hepatic glycogen, glucokinase, 6-phosphofructokinase and fructose-2,6-biphosphate. All the above decreased hepatic abnormalities are restored to normalcy in vanadate-dosed diabetic rats suggesting an insulin-mimetic effect of vanadate on hepatic intracellular events (Gil et al, 1988). Pederson et al (1989) reported that 3 week oral treatment of diabetic rats with vanadyl sulfate, results in the normalization of glucose tolerance and protection of islets from destruction by streptozotocin and these normalized conditions are retained even after 13 week of the withdrawal of vanadyl sulfate. Vanadyl sulfate is less toxic than vanadate but it is poorly absorbed. Further studies by Ramanadham et al (1989a, 1989b, 1990) have shown that vanadyl administered orally as aqueous vanadyl sulphate lowers blood glucose and blood lipids in streptozotocin-diabetic rats and prevents secondary complications of diabetes such as cataracts and cardiac dysfunction.

Vanadate administration also causes marked improvement in the disturbed glucose homeostasis in an animal model with severe insulin resistance and overt diabetes like in the insulin resistant Zucker fa/fa rats and diabetic ob/ob mice (characterized by their genetically transmitted obesity, hyperglycemia and marked hyperinsulinemia) [Blondel et al, 1990; Brichard et al, 1990; Meyerovitch et al, 1991].

In 90% pancreatectomized rats, vanadate improves the glucose tolerance and restores the disturbed glycogen synthesis in the liver and muscle (Rossetti and

This important aspect of vanadium to possess potent insulin-like properties has been further substantiated by recent reports that demonstrate the reversal of altered hepatic cytochrome P-450 isozymes in diabetes (Donahue and Morgan, 1990) and restoration of glucose transporter expression in the liver and skeletal muscle (Strout et al, 1990; Brichard et al, 1993; Valera et al, 1993).

Bradley (1992) envisaged the possibility of the future treatment of diabetics by vanadium compounds in the form of oral pill. In a novel attempt, McNeill et al (1992) chemically modified the vanadyl form and prepared bis(maltolato) oxovanadium (+4) compound which is proved to be 50% more potent and effective than vanadyl sulfate. This compound is well absorbed across the gastrointestinal membranes in contrast to vanadyl sulfate which is poorly absorbed.

The mechanism of the insulin-like actions of vanadate remains unclear at this stage. Further extensive research is required to explore its other insulin-mimetic properties and antidiabetic effects, which may also elucidate the mechanism of vanadate action and will provide better therapeutic interventions in the treatment of diabetes and its complications.