REVIEW OF LITERATURE
2.1 DIABETES MELLITUS

Diabetes mellitus as presently understood is almost certainly more than a single disease (Rotter and Rimoin, 1978; Fajans et al., 1978) as it is associated with derangement in metabolism of carbohydrates, proteins and fats. It is a complex disease characterized primarily by relative or absolute insufficiency of insulin secretion and concomitant insensitivity or resistance to the metabolic action of insulin on target tissues. Hyperglycemia results as a consequence of the defects in insulin secretion and action. Ultimately, in the diabetic process, there may be widespread involvement of virtually every organ system.

CLASSIFICATION

Over the years, a variety of classification and diagnostic criteria for diabetes mellitus have been proposed. A classification of diabetes and other categories of glucose intolerance, based on contemporary knowledge of this heterogenous syndrome was developed by an international work group sponsored by the national diabetes data group of the US National Institute of Health (NIH) in 1979. Although diabetes mellitus is primarily a disease of inadequate insulin secretion and action, measurement of abnormal insulin action in terms of serum or blood glucose are used almost exclusively to diagnose diabetes mellitus. In most formulations, basal euglycemia in the fasting state and an unimpaired ability to dispose off a carbohydrate load have been most widely used to assess the presence or absence of diabetes. Conditions other than diabetes mellitus also are associated with abnormal carbohydrate metabolism, carbohydrate intolerance, and even fasting hyperglycemia.
A recent attempt has been made to resolve these differences and to organize the available clinical data to provide a scientific basis for interpretations of fasting serum or plasma glucose concentrations and also of serum glucose responses during glucose tolerance tests. Three major types of patients with diabetes mellitus are now recognized (Garber, 1994).

**TYPE I (INSULIN-DEPENDENT DIABETES MELLITUS)**

Patients with type I diabetes mellitus have little or no endogenous insulin secretion. The onset of the disease is usually clinically abrupt. It can occur at any age. Incidence most commonly peaks in the middle of the first decade and again during the period of the growth acceleration of adolescence. As with all other forms of primary diabetes mellitus, a genetic predisposition appears to underlie the pathogenesis of type I diabetes mellitus. Patients with type I disease have in common one of several human histocompatibility antigens. Islet cell antibodies are frequently observed at the time of diagnosis. A viral etiology involving one of several enteroviruses such as coxsackievirus B4 and mumps has been proposed in these patients. Recently, evidence has developed which suggests that an ongoing, autoimmune destruction of pancreatic beta cells may underlie type I diabetes mellitus (Dahlquist, 1994).

**TYPE II (NON-INSULIN DEPENDENT DIABETES MELLITUS)**

Patients with type II diabetes present overt abnormalities of glucose homeostasis, including fasting hyperglycemia and/or carbohydrate intolerance despite the presence of some endogenous insulin secretory capability. These patients generally demonstrate marked resistance or
insensitivity to the metabolic actions of endogenous as well as exogenous insulin (Flier et al., 1979) in part as a result of decreased insulin receptors. A failure of post receptor coupling and of intracellular insulin action is a more important cause of insulin resistance. Obesity is a major risk factor for the development of this type of diabetes mellitus. A primary insufficiency of insulin secretion is the pathologic prerequisite for the clinical development of type II diabetes mellitus. Nevertheless, because of the relationship between pre-existing obesity and the development of type II diabetes, environmental influences clearly play a strong role in development of disease.

SECONDARY DIABETES MELLITUS

It constitutes a heterogenous group of patients for whom a designation of either of the traditional forms of diabetes mellitus appears inappropriate. Although abnormal glucose metabolism may be demonstrated by fasting hyperglycemia or an impaired ability to dispose off a carbohydrate load, genetically determined inadequacies of insulin secretion or action are not the primary pathogenic abnormality in secondary diabetes. In general, any disease resulting in antagonism to insulin action can be associated with secondary diabetes mellitus (Nestler and McClanahan, 1992; Sharp et al., 1992). A variety of drugs have also been associated with carbohydrate intolerance and secondary diabetes mellitus.

2.2 EXPERIMENTAL DIABETES

DISCOVERY: VonMering and Minkowski (1890) first discovered diabetes by totally extirpating the pancreas. Baumann and Marine (1932), Houssay et al. (1932) and Evans et al. (1939) reported the production of
experimental diabetes by injecting pituitary anterior extracts. The discovery of alloxan, which introduced the term ‘Chemical diabetes’ happened quite unintentionally by findings of Dunn et al. (1943a) who were studying the effect of a series of uric acid derivatives including alloxan with regard to kidney damage. Histological examination revealed early signs of expected kidney damage, but the surprising finding was the partial or even total necrosis of the pancreatic islets. Other tissues showed no damage. This and the following report (Dunn et al., 1943b) on selective necrosis of the islets of Langerhans provoked by alloxan led to investigations in several laboratories. In the same year alloxan injection was shown to result in a diabetic state in the rat by Dunn and McLetchie (1943), in the rabbit by Bailey and Bailey (1943) and Brunschwig et al. (1943) and in the dog by Goldner and Gomori (1943). By the year 1948 it was known that alloxan produces diabetes in the rabbit, rat, dog, hamster, cat, sheep, monkey, man, turtle, pigeon (Lukens, 1948). The alloxan diabetes showed the classical signs of human diabetes i.e. hyperglycemia, glycosuria, polydipsia and polyuria.

Since the discovery of alloxan diabetes, a variety of chemical substances have been discovered to be diabetogenic. Because none of the diabetogenic drugs subsequently discovered until 1960 exert an equally specific action, alloxan alone remained the most widely used diabetogen until 1963, when streptozotocin was reported to be diabetogenic and comparable to alloxan with regard to islet β-cell specificity (Rakieten et al., 1963).
2.2.1 ALLOXAN DIABETES

Alloxan, also called mesoxalylurea has got the following structure:

\[
\begin{align*}
\text{H-N} & - \text{C}=\text{O} \\
\text{O}=\text{C} & - \text{C}=\text{O} \\
\text{H-N} & - \text{C}=\text{O}
\end{align*}
\]

It is reduced by ascorbic acid \textit{in vitro} to dialuric acid, which in turn spontaneously autoxidises to form three highly reactive species $\text{H}_2\text{O}_2$, $\text{O}_2^\cdot$, and $\text{OH}$ (Cohen and Heikkila, 1974). $\text{H}_2\text{O}_2$, $\text{O}_2^\cdot$, and $\text{OH}$ have been postulated to be cytotoxic in a variety of biological systems, it appears likely that $\text{H}_2\text{O}_2$, $\text{O}_2^\cdot$, and $\text{OH}$ could be responsible for the destruction of the $\beta$-cells of the pancreas by dialuric acid and alloxan. Ethanol and related alcohols, thiourea and dimethyl sulphoxide all of which are very good hydroxyl radical scavengers, are shown to protect against alloxan diabetes in mice indicating a role for the hydroxyl radical in alloxan induced diabetes (Heikkila \textit{et al.}, 1974, 1976; Heikkila, 1977).

Development of alloxan diabetes

Alloxan diabetes ensues in three phases; initial hyperglycemia phase which is followed by hypoglycemic phase and finally chronic hyperglycemic phase results.

Histological changes in alloxan diabetes

After a diabetogenic dose of alloxan, a massive necrosis of the $\beta$-cells in the islet of Langerhans is observed (Lazarus and Volk, 1962; Frerichs and
Creutzfeldt, 1969). This effect of alloxan is so specific that besides slight and mostly reversible changes in the kidney (Bailey et al., 1944; Duff, 1945) and possibly the adrenal medulla (Hard and Carr, 1944), no other histological changes are encountered.

Severity and duration

A high dose of alloxan results in an immediate destruction of nearly all β-cells, the ensuing diabetes is very severe, death is a usual consequence. Insulin treatment is required to keep animals alive for longer periods. Doses causing subtotal destruction of the β-cell population yield different pictures according to species. Spontaneous recovery is particularly evident in rats and mice (Bunnag et al., 1967; Rerup, 1968). The recovery from diabetes is believed to be the consequence of either a multiplication of the β-cells that survived the alloxan poisoning or a new formation of β-cells (Bunnag et al., 1967).

2.2.2 STREPTOZOTOCIN DIABETES

Streptozotocin, is a broad-spectrum antibiotic which has a highly specific diabetogenic effect (Rakieten et al., 1963). Streptozotocin consists of 1-methyl-1-nitrosourea linked to position C2 of D-glucose.

\[
\text{Structural formula of streptozotocin}
\]
Streptozotocin is known to induce diabetes in the rat, dog, hamster, monkey and guinea pig (Rakieten et al., 1963; Schein et al., 1967; Brodsky and Logothetopoulos, 1968). The mechanism by which STZ exerts diabetogenic action on pancreatic β-cells is still not clearly understood. Evidence to suggest that STZ also mediates toxicity by free radical mechanism like alloxan is inconclusive (Slonim et al., 1980). Robbins et al. (1980) and Marklund and Granqvist (1981) have shown that the superoxide anion scavenging enzyme, superoxide dismutase, partially protects the islets when administered before STZ in vivo. Sandler and Andersson (1982) found dimethyl urea, a hydroxyl radical scavenger to partially protect against STZ-induced diabetes. The partial protection, afforded by free radical scavengers indicates that free radicals mediate STZ diabetes.

Development of STZ diabetes

The triphasic pattern of blood glucose level fluctuations observed after diabetogenic doses of alloxan is also seen after streptozotocin (Schein et al., 1967; Schein and Bates, 1968).

Histological changes in STZ diabetes

STZ, like alloxan in diabetogenic doses induced necrosis of β-cells in the islets of Langerhans (Junod et al., 1967). Except, the differences with regard to the time of occurrence of certain phenomena the majority of histological changes resemble in both STZ and alloxan diabetes (Lazarus and Volk, 1962).

Severity and duration

Hypoglycemia is more severe with STZ than with alloxan, and accordingly fatal convulsions are more frequent. STZ diabetes, unlike
alloxan diabetes, does not show spontaneous recovery from this chronic diabetic condition (Rerup and Tarding, 1969) however, apparent reversibility of islet β-cell damage with lower doses is reported (Junod et al., 1969).

2.3 INSULIN

INSULIN AND GLUCOSE HOMEOSTASIS

Diabetes mellitus is chiefly associated with hyperglycemia resulting from decreased entry of glucose into cells (Bogardus et al., 1984) and increased hepatic output (Revers et al., 1984; De Fronzo et al., 1985). In non-diabetic humans, the rapid increase in insulin concentration occurs after food ingestion which results in prompt suppression of hepatic glucose release (Rizza et al., 1981; Firth et al., 1986) and stimulation of glucose uptake (Radzuik et al., 1978; Ferrannini et al., 1988). Insulin is believed to stimulate glucose uptake in muscle by increasing the number or activity of plasma membrane glucose transporters (Kahn, 1992). Prager et al. (1986) have reported impairment in rate of insulin-induced stimulation of glucose uptake in individuals with insulin resistance in obese. The slower rate of glucose disappearance in diabetic subjects was observed by Calles-Escandon and Robbins (1987). Turk et al. (1995) have observed lower glucose disappearance and higher hepatic glucose release in diabetic than nondiabetic subjects after an overnight insulin suggesting additional defects at the level of insulin binding and or intracellular signaling. Such defects may be intrinsic to NIDDM or may be influenced by factors associated with the diabetic state.

INSULIN AND LIPID METABOLISM

Insulin plays a critical role in the production and clearance of
triglycerides in diabetics (Nikkila et al., 1977; Brunzell and Bierman, 1978). In insulin deficient state, hypertriglyceridemia can result due to a decreased activity of lipoprotein lipase and increased supply of free fatty acids (Steiner et al., 1975 and Chen et al., 1979). In diabetics with hyperinsulinemia, hypertriglyceridemia may result from enhanced triglyceride synthesis (Streja et al., 1977).

**INSULIN AND PROTEIN METABOLISM**

Insulin stimulates amino acid uptake and protein synthesis in peripheral tissues. Amino acids taken up by the liver are used for hepatic protein synthesis as well as for hepatic gluconeogenesis. Impaired protein metabolism has been reported (Morano et al., 1994).

**2.4 HYPERGLYCEMIA AND CONSEQUENCES**

Diabetes is a major health problem. It is a chronic disease that has no cure. The hallmark of diabetes is elevated blood glucose levels. The present medical interventions aim to maintain glucose homeostasis. Glucose is a molecule essential for life, particularly for function of the nervous system, its concentration must be maintained within a narrow window because of the powerful adverse effects of both too much and too little glucose. Most diabetic patients eventually experience one or more of the long term complications of the disease. Exactly how hyperglycemia causes these complications has been debated for years, but the recent (1993) National Institute of Health-sponsored diabetes control and complications trials (DCCT) clearly implicate glucose as a potentially toxic molecule.

Alterations in metabolic pathways due to hyperglycemia independent of insulin status have direct toxic effects. Elevated glucose levels result in
activation of aldose reductase pathway (Stevens et al., 1995), increase in non enzymic protein glycosylation (Brownlee, 1992; Taniguchi, 1996), autoxidation of glucose (Wolff and Dean, 1987), changes in energy metabolism, changes in the levels of inflammatory mediators (Raheja, 1994) and localized tissue damage resulting from hypoxia and ischemic reperfusion injury. These metabolic alterations eventually disturb redox state of the organism. Higher glucose levels lead to a direct (autoxidation of glucose) or an indirect generation of free radicals (Derlypere, 1994).

**ALDOSE REDUCTASE PATHWAY**

One of the consequences of hyperglycemia in human diabetes mellitus is increased metabolism of glucose through sorbitol (polyol) pathway (Taylor and Agius, 1988; Yue et al., 1989). This involves the reduction of glucose to sorbitol catalysed (Tomlinson, 1993) by aldose reductase and the oxidation of the sorbitol to fructose by sorbitol dehydrogenase. Conversion of glucose to sorbitol by aldose reductase requires nicotinamide adenine dinucleotide phosphate (NADPH) and thereby competes with other NADPH-requiring reactions. NADPH is required for the conversion of oxidized to reduced glutathione and for fatty acid and cholesterol biosynthesis.

**PROTEIN GLYCOSYLATION**

It is well known that enzyme activities are modulated by the post-translational modification reactions. The enzymes undergo post-translational modification reactions such as phosphorylation and acetylation reactions (Taniguchi and Meister, 1978; Kondo et al., 1984; Matsumoto et al., 1984; Fujita et al., 1985). Such reactions are expected to occur in different kinds
of proteins under physiological conditions. In the case of RNAse, the incorporation of glucose into the protein resulted in a reduction in its activity (Ahmed et al., 1986).

The glycation proceeds through the formation of a Schiff base between glucose and an α- or ε-amino group in a protein together with Amadori rearrangement (Scheme I) Schiff base adducts or Amadori products produced through the glycation reaction generate reactive oxygen species.

\[
\begin{array}{cccc}
\text{HC} = \text{O} & \text{O}_2 & \text{H}_2\text{O}_2 & \text{HC} = \text{NR} \\
\text{HCOH} & \text{Mn}^+ & \text{HC} \longrightarrow \text{NHR} \\
\text{(CHOH)}_3 & \text{RNH}_2 & \text{(CHOH)}_3 & \text{(CHOH)}_3 \\
\text{CH}_2\text{OH} & \text{Protein} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
\text{Glucose} & \text{Schiff} & \text{Amadori Product} & \text{AGEs}
\end{array}
\]

Scheme 1: General scheme of glycation reaction.

Hicks et al. (1988) proposed lipid hydroperoxides could react with Amadori adducts and produce reactive species which can further initiate peroxidative chain reaction (Scheme 2).
Involvement of glycation reaction in the pathogenesis of diabetic complications has been suggested (Brownlee, 1992; Taniguchi, 1992; Wolff, 1993; Taniguchi et al., 1996). Brownlee and Cerami (1981) proposed elevated glucose causes slow but significant nonenzymic glycosylation of proteins in diabetes. Kumari and Sahib (1993) have shown glycosylation to increase linearly as a function of duration of hyperglycemia. Glycation and inactivation of human CuZn-SOD has been reported (Arai et al., 1987; Ookawara et al., 1992). Picard (1995) has suggested hyperglycemia induced glycation of lipoproteins, particularly low-density lipoproteins (LDL) in diabetes as a detrimental factor in pathogenesis of atherosclerosis. Glycation of LDL prevents its recognition by the specific receptor and results in LDL accumulation in macrophages followed by its oxidation. Glycated LDL are more susceptible to oxidative insult as evidenced by the property of glycated proteins to react with lipid hydroperoxides (Hicks et al., 1988; Sakurai and Tsuchiya, 1988; Hunt et al., 1990).
The advanced glycation end products (AGEs) which are non enzymatically glycated and oxidized proteins generate oxygen-free radicals and mediate their action by binding to specific receptor proteins. Isolated AGE albumin from diabetic plasma and infused into normal animals led to the appearance of malondialdehyde determinants in the vessel wall and increased TBARS in the tissues, activation of nuclear factor-kappa B, and induction of haem oxygenase mRNA (Yan et al., 1994). These data indicate that interaction of AGEs with a cellular target such as endothelial cells, leads to oxidant stress resulting in changes in gene expression and other cellular properties.

**GLUCOSE AUTOXIDATION**

In addition to direct glycosylation reactions, glucose can enolize and thereby reduce molecular oxygen under physiological conditions, yielding α-ketoaldehydes and free radical intermediates (Scheme 3).
The enol form of glucose can react with lipid hydroperoxides and result in the formation of reactive species which can further initiate peroxidative chain reaction (Scheme 4) (Hicks et al., 1988).

\[
\begin{align*}
O & \quad O \\
\quad | & \quad | \\
R - C - C - H + LOOH \rightarrow & R - C = C - H + LO^* + H_2O \\
\text{(Dicarbonyl) Hydroperoxide}
\end{align*}
\]

Curcio et al. (1996) have observed that lipid peroxide products increase in cultured human endothelial cells on raising the glucose concentration in the culture medium from 5 mM to 20 mM and speculated autoxidation of glucose as source of free radicals and subsequent increase in peroxide products. Giugliano et al. (1994) found elevated oxygen derived free radicals in red blood cells and Starosel'tseva et al. (1986) in plasma of diabetic animals and patients which was correlated with metabolic control in patients.

2.5 REACTIVE OXYGEN SPECIES

In aerobic organisms, biochemical redox reactions involving oxygen are important sources of free radicals. A free radical is a chemical species with an unpaired electron. The presence of unpaired electron entails a species reactive. Both endogenous and exogenous factors may lead to the production of free radicals (Halliwell, 1991).
Aerobic organisms use oxygen to burn carbon and hydrogen rich molecules to obtain the chemical energy and heat necessary for life. In the process, the oxygen molecule is reduced to water. However, in mitochondrial transport chain there are potential sites from where leakage of single electrons occur, leading to the partial reduction of \( \text{O}_2 \) to superoxide anion \( (\text{O}_2^-) \) (Kehrer and Smith, 1994) superoxide can be further reduced by the addition of a second electron to hydrogen peroxide \( (\text{H}_2\text{O}_2) \) (Boveris et al., 1979). The addition of a third electron leads to the formation of the hydroxyl radical \((\text{OH}^*)\), which is most aggressive of the various reactive oxygen species (ROS). In addition to \( \text{O}_2^- \), \( \text{H}_2\text{O}_2 \) and \( \text{OH}^* \), there is another type of ROS called singlet oxygen \((\text{O}_2^*)\). Singlet oxygen can be formed from ground state oxygen in the presence of a photosensitizer and light.

In addition to the mitochondrial electron transport system there are further endogenous sources of \( \text{O}_2^- \) production; soluble oxidase enzymes such as NADPH oxidase in phagocytic cells (neutrophils and monocytes) (Babior, 1978; Smith et al., 1991; Stamler et al., 1992) and Xanthine oxidase (Marx, 1987); epinephrine and quinoid substrates such as coenzyme \( \text{Q}_{10} \) and vitamin K that can undergo redox cycling and the cytochrome \( \text{P}_{450} \) system (Ames et al., 1993). Under normal conditions superoxide can be produced from all these endogenous sources, and it has been estimated that each cell in human body is exposed to about \( 10^{10} \) molecules of \( \text{O}_2^- \) each day. Once formed from various endogenous sources \( \text{O}_2^- \) can react further and dismutate to \( \text{H}_2\text{O}_2 \) and \( \text{OH}^* \). In the presence of catalytic amounts of iron or copper, \( \text{H}_2\text{O}_2 \) can form \( \text{OH}^* \) in the metal
catalysed (Haber-Weiss reaction) or $H_2O_2$ can directly form $OH^*$ in the presence of iron or copper (Fenton reaction) (Halliwell and Gutteridge, 1984).

In addition to the above listed endogenous sources, a number of exogenous factors such as diet, pesticides, cigarette smoke, ozone, X rays, UV light etc. also contribute to the production of ROS.

### 2.6 OXIDATIVE DAMAGE IN DIABETES

ROS generated can cause oxidative damage to various biological macromolecules (Pryor, 1994) including proteins, carbohydrates, lipids and DNA. $OH^*$ can damage cellular membranes and lipoproteins by a process called lipid peroxidation. Proteins are also damaged by ROS, leading to structural changes or loss of enzyme activity. Oxidative damage to DNA leads to DNA lesions and mutations (Ames et al., 1993). There is no evidence that once oxidative damage occurs it may be reversed. In the case of DNA, repair enzymes act by excision and replacement of modified base or nucleotide. For proteins, lipids and RNA, the kinetics of the turnover of the molecule appears to be the critical factor (Baynes, 1991).

#### OXIDATIVE DAMAGE TO LIPIDS

Polyunsaturated fatty acids (PUFA) which are predominantly located in cell membranes, are particularly susceptible to free radical attack (Niki et al., 1991). When $OH$ is formed adjacent to a membrane it is able to abstract a hydrogen atom ($H^*$). Although the original $OH^*$ is inactivated, a lipid radical is formed which after molecular rearrangement can react with oxygen to give the peroxy radical. The lipid peroxy radical can react with membrane PUFA, forming more lipid radicals, while itself being converted
to a lipid hydroperoxide, which in the presence of various metal complexes can decompose into more radicals i.e. the peroxidation is propagated. Formation of lipid peroxide products by the action of free radicals on unsaturated fatty acids has been implicated in the pathogenesis of atherosclerosis and vascular disease (Dormandy, 1983; Steinberg et al., 1989; Stringer, 1989). Enhanced levels of lipid peroxides have been found secondary to cancer, ischemia-reperfusion injury, autoimmune disease, muscular dystrophy, diabetes and ageing process.

Lipid peroxide product levels have been found to increase in serum (Olczyk et al., 1994; Ozdemirler et al., 1995), plasma (Sato et al., 1979; Gallou et al., 1993; MacRury et al., 1992), erythrocytes (Uzel et al., 1987; Jain et al., 1989; Olczyk et al., 1994) and lipoprotein fractions (Nishigaki et al., 1981) in diabetic patients. Rud’Ko et al. (1994) found increased MDA, in platelets and red cells of type I diabetic patients. Tsai et al. (1994) have observed increased susceptibility of low density lipoproteins to oxidation in poorly controlled IDDM subjects.

Young et al. (1995) reported enhanced lipid peroxide (LP) products in plasma, red blood cells and urine of STZ-diabetic rats. Previously, increased levels of lipid peroxidation products have been reported in STZ-diabetic rats (Higuchi, 1982; Karpen et al., 1982). Morel and Chisolm (1989) have reported increased lipid peroxidation products in a lipoprotein fraction from the STZ-diabetic rats. Armstrong and al-Awadi (1991) have reported several fold elevation of LP at 10 days and 22 days post-induction of STZ diabetes and a dramatic fall below baseline values at 39 days.
OXIDATIVE DAMAGE TO PROTEIN

Proteins like lipids are susceptible to free radical damage. Aromatic amino acids, cysteine, disulphide bonds and peptide bonds all are affected resulting in fragmentation and altered structure and function (Wolff and Dean, 1986). For protein turnover of the molecule appears to be the critical factor limiting the accumulation of oxygen radical damage. For long lived irreparable molecules products of oxygen reaction may accumulate with time and through alterations in protein structure and function, these oxidation products may contribute to the development of pathology.

In diabetes modifications of long lived extracellular proteins such as crystallins, collagens, elastins, laminin, myelin sheath proteins and structural changes in tissues rich in these proteins (lens, vascular wall, basement membranes) are associated with the development of complications such as cataracts, microangiopathy, atherosclerosis, and nephropathy (Baynes, 1991). Indirect evidence suggests that collagen is oxidatively modified in diabetes (Sell et al., 1990). Enhanced carbohydrate-derived oxidation products were reported in collagen from diabetics by Ahmed et al. (1988), Kaanane and Labuza (1989) and Sell et al. (1990).

The increased levels of glycated enzyme CuZn-SOD is reported in erythrocytes of patients with diabetes mellitus (Arai et al., 1987; Kawamura et al., 1992). Ookawara et al. (1992) have reported that glycation of CuZn-SOD (Protein) resulted in fragmentation of the molecule.

OXIDATIVE DAMAGE TO DNA

Oxidative damage to DNA has been reported (Cadet, 1994). Several products of DNA damage have been detected in urine (Ames, 1988), as
repair enzymes excise most of the DNA lesions to release a free base or deoxynucleotide which can be excreted. Levels of 8-hydroxydeoxyguanosine a product of DNA oxidation, is taken as index of oxidative damage to DNA. Recently Dandona et al., 1996 have reported increased DNA oxidation products in monocytes from both type I and type II diabetic subjects. In vitro studies using cloned DNA fragments and reducing sugars, in the presence and absence of radical scavengers by Kaneto et al. (1994) indicate increased DNA cleavage.

**OXIDATIVE DAMAGE TO CARBOHYDRATES**

Little is known regarding the effects of free radical action on carbohydrates, although carbohydrate polymers such as hyaluronic acid may be susceptible to oxidation (Greenwald and Moy, 1980; Greenwald and Moak, 1986). The effect of diabetes on oxidative damage to carbohydrates appears to be unexplored.

**2.7 ANTIOXIDANT MECHANISMS AND DIABETES**

Life is believed to have arisen from a collection of chemicals through a progressive series of chemical reactions. Free oxygen, if present would have destroyed them by oxidation and it is generally agreed that at that time the atmosphere of the earth was a reducing one. During the course of evolution mutations occurred in certain cells enabling certain cells to liberate oxygen.

Through the course of evolution from anaerobic to aerobic life, organisms have adapted themselves and developed defense mechanisms to escape deleterious effects of oxygen. An important aspect to this is development of antioxidants. An antioxidant is any substance that, when
present at low concentrations compared with those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1991). The antioxidant defense systems in human body are extensive and consist of multiple layers that protect at different sites and against different types of ROS (Krinsky, 1988; Stocker and Frei, 1991; Retsky and Freeman, 1993).

2.7.1 ENZYMATIC ANTIOXIDANTS

An important part of the intracellular antioxidant systems are antioxidant enzymes, such as superoxide dismutase, catalase and peroxidases.

SUPEROXIDE DISMUTASE

SOD catalyzes the dismutation of $O_2^\cdot\cdot$ into $H_2O_2$. Mammalian tissues contain at least three different forms of SOD. CuZn-SOD found mainly in the cytoplasm of cells (McCord and Fridovich, 1969), Mn-SOD found mainly in the mitochondria (Halliwell and Gutteridge, 1985) and high molecular weight CuZn-SOD found in extracellular fluids. Alterations in SOD concentration has been reported in diabetes (Loven and Oberley, 1985). In some tissues of untreated diabetic rats, the activity of cytosolic form of enzyme is reported to decrease (Crouch et al., 1978, 1981; Matkovics et al., 1982) and the activity of mitochondrial form to increase (Loven, 1982). SOD activity was reported to increase in myocardium and remain unaltered in kidney in acute diabetic rats (Valkovova et al., 1993) and decrease in liver (Sekar et al., 1990; Saxena et al., 1993). Olczyk et al. (1994) found no change in erythrocyte SOD activity from diabetic patients whereas Rema et al. (1995) reported decline in SOD activity in type II diabetic patients.
CATALASE

Catalase consists of four protein subunits each with a haem group bound to its active site. Catalase is mainly located in peroxisomes and is present only in small amounts extracellularly (Halliwell and Gutteridge, 1985; Stockes and Frei, 1991). It catalyzes the conversion of $H_2O_2$ to $H_2O$ and $O_2$. The activity of catalase has been shown to decline in liver (Saxena et al., 1993) and increase in myocardium and remain unaltered in kidneys of diabetic rats (Volkovova et al., 1993). Catalase activity has been shown to increase in diabetic patients by Rema et al. (1995).

GLUTATHIONE PEROXIDASE

Glutathione peroxidase is a selenium dependent enzyme found in the cytosol and mitochondria of animal tissues. It is a tetramer consisting of four identical subunits with an atom of selenium (Flohe, 1979; Neve et al., 1985). Glutathione peroxidase like catalase, reduces $H_2O_2$ to $H_2O$ and $O_2$ and can also convert lipid hydroperoxides (LOOH) to corresponding alcohols, with the help of reducing substrate, usually glutathione. The activity of GPx has been shown to decrease in liver (Saxena et al., 1993) increase in kidney and remain unaltered in myocardium (Volkovova et al., 1993) of diabetic rats. Rema et al. (1995) reported GPx activity to increase in type II diabetic patients whereas Olczyk et al. (1994) found no alteration in erythrocyte GPx activity in diabetic patients.

GLUTATHIONE REDUCTASE

This widely distributed glycoprotein catalyzes regeneration of reduced glutathione and accounts for high GSH : GSSH ratios found in cells (Beutlar, 1984). The activity of GR has been found to be altered in various
pathological states. The erythrocyte GR activity has been found to be decreased in diabetic patients (Murakami et al., 1989). Wohaieb and Godin (1987) reported elevated heart and pancreatic GR activity in STZ-diabetic rats. The GR activity is reported to be increased (Heath et al., 1963) or unaltered (Wohaieb and Godin, 1987) in diabetic liver.

**GLUTATHIONE-S-TRANSFERASES**

The physiological functions of GSTs are diverse. They are involved in ethanol metabolism, drug metabolism and reduction of peroxides (including lipid peroxides) (Habig et al., 1974; Meister and Anderson, 1983). GSTs occur in substantial quantities in liver and other mammalian tissues. These comprise 10% of soluble protein of rat liver. Caronovale et al. (1990) have found a direct relationship between plasma insulin levels and GST in liver, renal cortex and small intestine. They found that in STZ or alloxan treated rats, when insulin-glucose levels did not change GST activity remained unaltered, however, when insulin-glucose levels were modified there were changes in GST activity in all tissues. Gupta et al. (1992) reported a compensatory increase in GST activity in erythrocytes.

**GLUCOSE-6-PHOSPHATE DEHYDROGENASE**

It is a key enzyme of hexose monophosphate shunt or pentose phosphate pathway, an alternative route for the oxidation of glucose and a major source of NADPH in most tissues. NADPH is required for the regeneration of reduced glutathione, a powerful antioxidant, and for fatty acid and cholesterol biosynthesis. It has been demonstrated practically in all animal tissues. Adipose tissue, lactating mammary glands and blood cells (Waller, 1959) are especially rich sources of the enzyme. Some researchers
have reported increased G6PD in kidney cortex in experimental diabetes (Sochor et al., 1979) and others have reported decreased G6PD activity in erythrocytes (Drel et al., 1976) and liver (Bode et al., 1993). Muggeo et al. (1993) found reduced activity of G6PD in mononuclear leukocytes from type II diabetic subjects.

2.7.2 NONENZYMATIC ANTIOXIDANTS

In addition to antioxidant enzymes, there are several small molecule antioxidants that also play an important role in antioxidant defense systems, particularly in the extracellular space, where antioxidant enzymes are absent or present in small quantities only (Frei et al., 1988; Stocker and Frei, 1991). The small molecule antioxidants can be separated into lipid soluble and water soluble antioxidants. The lipid soluble antioxidants are localized to membranes and lipoproteins, and water soluble ones are present in extracellular and intracellular fluids.

VITAMIN E

Vitamin E encompasses several closely related compounds which include \( \alpha \)-tocopherol as well as \( \beta \), \( \delta \), \( \epsilon \), and \( \nu \) tocopherols. The biological activity of tocopherols varies greatly. Structurally, vitamin E has got a phytanyl tail and chromanol ring. The different forms vary in the presence of methyl group on the chromanol ring (Ledvina, 1985).

\[
\begin{align*}
\text{HO} & \quad \text{CH} \quad \text{CH} \quad \text{CH} \\
\text{CH} & \quad \text{CH} \quad \text{CH} \\
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\( \alpha \)-tocopherol
Vitamin E is present in natural membranes and in association with lipoproteins. Studies have established that \( \alpha \)-tocopherol has close to optimal properties for trapping peroxyl radicals (Burton and Ingold, 1981; Burton et al., 1983). Vitamin E because of its hydrophobicity requires special transport mechanisms in aqueous milieu of the plasma, body fluids and cells (Bjornson et al., 1976). Unlike other fat soluble vitamins, vitamin E has no specific plasma transport protein, but rather is transported in plasma lipoproteins (Behrens et al., 1982; Ogihara et al., 1988). Vitamin E absorption requires the presence of bile acids and is secreted from intestine into chylomicrons (Bjørneboe et al., 1986, 1987) after uptake of chylomicron remnants by the liver, vitamin E is secreted into very low density lipoproteins (VLDL) and appears in plasma simultaneously in low and high density lipoproteins (LDL and HDL) respectively (Cohn et al., 1988; Traber et al., 1988). LDL uptake is an important mechanism by which tissues obtain \( \alpha \)-tocopherol (Dietschy et al., 1983; Traber and Kayden, 1984). Plasma \( \alpha \)-tocopherol concentrations is regulated by hepatic tocopherol-binding protein (Traber et al., 1990). Tocopherol binding protein salvages \( \alpha \)-tocopherol to prevent its excretion and facilitates its incorporation into VLDL for secretion into plasma. Undoubtedly plasma concentrations of tocopherols determine tissue concentrations, however, during deficiency states, vitamin E concentrations are maintained by mobilization of stored vitamin E (Traber and Kayden, 1987; Handelman et al., 1988). The recommended dietary allowance (RDA) for adults is 12-15 IU vitamin E.
The rate and mechanism of inhibition of peroxidation of lipids by tocopherols in homogenous solution are well elucidated. The α-tocopherol is a good antioxidant not only because of its high reactivity with peroxyl radical but also because of the high stability of the tocopheroxyl radical formed (Buettner, 1993).

Tocopherols scavenge both the oxygen radicals attacking from outside the membrane and the chain-carrying lipid peroxyl radicals within the membranes to terminate the free radical chain reaction (Barclay et al., 1985; Yamamoto et al., 1985; Niki, 1987; Niki et al., 1988) by donating the active, phenolic hydrogen atom. The resulting tocopherol radical may scavenge another peroxyl radical or may be reduced to regenerate the tocopherol. In membranes only vitamin E is a major chain breaking antioxidant (Esterbauer et al., 1992). All other antioxidants such as carotenoids and ubiquinols are less abundant. Previously Burton et al. (1983) have shown that vitamin E is certainly the major, and probably the only, lipid soluble, chain-breaking antioxidant in adult human blood plasma and red cells. Recently, Kontush et al. (1995) have envisaged that ubiquinone protects depletion of vitamin E and acts as a first line of defence to protect human LDL against lipid peroxidation. Based on these assumptions, antioxidant mechanisms of α-tocopherol and ubiquinone can be envisaged to operate individually or in tandem. Stoyanovsky et al. (1995) proposed regeneration of α-tocopherol by ubiquinone.
Currently three physiologically important reduction pathways are known for reduction of \( \alpha \)-tocopheroxyl radical. Packer et al. (1979) have shown that ascorbic acid reduces \( \alpha \)-tocopheroxyl radical. Kagan et al. (1990) and Mukai et al. (1990) have shown ubiquinol-10 to regenerate \( \alpha \)-tocopherol. Finally mitochondrial and microsomal electron transport processes have been demonstrated to reduce \( \alpha \)-tocopheroxyl radicals (Packer et al., 1989). Substantial evidence, exists which indicate regeneration of vitamin E by vitamin C (Niki et al., 1982, 1984; Barday et al., 1983, 1984) but whether such a mechanism is operative in vivo remains controversial (Burton et al., 1990; Frei, 1994).

Modification of chemically induced diabetes in rats by vitamin E (Slonim et al., 1983) and effect of vitamin E deficiency on pancreatic free radical scavenging systems (Asayama, 1986) suggest vitamin E to have antioxidant properties. Salonen et al. (1995) have reported low vitamin E concentration in patients with non-insulin dependent diabetes mellitus. Caballero (1993) and Paolisso et al. (1993) have reported vitamin E to improve action of insulin.

Triglyceride lowering effect of dietary vitamin E in streptozotocin induced diabetic rats (Pritchard et al., 1986) and effect of vitamin E deficiency on platelet aggregation in type I diabetic patients (Gisinger et al., 1988) and improvement in sciatic nerve dysfunction in STZ-diabetic rats with antioxidant treatment (Karasu et al., 1995) suggest beneficial role of vitamin E in diabetes.
VITAMIN C

Vitamin C is required for human health (Food and Nutrition Board, 1980). In human beings deprived of ascorbic acid, the deficiency disease scurvy develops and can be life threatening (Levine, 1986). Humans are unable to synthesize ascorbic acid, although most other mammals can synthesize it from glucose (Chatterjee, 1970). The RDA is 60 mg per day for adults. The biochemical importance of ascorbic acid in mammals is characterized extensively. Ascorbic acid is necessary for the regulation of many biochemical processes [Such as acting as a cosubstrate in procollagen (Hausmann, 1967), Catecholamine (Levine et al., 1985) and carinitine (Dunn et al., 1984) biosynthesis] and is a very effective scavenger of wide array of ROS and other oxidants (Frei, 1991; Retsky et al., 1993). The ability of the AA to scavenge free radicals involves electron transfer. AA is oxidized first to the ascorbyl radical and then to dehydroascorbate, in two one-electrons transfer steps.
The dehydroascorbate thus formed may be reduced back to ascorbate by the enzyme dehydroascorbate reductase, a reaction that also requires reduced glutathione. Alternatively, the semidehydroascorbate may be directly reduced to ascorbate by the enzyme NADH-semidehydroascorbate reductase (Bunker, 1992).

Abnormalities of ascorbic acid metabolism have been reported in experimentally-induced diabetes (Yew, 1983; Yew et al., 1989) and in diabetic patients (Jennings et al., 1987; Ali and Chakraborty, 1989). Mann in 1974 suggested that glucose and vitamin C might occupy the same transport system because of the structural similarity. Subsequent studies reported elevated glucose levels to interfere with cellular AA transport in erythrocytes (Mann and Newton, 1975). Others have observed inhibition by glucose of AA transport in vitro by human lymphocytes (Davis et al., 1983) and bovine endothelial cells (Kapeghian et al., 1983). Recently Fay et al. (1990) on their studies with cytochalasin B, a glucose transport inhibitor have shown that glucose and ascorbate share a common transport system. Chen et al. (1983) have reported hyperglycemia-induced intracellular depletion of ascorbic acid in human mononuclear leukocytes to be correlated to plasma glucose levels. Di Mattio (1992) has shown decreased ascorbic acid entry into cornea of streptozotocin-diabetic rats and guinea pigs.

Free radical mechanisms are increasingly being implicated in the pathogenesis of tissue damage in diabetes. Ascorbic acid, which may be a principal modulator of free radical activity, is shown to be consumed, presumably through free radical scavenging, thus preserving levels of other
antioxidants such as glutathione (Sinclair et al., 1992). Earlier Som et al. (1981) observed very low plasma ascorbic acid and significantly high dehydroascorbic acid irrespective of age, sex, duration of disease, type of treatment and glycaemic control. The erythrocyte reduced glutathione levels and glucose-6-phosphate dehydrogenase activities, which regulate the dehydroascorbate reduction, were similar in normal and diabetic subjects. Their experiments with diabetic rats indicated that the increased turnover of ascorbic acid was probably due to increased oxidation of ascorbate to dehydroascorbate. Banerice (1982) also found high levels of dehydroascorbic acid in diabetic patients and non-diabetic siblings of diabetic patients and suggested that in persons having an hereditary predisposition to diabetes, high blood dehydroascorbic acid levels may be used as a marker for early detection of the disease. Ali and Chakraborty (1989) and Chakraborty (1992) have reported lower ascorbic acid levels in diabetic subjects with complications as compared diabetic subjects without complications. Young et al. (1995) have reported combination of ascorbate and desferrioxamine in diabetic rats to reduce lipid peroxidation products and restore antioxidant vitamins to control values. Chronic vitamin C supplementation has been shown to have beneficial effects upon glucose and lipid metabolism in aged type II diabetic patients (Paolisso et al., 1995). Mekinova et al. (1995) have reported improvement of antioxidative status of kidneys of rats with streptozotocin-induced diabetes on intake of exogenous vitamins C, E and beta-carotene. Ting et al. (1996) have reported that supplemented vitamin C in patients with non-insulin-dependent diabetes improves endothelium-dependent vasodilation.
GLUTATHIONE

This ubiquitous tripeptide, l-γ-glutamyl-L-cysteinyl glycine, usually most prevalent intracellular thiol is known to function directly or indirectly in many important biological phenomena, including the synthesis of proteins and DNA, transport, enzyme activity, metabolism and protection of cells (Meister and Anderson, 1983). Glutathione also protects cell against the effects of free radicals and of reactive oxygen species.

Glutathione is synthesized intracellularly by the subsequent action of enzyme γ-glutamylcysteine synthetase and glutathione synthetase on precursor amino acids. Intracellular GSH is converted to GSSG by GSH peroxidase, which catalyzes the reduction of $H_2O_2$ and other peroxides (Chance et al., 1979) and thus is important for protection of cells against oxidative damage (Flohe, 1979; Tate et al., 1979). Glutathione acts as a substrate for glutathione-S-transferases (detoxification) (Boyland and Chasseaud, 1969) and γ-glutamyl-transpeptidase (transport). Reduction of GSSG to GSH (Griffith and Meister, 1979; Meister, 1981) is catalysed by widely distributed enzyme glutathione reductase. Repletion of intracellular GSH takes place by the enzymatic degradation of external GSH, uptake of the products, and intracellular resynthesis of GSH (Jensen and Meister, 1983).

Bono et al. (1987) reported a decrease in the concentration of GSH in erythrocytes from diabetic patients. Costagliola (1990) found higher GSSG levels in plasma from diabetic patients than those from controls and attributed it to reduced activity of G6PD (Source of NADPH) and activation of polyol pathway (uses NADPH) which reduces levels of NADPH for
glutathione reductase enzyme. Earlier, Murkami et al. (1989) proposed that decrease in levels of GSH in diabetics might be brought about by the decrease in the synthesizing step of GSH and an increase in levels of GSSG to the decrease in transport of GSSG and decrease in the regenerating activity of glutathione reductase. Saxena et al. (1993) found decreased GSH levels in alloxan diabetic rat liver in the presence of normal GSSG and GR activity.

**URIC ACID**

Uric acid has been reported to be a moderately effective antioxidant (Wayner et al., 1987) and the much increased level of urates in primates, in comparison with other animals, has been described in response to a low level of ascorbate (Ames et al., 1981). A double gene mutation was proposed wherein the loss of ascorbate synthetase was accompanied by a loss of uricase (accumulation of uric acid). Uric acid is a metabolic product of purine metabolism and serves as a potent antioxidant by means of radical scavenging and reducing activities (Smith and Lawing, 1983; Wilson et al., 1985). Urate not only behaves as a radical scavenger but also stabilizes ascorbic acid in biological fluids by inhibiting iron-catalysed oxidation of ascorbate (Hatch and Sevanian, 1984; Davies et al., 1986; Sevanian et al., 1991).

In addition to the above discussed antioxidants, a number of minerals (selenium, copper, zinc, mangenese) vitamin A, bilirubin and plasma proteins lie functionally at the heart of antioxidant protective mechanisms.