INTRODUCTION
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[1] DIABETES MELLITUS

Diabetes mellitus is commonly found all over the world and affects about 20-30 million people. Prior to the discovery of insulin, diabetes was thought to be a serious disease. Now diabetes can be controlled by different antidiabetic agents which lower different degrees of severity of the disease. Main causes of diabetes are genetic, obesity, and virus induced. Research is going on all over the world to find different means to control this metabolic disorder.

The major symptoms of diabetes mellitus are hyperglycemia, glucosuria, polydipsia, polyuria ketoacidosis and many other complications. The diagnosis of diabetes mellitus is done by measuring hyperglycemia, glucose tolerance test and glycosylated hemoglobin, central features and major causes of several other complications in diabetes mellitus (Rowland and Bellish, 1989). Various definitions and classifications of diabetes mellitus have been proposed. The WHO report (1985 : 8) recommended that the following three major clinical subclasses of diabetes should be recognized:

a) Insulin Dependent Diabetes Mellitus (IDDM) or Type I.
b) Non-Insulin Dependent Diabetes Mellitus (NIDDM) or Type II.
c) Gestational Diabetes Mellitus (GDM).

Since alloxan induced diabetes is similar to Type I diabetes, the emphasis in this thesis is on Type I diabetes.

(A) Insulin Dependent Diabetes Mellitus or Type I Diabetes

Diabetes mellitus represents a group of syndromes, all characterized by abnormal glucose tolerance due to impaired insulin secretion and function. IDDM afflicts only a relatively small proportion of all diabetic patients. IDDM
predominantly develops in children and young adults, and it sometimes leads to an increased risk of premature death (Reaven, 1988).

In IDDM, the β-cells of pancreas get destroyed resulting in relative or absolute insulin deficiency. The loss of insulin secretion appears as a result of an autoimmune process, directed at the insulin producing beta cells of the pancreas, which ultimately leads to their destruction and the development of diabetes (Brownlee and Cerami, 1981). This autoimmune process itself is probably triggered by some environmental factors in genetically susceptible individuals. The exact nature of these factors is unknown, but animal studies suggest viruses, nutritional factors or toxins (like streptozotocin, alloxan, dithiozone, pesticides, industrial toxins and drugs) as likely candidates (Leslie, 1993) (Figure A).

Alloxan and streptozotocin are among the most commonly used β-cell cytotoxin to induce experimental diabetes. Varying severity of diabetes could be obtained by their single injection in rats or multiple injections in mice (Junod et al., 1969). For the most part, the diabetes induced by these two chemicals is a reasonable analogue to the clinical conditions characterized by hyperglycemia, glucosuria, polydipsia, polyuria, loss in body weight, besides polyphagia, hyperlipidemia, ketonuria and acidosis. These metabolic alterations are shown to be reversed by exogenous insulin treatment which suggests that there is a minimum of non-specific damage to other organs by these two agents (Rowland and Bellish, 1989).

The possible mechanism for the β-cell destruction by these diabetogenic 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 ribosynthase, an enzyme depleting NADP⁺ in β-cells, and (iii) inhibition of active calcium transport and calmodulin
Modulated by HLA and other genetic factors

Environmental factors (viruses, toxins)

Antigenic perturbation of β cells

Immune response

T lymphocytes

B lymphocytes

Cytotoxic lymphocytes

Islet cell antibodies

Islet cell surface antibodies

β-cell damage

Diabetes

FIGURE A. Possible mechanism of beta cells destruction in diabetes mellitus.
dependent protein kinase activity (Rowland and Bellish, 1989 and Yoon et al., 1987). Many other compounds are experimentally used to induce diabetes in animal model including Chlorozotocin, vacor and hydroprolepatadine (Leslie et al., 1993).

[2] COMPLICATIONS OF DIABETES

Nearly 75 years have passed since Banting and Best (1922) made the breakthrough in the treatment of Insulin Dependent Diabetes Mellitus. Prior to 1922, diabetic children had the life expectancy of 2-3 years and 90 per cent died from ketoacidosis (Joslin, 1928). However, the introduction of insulin unmasked the second consequences of diabetes - the late diabetic complications. All complications are thought to be preceded by hyperglycemia. These complications can be studied by categorizing them as (i) Specific microvascular complications, including thickening of capillary basement membrane, retinopathy and nephropathy (ii) Macrovascular disease i.e. accelerated atherosclerosis, and (iii) a variety of other complications including neuropathy, complicated pregnancy and an increased tendency to infection (Ronald, 1994).

Diabetic nephropathy is relatively a common and very serious microvascular complication. Diabetes mellitus is indicated by the presence of persistent proteinuria in diabetic patients with concomitant retinopathy and elevated blood pressure, but without urinary tract infection, other renal diseases or heart failure. It has been shown that the control level of hyperglycemia during the first 15 years of diabetes has positive relation to the risk of persistent proteinuria. However, the incidence of nephropathy rapidly declines after 15 years of diabetes (Krolewski et al., 1985). The persistent proteinuria leads to the onset of the end stage renal disease or failure. The development of proteinuria and the end stage of renal disease depends on the age
and duration of diabetes occurrence reported in Joint working party on diabetic renal failure, 1985.

The other symptoms of nephropathy are (i) an increase in Glomerular Filtration Rate (GFR) and Renal Plasma Flow (RPF), (2) Microalbuminuria and (3) Arterial blood pressure, apart from retinopathy and cardiovascular diseases (Scandling and Myers, 1992). The GFR and RPF are elevated on an average by 20-40%. This is related to the control of blood glucose level. The increase in GFR and RPF is accompanied by an approximate 20% increase in kidney size (Osterby, 1992). The level of albumin increases in diabetes, which is an early indication of proteinuria and the onset of microalbuminuria, depends on the glycemic control in diabetic animals (Viberti et al., 1982). The arterial pressure is almost invariably elevated in diabetic patients with established nephropathy. The changes in all these physicochemical processes lead to the end stage renal failure, of which Ureamia is a common characteristic and takes maximum toll in diabetes (Tarsio et al., 1988).

Apart from nephropathy; retinopathy, neuropathy and cardiovascular diseases are common complications in diabetes. In retinopathy, retinal capillary damage, edema and hemorrhage makes blindness 25 times more common among diabetic patients. Opacity of the ocular lens and early cataract occurrences are more frequent in patients with diabetes (Brownlee and Cerami, 1981). In neuropathy, axonal dwindling and segmental de-myelination are associated with a very high prevalence of motor, sensory and autonomic dysfunction, including impotency which effect 40% of diabetic males (Brownlee and Cerami, et al., 1981). Diabetes is also a risk factor for atherogenesis and arterial diseases. Increased atheromata in medium and large arteries makes coronary artery disease and stroke twice as common and symptomatic peripheral arterial disease 4-5 times more common among diabetic patients (Taylor and Agius, 1988). Accelerated large vessel disease in diabetes is
due to synergistic pathological mechanisms involving hyperlipidemia, altered platelet behavior and abnormalities in arterial wall function. Elevated levels of very low density lipoproteins (VLDL), triglycerides, low density lipoprotein (LDL) and cholesterol have been observed in all major classes of diabetic patients (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. All the complications are due to insulin deficiency and its metabolic consequences are the primary etiological events in the pathogenesis of diabetic complications.

[3] HYPERGLYCEMIA

The major characteristics of diabetes is hyperglycemia, which results from (Hue, 1987):

1. decreased entry of glucose into cells,
2. decreased utilization of glucose by insulin dependent tissues and
3. increased production of glucose (gluconeogenesis) by liver.

The concentration of glucose in blood depends on its production and utilization in normal as well as in diabetic conditions by different tissues. The major site for glucose production is liver and its level is regulated by the ratio of insulin and glucagon. In diabetes, the ratio of insulin/glucagon decreases, which leads to an increase in both glycogenolysis and gluconeogenesis (Cherrington et al., 1987) and thereby in blood glucose level. Apart from the hormone level, substrate levels which control gluconeogenesis also increase in diabetes. Lipolysis is increased in diabetes and contributes to the increase in the level of non-esterified fatty acid (NEFA) (Randle et al., 1963), which also stimulates gluconeogenesis in the liver.
In the prolonged state of diabetes, the illness is precipitated as diabetic ketoacidosis and ketone bodies are utilized by non-muscle tissues such as brain, intestine and kidneys and inhibits glucose uptake and this also contributes to hyperglycemia in diabetes (Johnston et al., 1984).

All these alterations in metabolic processes lead to increased plasma glucose level (more than 180 mg/dl). This elevated level of plasma glucose crosses the threshold value of renal tubular reabsorption leading to glucosuria. The urine volume increases owing to osmotic diuresis and coincident obligatory water loss (polyuria), and this in turn leads to dehydration (hyperosmolarity) and increased thirst (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 inspite of increased appetite. Thus polyuria, polydipsia and weight loss in spite of adequate calorific intake are the major symptoms of insulin deficiency (Hunt et al., 1990).

**Glycation and Diabetic Complications**

Glycation comprises the spontaneous non-enzymatic reaction of glucose with proteins. In diabetes, elevated levels of glucose are present not only in plasma, but also in interstitial fluid and within cells where glucose entry is insulin independent. As a result, glycation of a wide variety of proteins is enhanced, and this has led to a hypothesis to explain the pathogenesis of diabetic complications (Strowig and Raskin, 1992).

The glycation of proteins may be regarded as a two-step process. In the initial step, covalent bonds are formed between aldehyde groups of glucose molecule and reactive amino groups of proteins. The second step is a complex process and leads to the formation of a stable end product. The reactions are collectively termed as the Browning Reactions (Lyons et al., 1992).
It has been suggested that the processes of glycation and free radical oxidation are closely linked. It appears that the products of combined glycation and oxidation (glycoxidation) may be important determinants for the development of diabetic complications. This knowledge of glycation and oxidative state of the body for the management of diabetic complications is important (Cerami et al., 1987).

Glycoxidation may have its greatest pathogenic potential in long-lived proteins as compared to short-lived proteins. Evidence is accumulating that glycated, oxidized and glycoxidised lipoproteins may contribute to the development of both macro and microvascular diabetic complications (Lyons, 1991a). Plasma proteins are exposed to elevated glucose level in diabetes and get glycosylated. This process is important in case of lipoproteins which are responsible for atherosclerosis. Of the major classes of lipoproteins (VLDL, LDL, HDL), LDL is the most studied class and it has only one apoprotein domain (Sasaki and Cottam, 1982; Pugazhenthii et al., 1991).

The glycosylated LDLs are not recognized by their receptors which are responsible for their degradation, and this contributes to the elevation of plasma-cholesterol levels in diabetes. The increased level of glycated LDL may influence the platelet aggregation and also contributes to the initiation of retinopathy by injuring endothelial cells, thereby causing capillary damage (Cushing et al., 1990).

The long lived structural proteins like lens crystallin and collagen are adversely affected by glycation and glycoxidation. The cataract formation, a feature of ageing, tends to occur early in diabetic than non diabetic patients. It was suggested that the glucose barrier in the lens gets destroyed in diabetes. The glucose level in lens increased and lens crystallin FL (Fructose-lysine) also increases which participates in cataract formation (Lyons et al., 1991; Makahama et al., 1993).
Collagen, a another structural protein, as people age, become increasingly insoluble, thermally stable, and resistant to enzyme attack (Hamlin and Kohn, 1971). These changes in collagen get accelerated in diabetic conditions. The collagen confined in glomerular basement membrane gets glycosylated and is thought to be the major cause of microalbuminuria, which is an important indicator of nephropathy (Cohen et al., 1980; Schneider and Kohn, 1980).

**AMINO ACID METABOLISM IN DIABETES**

Diabetes mellitus is characterized by derangements in the metabolism, not only of glucose and fat but also of protein and amino acids (Atchley et al., 1953). However, protein has always received less attention than fat and glucose, both in terms of alterations in its metabolism and in its nutritional implications. Although hyperglycemia and its consequences have always been the hallmark of the disease and plasma glucose the main index of diabetic control, altered protein metabolism was recognized even in the pre-insulin era because of the severe muscle and other protein depletion that occurred, even with an apparently adequate protein intake (Zeller, 1991). There are clear experimental evidences that the diabetic state affects protein metabolism and that insulin is an important regulator. Data obtained from nitrogen (N) balance studies have shown that insulin deficiency is associated with an increase in urinary nitrogen loss, which is reversed by insulin therapy (Kanter, 1976).

The increased level of protein catabolism in diabetes is achieved by the activation of many enzymes (Jefferson et al., 1974) (Figure B). Aspartate aminotransferase, alanine aminotransferase (AlaAT) and glutamate dehydrogenase (GLDH) are the important enzymes of amino acid metabolism and were chosen to study in diabetes. Aspartate aminotransferase (AspAT) is an important enzyme of
amino acid metabolism which channeled the amino group, from aspartate to glutamate. Alanine aminotransferase transfers the amino group from alanine to glutamate and it is also an important enzyme of Glucose-Alanine cycle. Aminotransferase accumulate amino groups in form of glutamate which further metabolise with glutamate dehydrogenase (GLDH) producing ammonium ions. The free ammonium ions are excreted in the form of Urea. Arginase, an enzyme of Urea cycle plays an important role in excretion of Urea in liver and kidney (Table A). Arginase also participates in the synthesis of polyamines and arginine in the kidney.

A. GLUTAMATE DEHYDROGENASE

Introduction

Glutamate dehydrogenase (GLDH) (EC 1.4.1.3) is a mitochondrial enzyme that catalyses the oxidative deamination of glutamate to yield 2-oxoglutarate and an ammonium ion using NAD+/ or NADP+ as cofactors (Lai et al., 1975). The enzyme links the L-amino acids with the Krebs cycle, which provides a major pathway for the metabolic interconversion of \( \alpha \)-amino acids and \( \alpha \)-keto acids. Substantial amount of glutamate dehydrogenase activity is known to be present in different mammalian organs such as liver, brain, and leukocytes (Eisenberg et al., 1976; Kazmi et al., 1985 and Sochor et al., 1985).

The purification and properties of liver and brain GLDH, and its interaction with metabolic effectors has been extensively studied (Schoolwerth et al., 1978 and Colon et al., 1986). The rat brain contains two different forms of GLDH which differ significantly in their susceptibility to GTP inhibitors in the presence of 1 mM ADP (Colon et al., 1986). Glutamate dehydrogenase uses both NAD and NADP with comparable facility, and shows negative co-operativity in its catalytically active form (Alex and Bell, 1980). Dhanakoti et al. (1983) showed that bicarbonate
Table A:- Activity of the alanine aminotransferase, aspartate aminotransferase, arginase and glutamate dehydrogenase in cytosolic (Cyto) and mitochondrial (Mito) fractions of rat kidney and liver.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Tissue</th>
<th>Fractions</th>
<th>Activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine aminotransferase</td>
<td>Kidney</td>
<td>Cyto</td>
<td>2.48</td>
<td>Sochor et. al. 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mito</td>
<td>2.77</td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>Liver</td>
<td>Cyto</td>
<td>8.60</td>
<td>Sochor et. al. 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mito</td>
<td>3.20</td>
<td></td>
</tr>
<tr>
<td>Arginase</td>
<td>Kidney</td>
<td>Cyto</td>
<td>24.45</td>
<td>Sochor et. al. 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mito</td>
<td>12.22</td>
<td></td>
</tr>
<tr>
<td>Glutamate Dehydrogenase</td>
<td>Kidney</td>
<td>Cyto</td>
<td>27.42</td>
<td>Dhanakoti et. al. 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mit</td>
<td>48.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Cyto</td>
<td>216.8</td>
<td>Iwase et. al. 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mito</td>
<td>23.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>Mito</td>
<td>6.20</td>
<td>Sochor et. al. 1985</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Mito</td>
<td>8.84</td>
<td>Sochor et. al. 1985</td>
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</table>

Units are expressed as Units/gm fresh tissue weight.
and ADP exert stimulatory effects on the ammonia formation via glutamate dehydrogenase in kidney.

The free energy change (at pH 7.0) for NAD⁺ and NADP⁺ linked glutamate dehydrogenase reactions is large (16.5 and 16.7 KCal respectively), suggesting that the glutamate dehydrogenase reaction might be poised for net reductive amination of α-ketoglutarate rather than for oxidative deamination, but experimental evidences indicate that the glutamate dehydrogenase is not a major route for glutamate formation (Newsholme and Start, 1973). McGiven and Chappell (1975) proposed that the purine nucleotide cycle may be a significant pathway of hepatic ammonia production. It was also experimentally shown that the purine nucleotide cycle also plays a significant role in ammonia production in kidney. It acts as an alternative source of ammonia in kidney (Alex and Bell, 1980).

Distribution

Glutamate dehydrogenase exists in four isoforms, distributed widely in different tissues. Liver is the richest source of this enzyme, followed by kidney and brain. Heart has a comparatively low activity of this enzyme (Eisenberg et al., 1976). The brain GLDH is known to be a hexamer composed of six identical subunits of 50 Kd each (Rajas and Rousset, 1993).

Glutamate dehydrogenase has been held as a mitochondrial marker enzyme. However, its occurrence in purified nuclear fractions form in ox liver has also been shown. Submitochondrial studies have shown that the enzyme is distributed almost equally between matrix and the inner membrane (Landriscina et al., 1970). The specific activity of both NAD and NADP⁺ linked enzyme has been found to be higher in synaptic mitochondria than in the free mitochondria (Lai et al., 1975).

Compartmentation studies in kidney have shown that glutamate dehydrogenase is preferentially localized in all the three segments of the proximal
tubule, intermediate in distal convoluted tubule, cortical and medullary thick ascending limbs, connecting tubules of cortical and medullary collecting ducts and in medullary thin limbs. During chronic acidosis GLDH activity is increased three fold, but only within the S₁ and S₂ segments of the proximal tubule (Seyer-Hansen, 1983). Blotting analysis of RNA from human, monkey, rabbit and rat showed that glutamate dehydrogenase mRNA is present in various amount in different tissues e.g. liver, lungs, pancreas, heart tissue, adrenal and kidney (Mavrothalassitis et al., 1988).

Substrate specificity and regulation

The catalytic activity of glutamate dehydrogenase is not restricted to glutamate and 2-oxoglutarate as substrate. The enzyme can catalyze the interconversion of a few other α-amino acids and their α-keto analogues, though with less reactivity (Smith et al., 1975). Alanine and glutamine have been reported as two alternative substrates for glutamate dehydrogenase reactions (Blumenthal et al., 1975). Glutamate dehydrogenase provides a route for the incorporation of nitrogen into organic compounds and therefore, maintains a link between carbohydrate and amino acid metabolism.

Considerable evidence suggests that glutamate dehydrogenase from various tissues is regulated either by allosteric effectors or by induction-repression effects. The regulatory factors can be classified into three groups:

a. purine nucleosides, di or tri-phosphates (ADP, GTP).

b. substrate and coenzymes (product inhibition).

c. certain hormones, lipids and leucine.

Glutamate dehydrogenase tends to form highly aggregate polymers with high activity towards glutamate, in the presence of ADP, and also ATP in certain cases.
The enzyme usually disaggregates with GTP and NAD(P)H (Fabien and Strmecki, 1969). Furthermore, liver glutamate dehydrogenase activity is dependent on intramitochondrial ATP/ADP ratio, the activity increases with decreasing ratio (Lenartowicz, 1979).

Leucine is a potent activator of the glutamate dehydrogenase activity of isolated mitochondria from liver (McGiven and Chappel, 1975). The Vmax in the direction of glutamate formation was increased ten fold, that in the direction of glutamate deamination two to three fold. Sodium ions may act as a product inhibitor for glutamate dehydrogenase in the direction of glutamate synthesis (Engel and Chen, 1975). Wanders et al. (1983) have shown that bicarbonate exerts stimulatory effects on the rate of ammonia formation via glutamate dehydrogenase in liver.

A variety of compounds have been shown to influence the activity of glutamate dehydrogenase through the process of induction/repression. Wolff (1962) reported inhibition of glutamate dehydrogenase by thyroxine, and concluded that the site of interaction was the allosteric site for the coenzyme. An increase in the activity of glutamate dehydrogenase has been reported during high protein diet and with certain glucocorticoid, while a decrease in the enzyme activity has been observed during high glucose diets and with adrenalectomy (Schimke and Doyle, 1970; Schepartz, 1973). The effects of insulin and thyroid hormones on the activity of liver and brain glutamate dehydrogenase (NAD⁺) have been reported earlier (Baquer et al., 1976; Ali et al., 1980 and Sochor et al., 1985).

Earlier reports have indicated an inhibition of glutamate dehydrogenase by norepinephrine, ACTH and serotonin (Kaur and Kanungo, 1970), and an activation by epinephrine and Dihydroxyphenylalanine (Julliard and Gautheron, 1972).

The liver GLDH isoprotein are quite complex and may be the products of different genes (Mavrothalassitis et al., 1988) and/or different mRNA species.
generated by alternative splicing. They could also arise by post-translation modifications.

AMINOTRANSFERASES

Aminotransferase are enzymes, which catalyse the transfer of an amino group from one molecule, generally an amino acid, to another, preferably a keto acid without the participation of ammonia. These enzymes occupy a significant place in amino acid metabolism, in the process of catabolism and also in the synthesis of non-essential amino acids, through the trans-amination of glutamic acid. These enzymes also provide mechanism for the conversion of amino acids like alanine, glutamate, aspartate to pyruvate, 2-oxoglutarate, oxaloacetate respectively linking the amino acid metabolism with that of carbohydrates (Benuck and Lajtha, 1974). The two most important aminotransferases in liver, kidney and other tissues are aspartate aminotransferase and alanine aminotransferase.

B. ASPARTATE AMINOTRANSFERASE

Aspartate aminotransferase (AspAT, EC 2.6.1.1) is an ubiquitous pyridoxal phosphate-dependent enzyme, present in microorganisms, plants and animals. Aspartate aminotransferase catalyses the interconversion of aspartate and 2-oxoglutarate with glutamate and oxaloacetate. In mammalian tissues, two isoenzymic forms have been identified, mitochondrial aspartate aminotransferase (mAspAT) and cytosolic aspartate aminotransferase (cAspAT). These isoenzymes play an important role in amino acid synthesis and degradation, as well as in the link between the Urea and tricarboxylic acid cycles. Moreover, the AspAT isoenzyme, coupled to the malate dehydrogenase isoenzyme, catalyzes the cytoplasmic and mitochondrial steps of the "malate-aspartate shuttle", which leads
to a net transport of reducing equivalents from the cytosol to the mitochondria (Krebs, 1967). Both isoenzymes are homodimers of 44,000 daltons each (Morino et al., 1970). Comparison of protein sequences of both isoenzyme from different species indicate an 80% identity. In contrast, the identity between mitochondrial and cytosolic AspAT protein sequences in the same species is approximately 50% (Garlatti et al., 1994).

The cytosolic AspAT is one of many enzymes involved in gluconeogenesis whereas mitochondrial AspAT is not directly involved in gluconeogenesis, which probably makes it differentially regulated in response to glucocorticoid insulin and cAMP (Feilleux-Duche et al., 1994).

Most of the available reports on aspartate aminotransferase are confined to pig heart (Sallach and Fahien, 1969; Braunstein, 1973). The aspartate aminotransferase also has been purified and characterized in other tissues like kidney (Horio et al., 1986), liver (Huynh et al., 1980) and heart (Morino et al., 1970). The enzyme from these tissues uses pyridoxal phosphate, with two molecules of coenzyme tightly bound to one molecule of the enzyme.

Distribution

Heart muscle has the highest activity of this enzyme, followed by liver and kidney (Hertzfeld and Greengard, 1971). The specific activity of the kidney enzyme is nearly 50% of the activity in liver (Knox, 1972). In liver and kidney, AspAT appears to be predominantly localized in mitochondria (80-90%), whereas heart and muscle have a larger amount of soluble enzyme (Salganicoff and De Robertis, 1965; Hertzfeld and Greengard, 1971). Characterization of submitochondrial forms has revealed its association with the inner mitochondrial membrane (Salganicoff and De Robertis, 1965; Orlicky et al., 1981). Aspartate aminotransferase is also present in mitochondrial preparations from synaptosomes (Lai et al., 1975).
The aspartate aminotransferase activity was detected in all parts of the nephron but mostly in the distal convoluted tubule and the thick ascending limb (Feilleux-Duche, 1994a). The gluconeogenic activity is localized in the proximal tubule of the kidney cortex.

Isoenzyme and Multiple Subforms

The presence of two distinct isoenzymes of aspartate aminotransferase, namely cytosolic (anionic) and mitochondrial (cationic), has been substantiated by extensive reports (for review, Please see Braunstein, 1973; Benuck and Lajtha, 1974). The isoenzymes differ in their electrophoretic mobility and other physicochemical as well as functional characteristics as given below:-

(a) The cytosolic isoenzyme has greater affinity for the keto acid substrate, whereas the mitochondrial isoenzyme prefers the amino acid substrate.

(b) Mitochondrial isoenzyme is much more labile to heat treatment than its soluble counterpart (Magee and Philips, 1971; Huynh et al., 1980).

(c) Inorganic phosphate is inhibitory only towards the mitochondrial isoenzyme (Magee and Philips, 1971).

(d) Brain mitochondrial enzyme has much higher amide nitrogen content than the cytoplasmic form, partially accounting for the former's cationic properties (Magee and Philips, 1971).

Besides the differences between the two isoenzyme, kidney enzyme differs from those of other sources, like liver, in induction by different conditions like high protein diet situation and diabetes. Kidney enzyme has a lower Km for 2-oxoglutarate (high substrate affinity) than the liver and heart enzymes (Krista and Fonda, 1973).
**Substrate Specificity and Inhibitors**

Glutamate and aspartate are the major amino acid substrate for aspartate aminotransferase. However, the enzyme can catalyse the transamination of some other amino acids, like alanine, methionine and phenylalanine, though at a lower rate (Benuck and Lajtha, 1974 and Kazmi et al., 1985a).

Glyceraldehyde-3-phosphate is a non-competitive inhibitor of brain and liver enzymes (Kopelvich et al., 1970; Magee and Philips, 1971). This inhibition raises the possibility of a rather long-range negative feedback control by a glycolytic metabolite. Amino-oxyacetic acid and cycloserine are two other well documented inhibitors for aspartate aminotransferase (John and Charteris, 1978; Janski and Cornell, 1981).

C. **ALANINE AMINOTRANSFERASE**

Alanine aminotransferase catalyses the inter-conversion of alanine and \( \alpha \)-ketoglutarate with glutamate and pyruvate. Liver exhibits the highest activity of this enzyme, muscle and heart have approximately 25% as much, whereas kidney contains about 5-10% of the liver enzyme activity in rat (Table A)(DeRosa and Swick, 1975). Physiological studies with liver enzyme indicate that the activity of alanine aminotransferase is under different hormonal and nutritional control, and that it plays a central role in the regulation of gluconeogenesis (Knox and Greengard, 1965; Swick et al., 1965; Fridrichs and Schoner, 1974).

**Distribution**

Extensive physicochemical and kinetic studies with purified kidney and liver enzymes have shown the existence of two forms of alanine aminotransferase, one present in mitochondria and the other in cytosol (Gatehouse et al., 1967; Sair and Genkins, 1967; DeRosa and Swick, 1975; Ruscak et al., 1982). Apart from the
variation, in the species AlaAT also showed a variation in different tissues of the same species. Per cent distribution of mitochondrial and cytosolic isoforms varies from species to species. In rat liver, the mitochondrial form is 25% of cytosolic one, whereas in chicken liver almost all the activity is present in mitochondrial fraction (Orlicky and Ruscak, 1977).

About 90 per cent of the total kidney alanine aminotransferase activity is localized in cytosolic fraction, whereas mitochondria has 10 % (Orlicky et al., 1981). The enzyme activity in the mitochondria is chiefly associated with the matrix, together with a substantial part of glutamate dehydrogenase which produced NADH (Orlicky et al., 1981).

Isoenzymes

The cytosolic and mitochondrial isoforms of alanine aminotransferase differ in their physicochemical and kinetic properties. Both the isoenzymes have different pH optima, pI values and thermostability constants (Ruscak et al., 1982). Further, the cytosolic isoenzyme has higher Km value for alanine than the mitochondrial form (Ruscak et al., 1982). The two isoenzymes also differ with respect to inhibition by anions. The mitochondrial isoenzyme is inhibited by low concentration of Cl⁻ and phosphate ions, unlike the soluble form (Orlicky and Ruscak, 1977). The cytosolic isoenzyme appears to be very specific for 2-oxoglutarate and alanine as substrate.

On the basis of the employed substrate concentrations (Km values) and the stationary level of metabolites entering the aminotransferase reactions, it has been proposed that in vivo, soluble isoenzyme is chiefly associated with the conversion of pyruvate to alanine, whereas mitochondrial enzyme catalyses the formation of pyruvate from alanine (DeRosa and Swick, 1975; Dieterle et al., 1978; Orlicky and Ruscak, 1977).
The occurrence of mitochondrial alanine aminotransferase in matrix, together with glutamate dehydrogenase, can be related to ammonia production and NADH reoxidation by joint reactions (Fabien et al., 1971). Furthermore, the alanine and 2-oxoglutarate produced from these joint reactions, may take part in the translocation of glutamate equivalents from mitochondria to extra mitochondrial space (Brand and Chappell, 1974; Ruscak et al., 1982).

Interaction of Coenzymes with Aminotransferase

Alanine aminotransferase contain pyridoxal phosphate as coenzyme, which is bound in Schiff base linkage to an ε-amino group of lysine residue (Hughes et al., 1962). Further studies have shown that phosphate moiety and pyridine ring may also contribute to the coenzyme binding (Fonda, 1971). The liver alanine aminotransferase showed considerable decrease in the activity when animals are maintained on pyridoxine deficient diet (Okada and Hirose, 1979).

Regulation of Aminotransferase Activity

Carbohydrate metabolism is closely related to the synthesis and breakdown of glutamate, the major routes for which are aminotransferase catalyzed reactions. Under conditions of restricted glucose metabolism, associated with gluconeogenesis and ureogenesis, the aminotransferases show higher activities to account for the catabolism of amino acids. Aminotransferases are increased in conditions such as fasting, diabetes, high protein diet and by administration of glucocorticoid and glucagon (Rosen and Nichol, 1964; Knox and Greengard, 1965; Shrago and Lardy, 1966).

Interaction between Glutamate Dehydrogenase and Aspartate/Alanine Aminotransferase

The glutamate dehydrogenase (GLDH) and the two aminotransferase (AspAT and AlaAT), included in the present studies, are known for their close functional
relationship in the onset and subsequent regulation of the metabolic pathways at the interface of carbohydrate and amino acid metabolism (Figure B). Several lines of evidences suggest that in liver, these three enzymes interact among themselves \textit{in vitro}, with a very strong possibility of the occurrence of such complexes \textit{in vivo} as well, as the enzymes concentrations required to obtain these interactions are almost similar or even lower than their physiological levels (Fahien \textit{et al.}, 1971; Bryce \textit{et al.}, 1976; Fahien \textit{et al.}, 1979; Manley \textit{et al.}, 1980; Salerno \textit{et al.}, 1982; Fahien and Kmiotek, 1983). In the presence of NADH, ammonium ions and keto acid, glutamate dehydrogenase and aminotransferase can combine to catalyze a sequence of reactions, leading to an enhanced rate of dehydrogenation and transamination events (Fahien \textit{et al.}, 1971). In these reactions, the aminotransferase does not function as catalyst but delivers the actual substrate, pyridoxal 5'-phosphate, to the active site of glutamate dehydrogenase (Fahien \textit{et al.}, 1971). Salerno \textit{et. al.} (1982) have also shown that if oxaloacetate is added to the system, it binds to and reacts with pyrodoxal-amine form of aminotransferase, presumably arising from glutamate dehydrogenase reaction.

The interaction of glutamate dehydrogenase and alanine aminotransferase could go in the following way (Fahien \textit{et al.}, 1971):

GLDH

\[ \text{NAD}^+ + \text{Glutamate} \rightleftharpoons \text{NADH} + 2\text{-oxoglutarate} + \text{NH}_4^+ \]

AlaAT

\[ \text{Glutamate} + \text{Pyruvate} \rightleftharpoons 2\text{-oxoglutarate} + \text{Alanine} \]

\[ \text{NADH} + \text{NH}_4^+ + \text{Pyruvate} \rightleftharpoons \text{NAD}^+ + \text{Alanine} \]
Figure B: Interrelationship between Tricarboxylic Acid and Urea Cycle

AS Lyase = Argininosuccinate lyase; AS Synthase = Argininosuccinate synthase
OTC = Ornithine transcarbamoylase; CPS-I = Carbamoyl phosphate synthase-I
2-OG = 2-Oxoglutarate; GLDH = Glutamate dehydrogenase
Such interactions among glutamate dehydrogenase, alanine aminotransferase and pyruvate could also be anticipated in kidney where the level of alanine aminotransferase is quite low and alanine metabolism is quite important. Churchich (1978) has presented two strong evidences in favour of such enzyme-enzyme interaction in kidney mitochondria:

(i) The conversion of pyridoxal form of aspartate amino transferase to pyridoxalamine form occurs when NADH is oxidized to NAD$^+$ in presence of glutamate dehydrogenase.

(ii) The rotational correlation time of aminotransferase is increased in presence of glutamate dehydrogenase.

Since very low concentration of glutamate dehydrogenase is required to achieve complete protection of malate dehydrogenase against Palmitoyl CoA (Fahien et al., 1979), the possibility of the occurrence of such a complex in other tissues can not be ruled out.

D. ARGINASE

Arginase (L-arginine amidohydrolase, E.C. 3.5.3.1) is the fifth enzyme of Urea cycle. It catalyses the conversion of arginine into Urea and ornithine. Ornithine re-enters the cycle and the Urea formed is eliminated as an excretory product. All the organisms capable of synthesizing arginine may possess the enzymes of Urea cycle but arginase is specific to those capable of synthesizing Urea (Ratner, 1973). It was first detected in mammalian liver and later VanElsen et al., 1975 reported the presence of arginase in extra-hepatic tissue like kidney and brain. Presence of arginase in the organs lacking Urea cycle enzymes reflects a selective repression of enzymes during their differentiation and development (Mora et al., 1965). Inspite of arginine's sole contribution to ornithine formation which, apart
from Urea cycle, takes part in the synthesis of putrescine, spermine and spermidine (Dhanakoti et al., 1983).

Arginine is known to participate in the arginine synthesis in kidney and as a nutrient to chicken, insects and cells in tissue culture. Recently Dhanakoti et al., 1983, have shown that the cytosolic arginase acts as a catabolic enzyme whereas mitochondrial form was involved in the synthesis of arginine, proline, and polyamines. The main form (more than 95%) in the liver is the cytosolic arginase A1, whereas mitochondrial enzyme A4 is the main form (about 90%) in kidney (Skrzypek-Osiecka et al., 1983). Arginine, the substrate of arginase, can also be converted into nitric oxide (NO) by nitric oxide synthase (NOS). Nitric oxide exhibits various biological functions such as neurotransmission, regulation of blood pressure, etc.. The cycle that constitutes NOS a cycle that can be regarded as bypassing the ornithine cycle and has been called the Citrulline-Nitrogen Oxide cycle (Wu et al., 1995).

Distribution and Isoenzymes

Arginase is present in hepatic as well as extra-hepatic tissues. The enzymes that feed amino acids into the Urea cycle particularly aspartate aminotransferase, glutamate dehydrogenase, carbamyl transferase are located in the mitochondria along with two enzymes of the Urea cycle namely, Carbamyl phosphate synthase and L-ornithine trans-carbamylase (Figure B). The last three enzymes, arginosuccinate synthase, arginosuccinase and arginase are localized in the cytosol of liver. The overall reaction of Urea cycle is as follows:

\[ 2 \text{NH}_3 + \text{CO}_2 + 3 \text{ATP} + 3\text{H}_2\text{O} \rightarrow \text{Urea} + 2\text{ADP} + \text{AMP} + 4\text{Pi} \]

This complex compartmentation of the Urea cycle reactions appeared to be necessary to prevent accumulation of free ammonia in blood which is highly toxic.
Arginase has been studied in the kidney of many organisms e.g. rat, pig and ox (Ratner, 1973). All the enzymes of Urea cycle are present in kidney, but in lower concentrations compared to the liver (Dhanakoti et al., 1983) and rat kidney contain even lower as compared to ox and pig. The liver arginase is localized only in the cytosolic fraction, whereas in the kidney a high proportion of the arginase is also found, bound to the mitochondrial fraction (Kaysan and Strecker, 1973).

The last part of the Urea cycle starting from citrulline to arginine to Urea has also been reported in rat brain (Sadasivadu et al., 1974). Frog and rat brain showed a high arginase activity and it was reported that considerable amount of Urea is formed in the brain of rat and frog (Murthy et al., 1983).

Kaysan (1973) was of the opinion that the arginase present in extra-hepatic tissues might be different from the liver tissue enzyme. The results of Stewart et al. (1977) indicated that the brain arginase differs from the liver arginase and resembles the arginase of other tissues in some respect. The non-reaction of purified kidney arginase with antisera, prepared against liver arginase, indicated that the two immunologically are different proteins (Kaysan et al., 1973).

Porembska (1973) reported four forms of different arginases, namely, A1, A2, A3 and A4, according to the order they emerged on DEAE cellulose column in liver, kidney, submaxillary gland and brain of rat. Arginase A1 was present in all tissues studied; A2 in submaxillary gland; A3 in liver and A4 in kidney. The arginase from lung tumor corresponds to A1 and A4 of kidney according to the above classification. In kidney A4 was the main form and A1 secondary, which accords with the lung tumor enzyme.

The list of other tissues containing the Urea cycle enzymes include spleen, muscle, skin, thyroid, thymus and lung which have one or two of the Urea cycle enzymes. The significance of these enzymes in kidney is yet to be understood.
Cellular and Molecular Regulation of Arginase

Arginase is a hormonally as well as chemically regulated enzyme. It is also known to be developmentally regulated and it is the first enzyme expressed during development in mammals. In the presence of high concentration of glucagon, the rate of amino acid metabolism was enhanced (Iwase et al., 1995). In diabetic condition the concentration of aspartate declined, whereas glutamate dehydrogenase and glutamate remains unchanged. This, together with increase in the ratio of NADH to NAD, suggested that the substrate and coenzyme were in part as a result of causative effect of the increased Urea output in diabetic condition (Nuzum and Snodgrass, 1971). Alloxan which destroys the β-cells, leaves the animal subjected only to glucagon. This absence of insulin exert dramatic effects on the cell (Morris et al., 1987).

In liver, arginase is regulated in a co-ordinated manner. The frog, thyroid hormone appears to trigger the induction of arginase and its mRNA accumulation (Helbing et al., 1994; Iwase et al., 1995). Postnatally, the enzyme genes are regulated nutritionally. A high protein diet intake (Schimke, 1962; Nuzum and Snodgrass, 1971) and starvation which augment ammonia production, tend to increase the activities of all ornithine cycle enzymes as well as mRNA levels of these enzymes in rat (Schimke, 1970). Glucocorticoid and glucagon enhance the enzyme activities (Morris et al., 1987) and the mRNA level in liver, primary cultured hepatocytes and hepatoma cell lines. Increase in mRNA level or rate of protein synthesis were also noted for arginase (Tanaka et al., 1995). The induction of arginase by glucocorticoid have been studied in detail and it was found that arginase is not directly effected by the glucocorticoid receptor complex, rather they are mediated by some protein factors synthesized \textit{de novo} in response to the hormone (Gotoh et al., 1994).
Transient transfection analysis, using the rat hepatoma cell line H4IIE, revealed an enhancer region that exhibits glucocorticoid responsiveness (Gotoh et al., 1994). The enhancer region resides in an approximately 200 bp segment around intron 7, located 11 kb downstream from the transcription start site. Induction of a reporter gene, under the control of the arginase enhancer exhibited a delayed time course compared with that under the control of the mouse mammary tumor virus promoter, which shows typical primary glucocorticoid responsiveness (Ringold, 1977). Therefore, the arginase enhancer seems to mediate the secondary glucocorticoid response. Four protein binding sites were detected in this enhancer region, two of which are recognized by C/EBP family members such as C/EBPx and C/EBPB. The C/EBPB gene in cultured rat hepatocytes was shown to be induced primarily by glucocorticoid. Induction of C/EBPB mRNA by glucocorticoid in a hepatoma cell line has also been reported (Baumann et al., 1992; Campos and Baumann, 1992). Besides the liver, studies on adipoblasts and adipocytes (MacDougald et al., 1994) have shown that the C/EBPB gene is primarily induced by glucocorticoid in these cells. Therefore the C/EBP family members can be regarded as candidates for factors that mediate the secondary response of the arginase gene. The availability of C/EBPB/NF-IL6 gene-disrupted mice (Tanaka et al., 1995) should facilitate the analysis of the role of this factor in the regulation of ornithine cycle enzyme genes.

[5] **Mechanism of insulin action**

Insulin is the primary hormone responsible for storage and utilization of glucose. It affects almost every organ of the body including kidney, muscle, liver and fat. Insulin acts both as an anabolic hormone activating transport system, involved in utilization and storage of glucose, amino acids and fatty acids as well
as catabolic hormone (inhibiting gluconeogenesis, glycogenolysis and protein breakdown). Glucose plays a central role in islets' metabolism and insulin secretion. A cell membrane localized glucose receptor, which is involved in the insulin secretory response to glucose (O'Brian et al., 1991). The molecular action of insulin is quite complex and its action can be studied at three different levels.

Level I begins with the binding of insulin to the \( \alpha \) subunit of its receptor and activation of the receptor tyrosine kinase. Level II is represented by a group of serine and threonine kinases and phosphatases which are centred around an intracellular enzyme termed MAP kinase. Level III represents the final effectors of insulin action responsible for activation and inactivation of cellular enzymes, glucose transporter, translocation and stimulation of gene expression, protein synthesis and cell growth. Kinetically, insulin effects on the cellular level can be classified as immediate, intermediate and long term based on the time course of their onset. The immediate effects occur within seconds or minutes after insulin stimulation. These include activation of excreting transport system, recruitment of intracellular proteins such as glucose transporters to the plasma membrane and covalent modification of pre-existing enzymes by phosphorylation such as pyruvate dehydrogenase, acetyl-coA carboxylase and glycogen synthase (German et al., 1995).

The intermediates effects require several minutes to few hours to be measured and involve the stimulation or inhibition of transcription of specific genes and new protein synthesis. These include stimulation of synthesis of enzymes like pyruvate kinase, malic enzyme and glucokinase and inhibition of phosphoenol pyruvate carboxykinase and fructose-1-6 biphosphate (O'Brien and Granner, 1991). The long term effects of insulin require many hours to several days and include stimulation of DNA synthesis, cell proliferation and cell differentiation. To what
extent all these actions of insulin share a single common pathway or where the branch points in insulin action might occur is as yet not clear.

[6] VANADIUM: PHYSIOLOGICAL RELEVANCE

Introduction

Vanadium is a rare but widely distributed element. It is the 21st most abundant element in the earth's crust, with an average concentration of 135 ppm (Nechay, 1984). Vanadium has been recognized 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 A.D. in Sweden by Nile Gabriel Sefstrom who named it after Vanadis, the Scandinavian goddess of beauty, (Dafiris et al., 1984). This is because vanadium containing crystals and salts give many striking colours in solution. Persons exposed to excess vanadium may acquire green colour on their tongues caused by deposition of green vanadium compound. During this century several actions of vanadium have been discovered, examined, or proposed without definite proof or identification of its functions. This had to do with nutrition, prevention of dental cavities, treatment of infections, diabetes, atherosclerosis and anaemia (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 a novel inhibitor of the Na+ K+-ATPase system and it may be a physiological regulator of cation pump by changing oxidation states with different inhibitory effectiveness. Its 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+ site outside the cell (Macara, 1980; Nechay, 1984). Vanadate also inhibits the Ca2+-ATPase in the
sarcoplasmic reticulum of skeletal muscle (Cantley et al., 1977) and heart (Nechay et al., 1986), H⁺K⁺-ATPase of the gastric mucosa and kidney tubule (Cantley 1977). Chemistry

Vanadium is a group V transitional trace element that gave five valency electron. It shows a complex chemistry which is attributed to (i) its multiple oxidation states (being a 3-d group element) (ii) hydrolysis and (iii) polymerization. The redox chemistry of this metal undoubtedly plays a role in its biochemical action (Macara, 1980). The most common oxidation states of vanadium are valences of +3, +4, +5; the most stable oxidation state is +4. Vanadium can also be present with oxidation states of -1, 0, +2. The first, V³⁺ is stable only in acidic solution (<pH 2.0) and in the absence of oxygen. 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, 1983; Smith, 1989; Wever and Kustin, 1990) (Figure C).

V⁴⁺ too is stable only in acidic solution, being rapidly oxidized by dissolved oxygen at physiological pH. Below pH 3.0 it exists as the blue vanadyl cation, VO²⁺. 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 (V⁴⁺) ion tend to undergo air oxidation to form vanadate (V⁵⁻). This susceptibility to oxidation makes biological studies with vanadyl (VO²⁺) more difficult than with vanadate (V⁵⁻) and probably accounts for the fact that vanadyl (VO²⁻), as yet has not been extensively studied as an inhibitor. Hydrolysed species of VO²⁺ are very prone toward air oxidation. When the VO²⁺ is chelated, however, oxidation is considerably retarded. Reduction of vanadate (V⁵⁻) to vanadyl (V⁴⁺) takes place in the presence
FIGURE C. Structural analogy linking phosphate and vanadate complexes.
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., 1977) 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 uM to 10mM 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, 1989).

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. Most tissues of higher animals contain intracellular vanadium at concentrations varying between 0.1 and 1 uM (Macara, 1980; Ramasarma and Crane, 1981). In humans the total body pool of vanadium is ~ 100 ug with a daily intake of 10-60 ug (Dafris and Sabatini 1994). 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 pg (Byrne and Kosta, 1978; Nechay, 1984). 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 cells.

In plasma, vanadium apparently exists as metavanadate (VO₃, +5) and inside cells in reduced form as vanadyl (VO₂⁺, +4) (Robinson, 1981). NMR spectroscopy confirms that vanadium in tissues exists in the +4 oxidation state (Sakurai et al., 1990). Erythrocytes transport vanadate from the environment by two mechanisms, one sensitive to anion transport inhibitors and the second 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 favors 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).

**Mechanism of Action of Vanadate**

The first physiological effect of vanadium was shown in 1977, when Cantley et al. (1977) showed that it inhibited 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 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 deadened transition state analog of inorganic phosphate by forming a stable inactive complex in a reversible reaction with the E₂ conformation of the enzyme (Hwang et al., 1991). Inhibition by vanadate requires a bivalent cation such as Mg²⁺, as does phosphorylation from ATP (Cantley et al., 1977).
Almost all the phosphoenzymes including ion transport ATPases, Ca\textsuperscript{2+}-ATPase of sarcoplasmic reticulum, gastric H\textsuperscript{+}K\textsuperscript{+}-ATPase and other ATPases are inhibited by vanadate (Frust and Solioz, 1985; Walderhaug et al., 1985). Acid and alkaline phosphatases and phosphotyrosyl protein phosphatase can also be inhibited by vanadate (Chasteen, 1983; Swarup et al., 1982). Interestingly, liver adenylate cyclase is stimulated six fold by 3 mM vanadate (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) (Table B).

**Vanadate: an insulin mimetic agent**

In recent years, there has been an increasing interest in the biological significance of vanadium since the demonstration that vanadium compounds possess potent insulin-like properties. Its insulin-mimetic effects were documented earlier in 1980 when it was shown that vanadate could stimulate glucose uptake and oxidation in rat adipocytes (Shechter and Karlish, 1980; Dubyak and Kleinzeller, 1980). Subsequently, several studies have amply indicated that vanadate is a novel and potent insulin-mimetic agent (Figure D).

The demonstration by Heylinger 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 aroused great interest. This discovery came as a breakthrough and several laboratories carried out extensive research to elucidate and explore other insulin-like or antidiabetic properties of vanadium compounds on various metabolic disorders.
Table B: Current list of the insulin-like actions mediated by vanadate in various tissues

<table>
<thead>
<tr>
<th>Activity</th>
<th>Direction of effect</th>
<th>Target Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexose transport</td>
<td>Stimulated</td>
<td>Skeletal muscle</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>Lypolysis</td>
<td>Inhibited</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>Glycogensynthase</td>
<td>Stimulated</td>
<td>Skeletal muscle</td>
</tr>
<tr>
<td>Mitogenic activity</td>
<td>Augmented</td>
<td>various cultured cells</td>
</tr>
<tr>
<td>Translocation of IGF-II</td>
<td>Stimulated</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>K+ uptake</td>
<td>Stimulated</td>
<td>Cardiac muscle cells</td>
</tr>
<tr>
<td>Ca+-Mg+-ATPas</td>
<td>Inhibited</td>
<td>Rat adipocytes</td>
</tr>
<tr>
<td>Ca+ 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>

(Shechter, 1990)
**Four Potential Sites of Vanadium Action**

**FIGURE D.** The cellular mechanism of insulin action and possible sites of vanadium action.
(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. 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, 1989). Shechter and Karlish (1980) reported that externally applied vanadate ($V^{+5}$), at low concentrations, mimic almost all 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 due to the inhibition of the 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 stimulate DNA synthesis in quiescent human fibroblast (Carpenter, 1981).

Tamura et al. (1984) demonstrated that like insulin, vanadate also 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) and glucose oxidation via glycolysis as well as HMP shunt (Degani et al., 1981; Brichard et al., 1990; Saxena et al., 1992).

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; Brichard et al., 1990). Clark et al. (1985) demonstrated that vanadate 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 in liver (Mooney et al., 1989). 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 fibroblast 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 ample evidences to firmly establish that vanadate possesses potent insulin-like properties and reverses kidney complication in experimental diabetes (Saxena et al., 1992).

(ii) In vivo studies

In an interesting extension of earlier reports regarding the in vitro insulin-mimetic activity of vanadate in isolated cells, Heylinger et al. (1985) for the
first time demonstrated that vanadate could be orally administered to diabetic rats. They found that vanadate like insulin administered to diabetic rats in drinking water (0.6 to 0.8 mg/ml), 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 therapeutic beneficial effects on diabetic state (Paulson et al., 1987; Gil et al., 1988; Bollen et al., 1990; Valera et al., 1993).

Oral administration of vanadate to experimentally induced diabetic rats not only effectively causes stable normoglyceamia but also improves many metabolic disorders related to diabetes, though the decreased level of insulin is not restored (Heylinger et al., 1985; Shechter, 1990). Vanadate therapy has been demonstrated to exert a prophylactic effect on the progressive cardiac depression 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 drinking water) maintains a long lasting 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 and Sakurai et al., 1990). However, the much used and convenient vanadate treatment route seems to be through drinking water (Heylinger 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-biphosphatase. 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 weeks of the withdrawal of vanadyl sulfate. Vanadyl sulfate is less toxic than vanadate but it is poorly absorbed. Further studies by Ramanadham et al. (1989) 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 cataract and cardiac dysfunction (Brichard et al., 1995).

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 per cent pancreatectomized rats, vanadate improves glucose tolerance and restores disturbed glycogen synthesis in liver and muscle (Rossetti and Laughlin, 1989). Subsequent reports further potentiate that vanadate therapy to diabetic rats effectively normalizes the impaired glycogen synthase activity and markedly improves the altered hepatic glycogen metabolism (Pugazhenthi and Khandelwal, 1990; Bollen et al., 1990).
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 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 diabetes by vanadium compounds in the form of an 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.

[7] OTHER ANTIDIABETIC AGENTS

Recently attention has been focussed on the insulin-mimetic properties of several trace metals and plants materials. Among trace metals, lithium and vanadate were of special interest for their antidiabetic activities. Many plant species were identified with anti-diabetic properties and Memordica charantia is relatively well studied for its insulin mimetic properties (Shibib, 1993).

(A) Lithium

Lithium salts are widely used in the treatment of psychiatric disorders. An insulin like effect of lithium and of other monovalent cations have been described
in *in vitro* studies (Nechay *et al*., 1986). Rossetti *et al*. (1990) have demonstrated that oral administration of lithium alone, to partially pancreatectomized rats improves glucose tolerance, normalized insulin mediated glucose uptake and stimulates muscle and liver glycogen synthesis. However, conflicting data have been reported regarding the effect of lithium on carbohydrate metabolism *in vivo* in animals and in human. It has been reported to impair (Defris *et al*., 1994) or improve (Mukherjee *et al*., 1992) glucose tolerance. Some of these contradictory results, reported in the literature concerning glucose tolerance, may depend on an independent effect of lithium on insulin secretion. The insulin-mimetic actions of lithium needs further characterization.

(B) *Memordica charantia*

*Memordica charantia*, known as "Karela" have been shown to have a hypoglycemic effects in animals (Mukherjee *et al*., 1992) and human (Khan *et al*., 1992). It lowered blood glucose by lowering its synthesis through decrease in the key gluconeogenic enzymes glucose-6-phosphatase and fructose 1,6-biphosphatase and by enhancing glucose oxidation by the shunt pathway enzyme G6PDH (Shibib *et al*., 1993). *Memordica charantia* extracts also lowered the increased level of cholesterol in experimental diabetes (Leatherdale *et al*., 1981; ). In all these cases, *Memordica charantia* neither affect the insulin level nor decrease the absorption of glucose by the intestine. Though several other reports are available (Leatherdale *et al*., 1981; Shabibi *et al*., 1993) for its hypoglycemic activity, yet its underlying biochemical mechanism remains unexplored.