Review of Literature
3.1 Insulin Resistance

Himsworth (1949), twenty-seven years after the discovery of insulin, pointed out that "in the diabetic patients insulin appears to vary in efficacy at different times". He suggested that diabetes could be differentiated into insulin sensitive and insulin insensitive types on the basis of the blood glucose response to insulin administered immediately following oral glucose load. Continuing this line of investigation Himsworth published the accumulated evidences in support of his notion that insulin insensitivity and not insulin deficiency was present in many patients with diabetes. He further suggested that the classification of patients with diabetes into two groups corresponds to the clinical forms of diabetes. Patients who are insulin sensitive tended to be ketosis-prone, while the middle aged insulin insensitive patients who are non-ketotic diabetics. Definitive proof for these ideas awaited the direct measurement of plasma insulin that became available in 1960's (Yalow & Berson, 1960). It was found that higher insulin levels existed in the subjects with the adult onset form of the disease and was concluded that the tissues of the maturity onset diabetics do not respond to insulin (Yalow & Berson, 1960). Rabinowitz and Zierter (1962) provided the first direct evidences of insulin resistance in man when they demonstrated that intra-arterial administration of insulin produced significantly less glucose uptake by forearm muscle in obese subjects than in normal individuals.

Insulin resistance is defined as a subnormal biologic response to a given concentration of insulin. Traditionally insulin resistance in the diabetic is considered to be present when more than 200 units of insulin per day are required to control hyperglycemia and prevent ketoacidosis (Foster, 1983). From physiological viewpoint insulin resistance is considered to be present whenever the therapeutic dose of insulin exceeds the secretory rate of insulin which in normal person is 0.4-0.5 units/kg body weight per day (Kahn, 1986). For practical purpose most physicians consider patients clinically resistant to insulin when the insulin dose exceeds 2.0 units/kg/day (Koffler et al., 1989).
Since insulin travels from the β cell, through the circulation to the target tissue, events at any one of these loci can influence the ultimate action of the hormone.

### 3.1.1 Causes of Insulin Resistance

Insulin resistance can be due to three general categories of causes (Olefsky & Molina, 1990).

1. Abnormal β cell secretory product
   - Abnormal insulin molecule
   - Incomplete conversion of pro-insulin to insulin
2. Circulating insulin antagonists
   - Elevated levels of counter regulatory hormones e.g. growth hormone, cortisol, glucagon, or catecholamines
   - Anti-insulin antibodies
   - Anti-insulin receptor antibodies
   - Amylin?
3. Target tissue defects
   - Insulin receptor defects
   - Post-receptor defects

#### 1. Abnormal β -Cell Secretory Product

(A) Abnormal insulin molecule

Several patients have been described who secrete a structurally abnormal biologically defective insulin molecule as a result of a mutation in the structural gene for insulin. The first reported mutation was a substitution of leucine for phenylalanine in the B25 position (Given et al., 1980; Shoelson et al., 1983). This abnormal insulin has only 1-4% of the receptor binding and biological activity of native insulin (Keefer et al., 1981; Wollmer et al., 1981; Kobayashi, 1982; Tager et al., 1985). The second of the mutant insulin has a serine for phenylalanine replacement at B24 (Haneda et al., 1983). This mutant insulin has only 0.5-2% of the biological activity of native insulin (Haneda et al., 1985).
The third identified insulin mutant has a leucine for valine substitution at position A3 (Nanjo et al., 1986). This insulin has even less biological activity, amounting to only 0.14% that of intact insulin when assayed using isolated rat adipocytes (Nanjo et al., 1987). Other potentially abnormal insulins have been reported but at this time the structural alterations are unknown (Seino et al., 1985).

The typical clinical presentation is high levels of circulating immunoreactive insulin but varying degrees of glucose intolerance or overt diabetes. The high levels of circulating immunoreactive material in these patients are due to a prolonged half-life and a decreased clearance from the plasma. Since insulin removal and degradation is primarily due to receptor binding, the reduced affinity for the receptor results in a markedly prolonged half-life and thus, accumulation in plasma. Although all of the reported mutant insulins have reduced biological activity, it is entirely possible that other mutations occur with relatively normal or even increased activity but have gone undetected.

**Figure 1.** Schematic representation of insulin action and possible sites for impaired action
(B) Incomplete conversion of proinsulin to insulin

Patients have been described with familial hyperproinsulinaemia, who demonstrated incomplete conversion of proinsulin to insulin within the cell secretory granule as a result of structural abnormalities at the proteolytic cleavage sites of the proinsulin molecule. The first reported defect is due to a substitution of Histamine for Argentine at the 65th residue. With the loss of the basic residue, incomplete conversion occurred, so that the plasma contained a proinsulin (Gabbay et al., 1976, 1979; Robbins et al., 1981, 1984). In the other defect, no changes in the connecting peptide regions were found and it was first felt to be an abnormality in the converting enzyme (Gruppuso et al., 1984). A subsequent study, however, demonstrated an abnormality in the insulin gene (Elbein et al., 1985) and Chan et al. (1987) isolated a DNA clone which revealed a single mutation in the codon for the B10 histidine which was replaced by aspartic acid. The failure of this mutant proinsulin to be converted to insulin is apparently due to three-dimensional changes in the folding of the protein.

2. Circulating Insulin Antagonist

Circulating antagonists can generally be grouped into hormonal and non-hormonal categories.

(A) Hormonal antagonists

These include all of the known counter-regulatory hormones such as cortisol, growth hormone, glucagon, catecholamines, TNF α, leptin.

(i) Glucocorticoids

It is well known that excess endogenous or exogenous glucocorticoids impair carbohydrate metabolism and this is often referred to as steroid diabetes. This effect is perhaps best exemplified in patients with Cushing’s syndrome. In these patients, carbohydrate tolerance is often impaired, the fasting blood glucose values in patients with Cushing’s syndrome are rarely elevated and the abnormalities of carbohydrate tolerance are generally limited to those, which can be elicited only through the stress of a glucose challenge. Analogous results are seen in patients who receive exogenous glucocorticoids. Analysis of
glucose tolerance tests after treatment with glucocorticoid reveals increased plasma insulin values accompanied by only mild to moderate increases in glucose concentrations (Berger et al., 1966; Perley & Kipnis, 1966). This pattern of glucose tolerance-increased glucose concentrations in the face of increased insulin concentrations is the characteristic of an insulin-resistant state.

A number of mechanisms exist for this decrease in insulin action since glucocorticoids counteract the effects of insulin at several steps in glucose homeostasis. Hepatic glucose output increases in some patients with Cushing’s syndrome (Nossadini et al., 1983) and after infusion of cortisol (Rizza et al., 1982a) and the liver becomes resistant to the normal suppressive effect of insulin on hepatic glucose output (Rizza et al., 1982a). Glucocorticoids increase the activity of key hepatic gluconeogenic enzymes (Wicks et al., 1974) and the release of gluconeogenic substrates i.e. amino acids (Wise et al., 1973) and lactate (Issekutz & Allen, 1972) from peripheral tissues. It seems likely that both increased substrate availability and increased hepatic capacity to produce glucose from substrates are involved in the glucocorticoid-induced increase in hepatic glucose production. Furthermore, glucocorticoid treatment raises plasma glucagon levels (Marco et al., 1973; Wise et al., 1973) which also augments hepatic glucose production. Glucocorticoids can also lead to a decrease in peripheral glucose utilization. Again, this effect is mediated through several mechanisms. Corticosteroids decrease the activity of the plasma membrane glucose transport system (Munck, 1971) by decreasing the transport Vmax (Olefsky, 1975). Glucocorticoids appear to exert this effect by inhibiting the ability of insulin to mediate the recruitment, or translocation of glucose transport proteins from the cell interior to the cell surface (Carter-Su & Okamoto, 1987). Additionally, some glucocorticoids can cause a decrease in insulin binding to receptors, both in vivo and in vitro; this is mediated through a decrease in both receptor affinity (Olefsky et al., 1975; Kahn et al., 1978) and number (Olefsky et al., 1975). In vivo, the effects of glucocorticoids to cause insulin resistance have been well documented using the glucose clamp technique; the agents reduce the effect of insulin at all insulin
concentrations (Rizza et al., 1982a) and also markedly slow the rate of activation of insulin’s in vivo biologic effects (Baron et al., 1987b).

(ii) Growth Hormone

Growth hormone is a well-recognized circulating humoral insulin antagonist and chronic excessive secretion of growth hormone can lead to carbohydrate intolerance (Fineberg et al., 1970). Acromegaly can be associated with hyperinsulinaemia, glucose intolerance and decreased effectiveness of exogenous insulin. In most cases acromegaly, compensatory hyperinsulinaemia is sufficient to prevent gross deterioration of glucose homeostasis. Thus, mild abnormalities of glucose intolerance are the rule and less than 15% of patient develop fasting hyperglycemia. The mechanism underlying the anti-insulin effects of growth hormone have not been clearly elucidated, and this is in part due to the multiplicity of cellular effects of growth hormone and heterogeneity of circulating forms of the hormone. Although excess growth hormone clearly leads to an insulin-resistant in the chronic situation, the acute effects of growth hormone on insulin action are less clear.

(iii) Catecholamines

Excessive levels of circulating catecholamines can also antagonize the effects of insulin and several mechanisms are involved. Catecholamines can stimulate glucagon secretion (α -adrenergic effect) and increases hepatic glucose production by direct stimulation of glycogenolysis and gluconeogenesis (α + β -adrenergic effect). In combination, these effects will tend to cause hyperglycemia and are opposite to the actions of insulin. Additionally, catecholamines directly inhibit peripheral glucose uptake (β-adrenergic effect), and this has been demonstrated both in vitro (Sacca et al., 1979) in isolated adipocytes and in vivo using the glucose clamp technique (Rizza et al., 1980; Deibert & DeFronzo, 1980). It is possible that in addition to direct insulin antagonistic effects, the β-adrenergic-induced augmentation of lipolysis
leads to a secondary fatty acid-induced decrease in glucose uptake, at least in vivo.

(iv) Glucagon

Glucagon influences glucose metabolism by augmenting hepatic glycogenolysis and gluconeogenesis and in this sense, glucagon can counteract some of insulin’s effects. However, glucagon has no influence on insulin’s ability to promote peripheral glucose metabolism and does not lead to a true state of insulin resistance.

(v) Tumor Necrosis Factor-α (TNF-α) (Iannello, 2000)

TNF-α is one of the proteins formed by adipocytes, whose production increases with increasing adipocyte mass (obesity). Indeed, TNF-α (as well as chronic hyperinsulinaemia that induces insulin resistance) triggers increased Ser/Thr phosphorylation of the insulin receptor and of its major receptor substrates IRS-1 and IRS-2, which may be a molecular mechanism for uncoupling insulin signaling, as enhanced Ser/Thr phosphorylation of IRS-1 and IRS-2 impairs their interaction with the juxtamembrane region of insulin receptor. Thus, the TNF-α produced by adipocytes may function as a local ‘adipostat’ to limit fat accumulation. Increased production of TNF-α by fat cells stimulates down regulation of the insulin-sensitive glucose transporter, GLUT-4 in adipocytes. TNF-α is overexpressed in the adipose tissue of obese rodents and humans and is associated with insulin resistance. The exact role of TNF-α, however, remains to be established.

(vi) Leptin (Iannello, 2000)

Leptin is a product of OB gene. This 16-kDa protein is produced by mature adipocytes and is secreted in the plasma. Its plasma levels are strongly correlated with adipose mass in rodents as well as humans. Leptin inhibits food intake, reduces body weight and stimulates energy expenditure. Leptin binds to a long-form of leptin receptor in the hypothalamus, thus stimulating the release of GLP-1 and decreasing the production of neuropeptide Y, a neuromediator (stimulator) of food
intake. Recent studies have shown that leptin inhibits insulin secretion and has anti-insulin effects on liver and adipose tissue. If these effects are confirmed, leptin could play a role similar to that of TNF-α and could participate in insulin resistance of obesity and type II diabetes. Serum leptin is increased in insulin-resistant offspring of type II diabetic patients.

**(B) Non-hormonal Antagonists**

**(i) Free Fatty Acids**

Randle *et al.* (1963) had hypothesized that elevated circulating levels of free fatty acids could impair peripheral glucose utilization. Fatty acids are taken up by cells and oxidized intracellularly. As a result of elevated cellular rates of free fatty acid oxidation, glycolysis and glucose uptake are inhibited and this would lead to antagonism of insulin action. Although *in vitro* evidence exists which is consistent with the idea that alterations in intracellular oxidation of fatty acids could affect glucose metabolism, no conclusive evidence has yet been generated to assign a pathophysiologic role for this mechanism in obesity or diabetes. *In vivo*, it has been shown that pharmacologic elevation of FFA levels can lead to a mild state of insulin resistance (Ferrannini *et al.*., 1983), but combined indirect calorimetry and glucose clamp studies have failed to detect a relationship between insulin resistance and the rate of fat oxidation (Lillioja *et al.*, 1985).

**(ii) Anti-insulin Antibodies**

Essentially all patients who receive animal derived insulin for a long enough period of time eventually develop anti-insulin antibodies. Even with completely pure beef or pork insulin, some antigenicity still exists, since there are structural differences between human and pork or beef insulin. With the more recent use of highly purified human insulin made by either recombinant or chemical methods, the problem of insulin antibodies had become even less of an issue, although low titers of insulin anti-bodies have been noted even in patients treated only with pure human insulin.
Although anti-insulin antibodies do not usually lead to a clinically significant insulin-resistant state, the presence of these anti-bodies alters the pharmacokinetics of insulin (Kurtz & Nabarro, 1980). High titers of high-affinity antibodies can act as a reservoir for insulin by binding the hormone when it initially enters the circulation with subsequently later release. This increases the half-life of circulating insulin and prolongs the time course of insulin action (Roy et al., 1980).

(iii) Anti-insulin Receptor Antibodies

In a few well-documented cases, circulating endogenous immunoglobulins directed against the insulin receptor have been described in insulin-resistant diabetic patients (Flier et al., 1975; Kahn et al., 1977). This syndrome is extremely rare.

(iv) Other

Recently, a new substance has been identified to have antagonistic activities. For many years it has been known that islets from NIDDM subjects contain characteristic interstitial amyloid deposits which were thought to represent, at least in part, insulin aggregates (Clark et al., 1987). Newer studies have shown that the protein component of this amyloid material is a 37-amino acid peptide termed diabetes associated peptide (DAP) or amylin (Cooper et al., 1987). This peptide has a high degree of homology to calcitonin gene related peptide (CGRP) and recent studies have shown that both amylin and CGRP can produce insulin resistance in vitro (Leighton & Cooper, 1988) and in vivo (Molina et al., 1990).

3. Target Tissue Defects

Insulin exerts its biologic effects by initially binding to its specific cell surface receptor (Czech, 1977; Kahn & Crettaz, 1985). After this binding event, the insulin receptor complex is formed and one or more signals are generated (Jarrett & Seals, 1976; Larner et al., 1979; Czech, 1981). The signal or "second messenger" of insulin action may involve the generation of a chemical mediator, a conformational change within
the plasma membrane, alteration in ion flux or other information transfer (Czech, 1977, 1980).

**Figure 2.** A schematic representation of events following binding of the insulin molecule to insulin receptor on target cells

Regardless of its precise physico-chemical nature, this signal(s) interact with a variety of effector units that mediate the entire host of biologic actions attributable to insulin. In many instances the effector unit consists of a series of steps such as a sequentially linked enzyme system (i.e. activation of glycogen phosphorylase) or a series of enzymes involved in the degradation of a particular substrate (i.e. glucose). Clearly, insulin action involves a cascade of events and abnormalities
anywhere along this sequence can lead to insulin resistance. Hence tissue abnormalities in insulin action can be categorized under the heading of receptor and post receptor defects.

(A) Insulin Receptor Defects

Decreased cellular insulin receptors have been described in a variety of pathophysiologic situations. The most common of these are obesity (Roth et al., 1975; Bar et al., 1976; Olefsky, 1976) and non-insulin-dependent (type II) diabetes (Olefsky & Reaven, 1974; Olefsky, 1976; Beck-Nielsen, 1978). Decreased insulin receptors have also been described in acromegaly (Muggeo et al., 1979), after glucocorticoid (Kahn et al., 1978) or oral contraceptive (Bertoli et al., 1980) therapy, and in several other less common conditions (Oseid et al., 1977; Bar et al., 1978). Since the first step in insulin action involves binding to the receptor it is apparent that a decrease in cellular insulin receptors could lead to insulin resistance. However, this potential relationship is not clear as it would seem, since the normal relationship between insulin receptors and insulin action is not straightforward due to the fact that cells possess spare receptors (Kono and Barham, 1971), a concept based on the observation that a maximum insulin effect is achieved at an insulin concentration that occupies less than the total number of cellular receptors. The only time a decrease in insulin receptors can lead to a decrease in maximum insulin action is if the reduction in receptors is great enough so that less than 10% of the normal receptor complements is present.

Numerous studies have demonstrated that insulin binding to monocytes and adipocytes from NIDDM patient and in patients with fasting hyperglycemia is reduced by 20% to 30% (Robinson et al., 1979; DePirro et al., 1980; Olefsky, 1981; Rizza et al., 1981; Pederson, 1984). This decrease in insulin binding is due to a decrease in number in insulin receptors without any alteration in the affinity of insulin for its receptors. Because monocyte and adipocyte binding has been shown to correlate insulin binding to hepatocytes (Baxer and Lazarus, 1975; Soll et al., 1975) and muscle (Forgue & Freychet, 1975; LeMarchand et al., 1978) in animals, it has been reasonable to assume that a decrease in
insulin binding contributes to the insulin resistance in some NIDDM subjects. However, Arner et al. (1986) have challenged this assumption by demonstrating normal insulin binding to hepatic membranes isolated from NIDDM patients. In contrast, a decrease in insulin receptor number can not be demonstrated in approximately one third to one half of all NIDDM subjects, especially those with high fasting plasma glucose levels (Okamoto et al., 1981; Bolinder et al., 1982; Hidaka et al., 1982; Nankervis et al., 1982; Seltzer, 1982; Kashiwagi et al., 1983b; Pedersen, 1984).

Lonnroth et al. (1983b), Olefsky & Reaven (1977) and others have been unable to find a correlation between insulin binding and the severity of insulin resistance in their NIDDM patients. In fact, many investigators have not been able to demonstrate any decrease in insulin binding in NIDDM patients (Okamoto et al., 1981; Bolinder et al., 1982; Hidaka et al., 1982; Nankervis et al., 1982; Seltzer, 1982; Kashiwagi et al., 1983b; Pedersen, 1984). Mandarino et al. (1984b) and Kolterman et al. (1981) when examining the dose-dependent relationship between insulin-mediated glucose disposal and plasma insulin concentration have provided evidence to support the existence of a severe post receptor defect in insulin action. In patients with impaired glucose tolerance and very mild diabetes, the dose response curve was shifted to the right, but glucose metabolism could be normalized at very high plasma insulin concentrations; this is consistent with a receptor defect, which was indeed documented in isolated adipocytes and monocytes. In contrast, in diabetic patients with moderate to severe fasting hyperglycemia, the dose-response curve was shifted to the right, and insulin-mediated glucose disposal could not be normalized at maximally effective plasma insulin concentrations. These results suggest the presence of a postreceptor defect in insulin action and indeed no decrease in insulin binding could be demonstrated in this group of diabetic patients. Taken together the above results indicate that a postbinding defect in insulin action must be responsible for the observed insulin resistance in diabetic patients with well-established fasting hyperglycemia.
(B) Post Receptor Defects

The overall insulin scheme represents multisteps sequences in which binding to receptor is only the initial event. A defect in any of the effector systems distal to receptor binding can also lead to impaired insulin action and insulin resistance (Figure 2).

A postbinding defect in insulin action could result from one of three metabolic disturbances: impaired generation of insulin's second messenger, diminished glucose transport into the cell or a postglucose transport defect in some critical enzyme involved in glucose utilization. It has been suggested that phosphorylation/ dephosphorylation of key intracellular proteins may be an important signaling mechanism that couples insulin binding to the intracellular action of insulin (Larner et al., 1979; Avruch et al., 1985). With regard to this, the α-subunit of the insulin receptor has been shown to contain the major insulin binding domain, whereas the β-subunit expresses insulin-stimulated kinase activity directed toward its own tyrosine residues (Czech, 1977, 1981; Kahn & Crettaz, 1985).

The most current popular theory suggests that insulin receptor phosphorylation, with subsequent activation of insulin receptor tyrosine kinase, may represent an important second messenger for the hormone’s action. Freidenberg et al. (1987) have shown that insulin-stimulated tyrosine kinase activity of partially purified insulin receptors from adipocytes of obese diabetic patients, but not nondiabetic obese subjects, was decreased by 50%. Similarly, Comi et al. (1987) has shown decreased tyrosine kinase activity in erythrocytes from obese NIDDM individuals. Caro et al. (1987) have also documented decreased insulin-stimulated tyrosine kinase activity in muscle and liver (Caro et al., 1986) from morbidly obese NIDDM subjects but not in weight matched non-diabetic subjects. The decrease in tyrosine kinase activity could not be explained by a decrease in insulin binding, which was normal in the latter three studies (Caro et al., 1986, 1987; Comi et al., 1987). To the extent that the insulin receptor tyrosine kinase represents an important mediator of
insulin action its decreased activity in NIDDM patients may represent a key intracellular mechanism for insulin resistance.

Once the second messenger for insulin action has been activated it enhances glucose transport into the cell. This effect is facilitated by the translocation of an intracellular pool of glucose transporters to the cell membrane (Cushman & Wardzala, 1980; Kono et al., 1982). Several studies have examined glucose transport activity in NIDDM and have found it to be uniformly diminished (Kolterman et al., 1981; Bolinder et al., 1982; Ciaraldi et al., 1982; Seltzer, 1982; Kashiwagi et al., 1983b; Mandarino et al., 1984b; Simpson & Cushman, 1986). Whether this is due to a decrease in the number of intracellular glucose transport units or to an inability of insulin to translocate them to the cell membrane and cause their activation has yet to be determined. Furthermore, it is unknown whether the defect in insulin stimulated glucose transport is a primary manifestation of insulin resistance, is secondary to impaired generation of insulin second messenger, or is the consequence of down regulation of the glucose transport system by hyperglycemia (Rossetti et al., 1987; L Rossetti RAD, Shulman GI, Cushman SW and Kahn BB, Unpublished observation).

Because glucose transport represents an early step in insulin action, if this were the primary cause of the insulin resistance, the two major metabolic pathways of glucose metabolism, i.e. glucose oxidation and glucose storage (non-oxidative glucose disposal), would be expected to be similarly impaired when expressed as a percentage of the total-body glucose disposal rate. In control subjects, after the ingestion of 100g of glucose, glucose storage accounted for 72 ± 1% of the oral load. In NIDDM patients with fasting plasma glucose concentrations <180 mg/dl, glucose storage represented 60 ± 3% of the ingested glucose load and was reduced further to 49 ± 3% in diabetic individuals with fasting glucose levels >180 mg/dl. Similar results have been reported by Bogardus et al., (1984a, 1984b) and colleagues (Lilloja et al., 1986). These results suggest that defects in enzymatic steps distal to glucose transport may become rate limiting for insulin mediated glucose-disposal in NIDDM subjects with moderately severe to severe glucose intolerance.
The other major pathway of glucose disposal, i.e., glucose oxidation, is also impaired in NIDDM individuals. A key regulator of glucose oxidation is pyruvate dehydrogenase, an enzyme regulated by insulin (Buffington et al., 1984). In one study, pyruvate dehydrogenase activity was examined in NIDDM patients and the ability of insulin to stimulate this enzyme complex in adipocytes was found to be impaired (Mandarino et al., 1986).

In summary, it appears that both binding and postbinding defects in insulin action contribute to the insulin resistance. However, diminished insulin binding occurs primarily in individuals with impaired glucose tolerance or very mild diabetes. In NIDDM patients with fasting glucose levels >140 mg/dl, diminished insulin binding is unusual and postbinding defects are primarily responsible for the insulin resistance. Several postbinding defects have been documented, including diminished tyrosine kinase activity, decreased glucose transport, impaired glycogen synthase activity and reduced pyruvate dehydrogenase stimulation.

Other Causes Of Insulin Resistance

The interaction of insulin with its receptor is also very sensitive to minor changes in pH accounting, in part, for the insulin resistance present in patients with type I diabetes with ketoacidosis (Kahn, 1985). Alterations in insulin action at the post receptor levels are present in type I diabetic patients, in whom the pool of transporters is reduced in number (Karnieli et al., 1981) and in type II diabetic patients, in whom both reduced numbers and impaired activity of glucose transporters (Amiel et al., 1986) lead to a decrease in the insulin responsiveness. These alterations are also present in the insulin resistance associated with excesses of growth hormone or glucocorticoids (Kahn, 1986) and they probably have a role in the resistance observed in women taking oral contraceptives and in patients of both sexes during puberty (Paulsen et al., 1979).

In 1979, a patient with poorly controlled diabetes was described in whom insulin was rapidly degraded in the subcutaneous tissue by
insulin-specific protease (Schade & Duckworth, 1986). Because many patients with type I diabetes have poor metabolic control despite intensive insulin therapy, the diagnosis of subcutaneous insulin resistance is very attractive as an explanation for the patients with metabolic instability. To establish this diagnosis three criteria must be fulfilled. (1) resistance to the hypoglycemic action of subcutaneously injected insulin but normal sensitivity to insulin administered intravenously; (2) a lack of increase in plasma free insulin level after large doses of insulin are injected subcutaneously; (3) increased insulin-degrading activity in the subcutaneous tissue (Schade & Duckworth, 1986). Little is known about the states in which increased degradation of insulin may occur in circulation (Kahn, 1986).

3.1.2 Site Of Insulin Resistance

Insulin controls glucose homeostasis through three coordinated mechanisms: suppression of hepatic glucose output (HPG), stimulation of glucose uptake by liver, stimulation of glucose uptake by peripheral tissues. Glucose uptake in turn depends on to major metabolic pathways: glucose oxidation and glucose storage.

Figure 3. Normal physiology of glucose homeostasis
Each of these processes may be a cause of the insulin resistance.

Figure 4. Metabolic defects due to insulin resistance leading to hyperglycemia

3.1.2.1 Hepatic Glucose Production (HGP)

With the use of radioisotope dilution techniques, basal hepatic glucose production in diabetics has been reported to be either normal (Kalant et al., 1963; Manougian et al., 1964) or slightly elevated (Forbath & Hetenyi, 1966; Bowen & Moorehouse, 1973; Kimmerling et al., 1976; DeFronzo et al., 1979a, 1982b). Likewise, when splanchnic glucose production has been measured with the hepatic vein catheterization technique (Bearn et al., 1951; Felig et al., 1978), normal rates of glucose production have been found. However, these “normal” rates of hepatic glucose production are inappropriately high for the ambient blood glucose concentration. Studies by Felig and Wahren (1971; 1974) have shown that in normal man a rise in blood glucose concentration of only 15-20 mg/dl, in association with a modest increase in peripheral plasma insulin levels, is sufficient to inhibit hepatic glucose output by 80-85%.
More recently, DeFronzo et al. (1978c) have shown that hyperglycemia, in the absence of an increase in plasma insulin concentration, is capable of effectively inhibiting hepatic glucose production in normal man. In contrast, the diabetic liver produces glucose at "normal" rates despite blood glucose concentration 100-200 mg/dl above basal levels. Fasting hyperglycemia may thus be viewed as an abnormality of hepatic glucose production in which there is a relative rather than absolute overproduction of glucose.

The ability of insulin to inhibit basal hepatic glucose production in diabetics with fasting hyperglycemia was examined by DeFronzo et al. (1979a) employing the insulin clamp technique. Basal glucose production was similar in controls and diabetics (2.3 ± 0.1 vs. 2.7 ± 0.3 mg/min.kg) and was suppressed by 90-95% in both groups following insulin infusion. In this study, euglycemia was maintained throughout the insulin clamp study and endogenous insulin secretion, estimated from peripheral C-peptide levels, was suppressed by 50-75% (DeFronzo et al., 1981b). Therefore, approximately equivalent increments (~100uU/ml) in plasma insulin concentration were achieved in the peripheral and portal circulation. Since this degree of portal hyperinsulinaemia is well within the range observed following intravenous or oral glucose administration (Blackard & Nelson, 1970), this finding implies that in diabetics hepatic glucose production can be normally inhibited by physiologic hyperinsulinaemia (DeFronzo et al., 1982b). Kalant et al. (1963) and Forbath and Hetenyi (1966) have also documented a normal suppressive effect of insulin and glucose plus insulin on endogenous glucose release in non-insulin dependent diabetics. Kimmerling et al. (1976), on the other hand, could not demonstrate a normal suppressive effect of insulin on hepatic glucose production in NIDDM patients. This study, however, was carried out with the use of the quadruple infusion technique (Shen et al., 1970; Reaven & Olefsky, 1977) and as previously discussed, the inability of insulin to inhibit hepatic glucose production under these experimental circumstances may be due to hypersensitivity of the diabetic liver to epinephrine (Shamoon et al., 1976).
3.1.2.2 Hepatic Glucose Uptake

In normal man Felig et al. (1975), employing the hepatic venous catheter technique, have demonstrated that the primary site of uptake of ingested glucose is the splanchnic area. According to this study, following oral glucose administration, approximately 60% of the load is retained in the splanchnic bed, 25% is utilized by the brain, and only 15% is available for insulin-mediated uptake by peripheral tissues.

However, the hepatic venous catheter technique only measures the net splanchnic glucose balance. This is the sum of hepatic and extrahepatic (intestinal) net glucose balances. Although the extrahepatic tissues are capable of utilizing glucose in the post absorptive state, their contribution amounts to only ~15-20% of the total tissue glucose uptake (Barrett et al., 1980). Therefore, net splanchnic glucose balance is likely to reflect, in large measure, hepatic glucose exchange.

Using indirect calorimetry, Felber et al. (1978) have arrived at similar figures for hepatic glucose disposal. Of a 100 g oral glucose load, 38% was oxidized and 62% was stored. Comparable results have also been obtained by Perley and Kipnis (1967) and Jackson et al. (1973), who estimated that 63% and 42% respectively of ingested glucose is disposed of by the liver. Thus, all four studies (Perley and Kipnis; 1967; Jackson et al., 1973; Felig et al., 1975; Felber et al., 1978) are in close agreement and indicate that the liver is the major organ responsible for the disposition of oral glucose in man.

When oral glucose was administered to diabetics with fasting hyperglycemia, the increment in splanchnic escape of glucose over the ensuing 3 hours was 100% greater than in normal subjects (Felig et al., 1978). This failure of the splanchnic bed to retain glucose accounted for 75% of the observed post pyramidal rise in blood glucose. Since in this study plasma insulin and glucose levels were consistently plasma insulin and glucose levels were consistently higher in diabetics than in controls, the impairment in net glucose removal by the diabetic liver cannot be attributed to inadequate glucose delivery or insulin lack.

Although the liver has long been known to play a central role in glucose homeostasis (Bondy et al., 1949; Madison, 1969; Perley & Kipnis,
1967; Felig et al., 1975, 1978), the factors controlling hepatic glucose uptake have remained controversial. Both hyperglycemia (Bergman, 1977; Soskin & Levine, 1952) and hyperinsulinaemia (Felig & Wahren, 1971; Madison, 1969) have been proposed as the primary signal responsible for the switch of the liver from a glucose-producing to a glucose-assimilating organ. Recent studies (DeFronzo et al., 1978a; Ferrannini et al., 1980) have indicated that although both hyperglycemia and hyperinsulinaemia are capable of enhancing net hepatic glucose uptake, their effects are small compared to the effect of the route of glucose administration, i.e. oral versus intravenous. In these studies, when physiologic hyperinsulinaemia (~100 µU/ml) was created while maintaining euglycemia (insulin clamp technique), splanchnic glucose balance reverted from a basal output of 1.9 mg/min.kg to a small net uptake of 0.5 mg/min.kg. Elevation of the plasma insulin concentration to ~1100 µU/ml with maintenance of euglycemia had no further effect on splanchnic glucose uptake (Ferrannini et al., 1980). When steady-state of hyperglycemia (233 mg/dl) was aerated (hyperglycemic clamp technique) (DeFronzo et al., 1979b), net splanchnic glucose uptake increased approximately two-fold, to 1.2 mg/min/kg. When extreme hyperglycemia (400 mg/dl) was created with intravenous glucose and an exogenous insulin infusion was added to produce insulin levels of ~900 µU/ml, litter additional uptake of glucose by the splanchnic bed was observed. Thus, despite the presence of supraphysiologic hyperglycemia and hyperinsulinaemia net splanchnic glucose uptake was always less than the post absorptive state rate of glucose output. In contrast, when oral glucose was administered, alone or in combination with intravenous glucose, net splanchnic glucose uptake was markedly stimulated (4.1 and 6.1 mg/kg.min, respectively). These results indicate that, in addition to hyperglycemia and hyperinsulinaemia, the route of glucose administration is a critical determinant of net splanchnic glucose uptake. This “gut effect” could simply reflect direct glucose uptake by intestinal tissues. Alternatively, it could be mediated by the release of some unidentified gastrointestinal hormone (DeFronzo et al., 1978b), activation of some neural reflex that specifically enhances insulin-stimulated
glucose uptake by the liver (Lautt, 1980), or perhaps in part by the increase in hepatic blood flow that accompanies oral glucose ingestion (Felig et al., 1975). A similar conclusion was reached by Lickley et al. (1975) from studies comparing plasma glucose responses following intraduodenal versus intravenous glucose administration in normal dogs and dogs with portacaval anastomosis. A greater enhancement of hepatic glucose uptake with oral than with intravenous glucose has also been demonstrated by Barrett et al. (1980) in the conscious dog.

Since in diabetics net splanchnic glucose uptake following glucose ingestion is substantially reduced (44-%) in the face of higher plasma glucose and insulin levels, it was logical to hypothesize (DeFronzo et al., 1978b) that these patients may lack the "gut effect" or entero-hepatic interaction that is brought into play by oral glucose and is presumably responsible for the enhancement of hepatic glucose uptake. Regardless of the underlying mechanism(s), it is clear that enhanced splanchnic escape of orally administered glucose is a major contributory factor in the glucose intolerance of NIDDM patients.

It should be emphasized that the net hepatic balance is the sum of two simultaneously ongoing processes, uptake and production. From a purely quantitative standpoint, it seems unlikely that failure of suppression of HGP could account for the observed increase in splanchnic glucose escape in diabetics after oral glucose (Felig et al., 1978). In fact, intravenous insulin and/or glucose (Kalant et al., 1963; Forbath & Hetenyi, 1966; DeFronzo et al., 1979a) induce a normal decline in hepatic glucose production in diabetics with fasting plasma glucose concentration in the 140-220 mg/dl range. However, a partial defect in hepatic glucose suppression following oral glucose cannot be excluded. Recently, Radziuk et al. (1978) and Pilo et al. (1981) have described a double isotope technique, which allows the quantitation of hepatic glucose production following oral glucose. Preliminary results (Ferrannini and DeFronzo, unpublished observations) would indicate that endogenous glucose release after glucose ingestion is not grossly abnormal in patients with NIDDM.
3.1.2.3 **Peripheral Glucose Uptake**

The studies by Zierler & Rabinowitz (1963), using the human forearm perfusion technique, and those of DeFronzo et al. (1979a), employing the insulin clamp technique, have both provided evidence for an impairment in peripheral glucose uptake in response to physiologic hyperinsulinaemia in diabetics. It should be recalled that, under the conditions of euglycemic hyperinsulinaemia imposed by a clamp study, hepatic glucose production is suppressed by over 90% and hepatic glucose uptake is negligible (DeFronzo et al., 1978a; Ferrannini et al., 1980). Therefore, the greatest part of glucose uptake occurs in peripheral tissues. More recent studies, in which the euglycemic clamp technique has been combined with leg catheterization, have directly confirmed that over 80-85% of insulin-mediated glucose uptake can be accounted for by peripheral tissues (DeFronzo et al., 1981a, 1981c). Of these, muscle would appear to be quantitatively responsible for the impairment in glucose uptake of diabetics, since adipose tissue accounts for the disposal of less than 2% of an oral or intravenous glucose load (Bjornorp et al., 1970, 1971). Kimmerling et al. (1976), employing the quadruple infusion technique, have come to the same conclusion concerning the site of insulin resistance in diabetics. With the use of indirect calorimetry Felber et al. (1978) have shown that following oral glucose ingestion, glucose oxidation, which primarily occurs in muscle tissue, is reduced by 30% in normal-weight, non-insulin-dependent diabetics. A similar impairment in glucose oxidation was found by Bowen and Moorehouse using $^{14}$C-glucose (Bowen & Moorehouse, 1973). Recalculation of the data of Felig et al. (1978) also indicates the presence of peripheral tissue insensitivity to insulin. In that study, since the total amount of glucose metabolized is known and since splanchnic glucose uptake was measured directly, the difference between these two must represent glucose uptake by peripheral tissues. This value was slightly decreased in NIDDM despite plasma glucose and insulin levels that were more than two-fold greater than in controls. Most recently, we have directly examined leg glucose uptake under euglycemic hyperinsulinaemic conditions in NIDDM (DeFronzo et al., unpublished observations). Compared to
controls, total body glucose metabolism was diminished by 41% and this decrease was paralleled by a similar decrease in leg glucose uptake.

In the post-absorptive state in which plasma glucose concentration remains relatively constant, glucose is taken up by all the body tissues at a rate equal to the rate of endogenous glucose production. Since the latter is usually normal in diabetics, it flows that glucose uptake is not reduced in these patients (Bowen & Moorehouse, 1973; DeFronzo et al., 1982b). This uptake, however, occurs at plasma glucose levels that are considerably higher than normal and is maintained by plasma insulin levels that are normal or slightly raised. In fact, if the fasting rate of glucose disposal is divided by the fasting glucose concentration, the value for glucose clearance so calculated is significantly decreased (by 30%) as compared to control subjects (DeFronzo et al., 1982a). Thus, the peripheral tissues in diabetics are “glucose intolerant”; the hyperglycemia can be considered as a compensatory mechanism that drives glucose by mass action into less permeable cells. It should be recalled that, in the post absorptive state, 50-70% of total glucose disposal takes place in insulin-independent tissues (Vranic & Wrenshall, 1968). Thus, the observed reduced efficiency of overall glucose clearance in the post absorptive state in diabetics could be located either in insulin-sensitive or insulin-independent tissues or both.

In summary, insulin resistance in diabetics is found in both the liver and the peripheral tissues. Hepatic resistance manifests itself as a relative increase in glucose production in the fasting state. Absolutely increased rates of glucose production are likely to be found inpatients with fasting hyperglycemia greater than 180 mg/dl (Bowen & Moorehouse, 1973; DeFronzo et al., 1982a). Peripheral insulin resistance is evidenced as impaired glucose uptake following insulin and, possibly, as reduced efficiency of plasma glucose clearance in the post absorptive state.
3.1.3 Insulin Resistance In Insulin-Dependent (type I) Diabetes Mellitus (IDDM)

Although absolute insulin deficiency represents the primary pathogenic abnormality in patients with insulin-dependent diabetes mellitus, it should be considered that chronic lack of insulin action may produce insulin resistance. Various studies in the literature also emphasize that most IDDM patients may be moderately to severely resistant to the actions of insulin both at onset and after long duration of diabetes (DeFronzo et al., 1982a; Hjollund, 1982; Del Prato et al., 1983; Lager et al., 1983; Lonnroth et al., 1983a; Proietto et al., 1983; Beck-Nielsen et al., 1984; Nankervis et al., 1984; Pernet et al., 1984; Yki-Jarvinen & Koivisto, 1984b; Yki-Jarvinen et al., 1984b; Hansen et al., 1985; Pederson & Hjollund et al., 1985; Simonson et al., 1985).

3.1.3.1 Insulin Resistance In Acute, Untreated, Insulin-deficient Diabetes Without Severe Acidosis

Studies in humans: Recently, in vivo insulin action has been characterized in untreated but nonacidotic newly diagnosed IDDM patients by applying a hyperglycemic insulin clamp at basal level and two different insulin steady-state levels in the physiologic plasma insulin range (Nankervis et al., 1984). The major findings were elevated basal hepatic glucose production, insulin insensitivity of hepatic glucose release, and resistance to the action of insulin in peripheral tissues. These findings are in accordance with previous studies with indirect calorimetry, in which both glucose oxidation and glucose storage were reduced in IDDM (Felber et al., 1977). After 1 wk of conventional insulin therapy, hepatic insensitivity to insulin disappeared, whereas peripheral glucose disposal only partially improved (Nankervis et al., 1984). Another group of investigators, who studies newly diagnosed IDDM 2 wk after start of insulin treatment with the euglycemic clamp at a single steady-state plasma insulin level in the physiologic range, also found insulin resistance of glucose utilization of peripheral tissues (Yki-Jarvinen & Koivisto, 1984a). Basal hepatic glucose output was significantly
increased and unaffected by 3 months of insulin therapy, which may be due to portal hypoinsulinaemia and not insulin resistance. Conversely, the insulin stimulated glucose clearance of peripheral tissues was normalized after 3 months.

In vitro approaches to characterize the insulin insensitivity of untreated newly diagnosed IDDM patients have demonstrated normal insulin binding to adipocytes, monocytes, and erythrocytes (Hjollund et al., 1985), whereas studies of glucose processing in adipocytes have shown very low activity of phosphofructokinase, (Galton & Wilson, 1971)) and severely depressed maximal insulin responsiveness of glucose transport and metabolism (Hjollund et al., 1985). Moreover, the basal conversion of glucose to lipids was impaired (Hjollund et al., 1985). In skeletal muscles from untreated diabetics the glycogen syntheses I activity is reduced, but the defect is reversed by insulin therapy (Roch-Norlund et al., 1972).

Studies in animals: The idea that absolute insulin deficiency with accompanying catabolism may cause insulin resistance is supported by studies in alloxan or streptozotocin treated dogs with either the epinephrine-propranolol insulin suppression-test (Reaven et al., 1977) or the insulin clamp technique (Caruso et al., 1983; DeFronzo et al., 1983). There is consensus that peripheral tissues in the diabetic dog are resistant to the action of insulin, whereas maximal insulin suppression of hepatic glucose production has been found normal or impaired. In alloxan-diabetic dogs the insulin resistance is partially reversed by 14 days of insulin treatment.

Rodents with experimental insulin-deficient diabetes exhibit increments of insulin receptor binding to fat cells (Schoenle et al., 1977; Kasuga et al., 1978; Kobayashi & Olefsky, 1979), liver membranes (Hepp et al., 1975; Davidson & Kaplan, 1977), and intact skeletal muscles (Le Marchand-Brustel & Freychet, 1979). In studies of insulin-stimulated glucose transport in diabetic rats, adipocyte glucose transport has been reported to be markedly reduced (Kasuga et al., 1978; Kobayashi & Olefsky, 1979; Karnieli et al., 1981) in all cases except one (Schoenle et al., 1977), and recently it was found that insulin resistance at the level of
glucose transport in streptozotocin-diabetic rats was due to depletion of intracellular transport (Kamieli et al., 1981). Additionally, studies of glucose metabolism in experimental diabetes have shown decreased activities of adipocytes (Schoenle et al., 1977; Kasuga et al., 1978; Kobayashi & Olefsky, 1979). Contrary to the adipocyte findings, experiments with isolated soleus muscle from streptozotocin-diabetic mice have shown increased insulin-stimulated glucose transport and glycogen synthesis at sub maximal insulin concentrations (Le Marchand-Brustel & Freychet, 1979).

*Pathogenetic considerations*: The available data from untreated, insulin-deficient diabetic humans do not support the hypothesis that impaired insulin-receptor binding is involved in the insulin resistance of this state. Again, it must be stressed that we lack results from *in vitro* studies of human muscle and hepatic tissues. In diabetic animals, insulin binding is increased, which has been considered an upregulation secondary to the prevailing hypoinsulinaemia. The disparity between receptor status in diabetic humans and animals is unknown but might be due to differences in etiological factors and the duration of the catabolic disorder. There is consistency between findings in adipocytes from diabetic humans and rodents at post-insulin-binding steps, suggesting abnormalities associated with glucose transporters as well as intracellular enzyme activities of glucose metabolism. On the other hand, it is currently unknown if the opposite changes in adipose and muscle tissues from diabetic rodents represent a real tissue difference. Further studies to clarify this problem are greatly needed.

The molecular mechanisms that trigger the insulin resistance in newly diagnosed insulin-deficient diabetics are also poorly understood, but it is natural to speculate that this type of insulin resistance is secondary to insulin deficiency, because it may be reversed by short-term insulin replacement therapy and because experimental hypoinsulinaemia may induce the same defects.

In summary, untreated IDDM patients have insulin resistance in peripheral tissues, and the major dysfunctions seem to be associated
with glucose transport, oxidation and storage. Also, the insulin effect on the liver may be impaired in the acute phase. The insulin resistance is reversible, and in well-treated IDDM the in vivo insulin action remains normal for at least 3 months. Patients who are in clinical remission ("honeymoon period") have significantly greater insulin action than patients who continue to depend on insulin therapy, despite no significant differences in endogenous insulin secretion between the two groups (Yki-Jarvinen & Koivisto, 1986).

3.1.3.2 Insulin Resistance In Long-standing Diabetes

By applying the combined oral glucose-intravenous insulin tolerance test, it was shown in 1939 that about one-third of normal-weight insulin-treated diabetics with onset of diabetes in early life were insulin insensitive (Himsworth & Kerr, 1939). Studies with the insulin-suppression test have confirmed this observation (Harano et al., 1981; Ginsberg, 1977a) and during the last few years the insulin-clamp method has been used in euglycemia at one or more steady state plasma insulin concentrations in a series of studies of patients with diabetes of long duration (DeFronzo et al., 1982a, 1982b; Del Prato et al, 1983; Lager et al., 1983; Proietto et al., 1983; Reaven, 1983; Beck-Nielsen et al., 1984; Pernet et al., 1984; Yki-Jarvinen et al., 1984b; Hansen et al., 1985; Simonson et al., 1985). These studies have consistently substantiated the presence of moderate to severe insulin resistance of the glucose disposal to peripheral tissues. In one study, insulin resistance of peripheral tissues could only be demonstrated in poorly controlled IDDM (Revers et al., 1984b). Basal hepatic glucose release in these diabetics is either elevated or normal, apparently depending on the ambient plasma concentrations of glucose and insulin. Hepatic glucose output is completely inhibited at peripheral plasma insulin concentration of 100 U/ml, suggesting normal or increased maximal suppression of insulin. However, preliminary data, with peripheral insulin infusions at low rates that give plasma insulin levels of 10-25 U/ml, allude to the possibility that the hepatic insulin sensitivity may be increased in long-term conventionally treated IDDM (Nielsen et al., 1986). At the cellular level,
insulin binding to monocytes and erythrocytes from long-term treated IDDM patients is normal (Pederson et al., 1978; Fantus et al., 1981; Pederson et al., 1982a), whereas insulin binding to peripheral fixed cells such as adipocytes is significantly impaired (Pederson & Hjollund, 1982). As a biological consequence of reduced adipocyte binding, it might be anticipated that the dose-response curves for insulin-stimulated substrate turnover were shifted rightward to express a decreased cellular sensitivity to insulin. This was the case in studies of glucose transport and antilipolysis, whereas the relative dose-response curves for insulin-stimulated glucose inversion to basal fat-cell glucose oxidation and lipogenesis were severely reduced.

In considering adipocyte results from both newly diagnosed and long-term treated IDDM patients, it is evident that conventional insulin therapy once or twice a day is associated with a normalization of maximal insulin responsiveness of glucose transport and metabolism, an unaltered impaired basal glucose metabolism, and an induction of depressed insulin receptor binding with reduced sensitivity of glucose transport and the antilipolytic effect of insulin (Pederson & Hjollund; 1982).

Pathogenetic mechanisms. IDDM patients with long-standing disease exhibit reduced glucose disposal to peripheral tissues. Because insulin resistance of newly diagnosed IDDM patients can be reversed by short-term treated IDDM patients might be a consequence of the unphysiologic way of insulin administration to subcutaneous tissue or the persistent diabetic state itself. Although there are difficulties in analyzing free insulin in these diabetics, there is now agreement that most conventionally treated IDDM patients have fasting hyperinsulinaemia in the peripheral circulation and postprandial and presumably fasting hypoinsulinaemia in the portal circulation. Assuming that the ambient insulin level is an effective regulator of insulin-receptor binding, the lack of correlation between adipocyte and blood cell insulin receptors may be explained by the abnormalities in circulating insulin concentrations. Thus, adipose tissue as a fixed peripheral tissue is exposed to a constant hyperinsulinaemia, which may cause downregulation of the adipocyte
insulin binding, whereas blood cells are exposed to peripheral hyperinsulinaemia and portal hypoinsulinaemia with unchanged receptor status. There is also solid experimental evidence that short-terms insulin infusion in normal humans causing moderate hypoinsulinaemia is associated with in vivo insulin resistance of peripheral tissue (Rizza et al., 1985) and that in vitro insulin resistance of adipocytes is localized to the glucose-transport step and the lipogenetic pathway (Mandarino et al., 1984a).

Alternatively, it may be hypothesized that chronic impairments in carbohydrate, lipid, and amino acid metabolism in conventionally insulin-treated IDDM patients may lead to irreversible charges at the level of target tissues in insulin action, e.g., glycosylation of regulatory peptides and enzymes, which might alter the post binding activities of insulin controlled metabolic pathways. This hypothesis has not yet been experimentally tested.

In summary, conventionally treated long-term IDDM patients have impaired insulin binding to adipocytes but normal insulin binding to blood cells. In adipocytes the insulin sensitivity of glucose transport and the antilipolytic pathway are reduced, and the basal glucose metabolism and, thereby, the insulin-mediated glucose metabolism are impaired. In vitro studies correspond within vivo experiments in which insulin resistance has been identified in peripheral tissues. Theoretically, subcutaneous insulin injections resulting in elevate peripheral plasma insulin levels may contribute to a desensitization of insulin action at both insulin-receptor and post. Binding steps. Moreover, portal hypoinsulinaemia with increased hepatic glucose release and elevated plasma levels of intermediary metabolites (e.g. FFA) may add to the reduced action of insulin on peripheral tissues.
3.1.3.3 *Insulin Resistance In Diabetic Ketoacidosis*

After the successful introduction of low-dose insulin treatment of diabetic ketoacidosis (Alberti *et al*., 1973), it may be asked if the role of insulin resistance in this state has been overemphasized. In fact, there is solid evidence from *in vivo* measurements of insulin action by several different techniques that resistance to the action of insulin on glucose metabolism is indeed present in diabetics with severe metabolic decompensation.

About 20 year ago, studies with insulin-tolerance tests in a few patients with diabetic ketoacidosis indicated the presence of impaired whole-body insulin effectiveness (Walker *et al*., 1963; Alford *et al*., 1971). Subsequent experiments under conditions of steady-state plasma insulin and glucose (achieved with the epinephrine-propranolol insulin-suppression test) revealed significant insulin resistance of peripheral glucose disposal in diabetics during ketoacidosis compared with the situation after a few weeks of insulin treatment (Ginsberg, 1977b).

In another clinical investigation the half-time of the fall in plasma glucose during low-dose insulin therapy of ketoacidotic diabetics was monitored (Barrett *et al*., 1982). The main finding was average rate of decline in plasma glucose level that was only 8% of that in control subjects rendered equally hyperglycemic by a combined infusion of somatostatin and glucose. Whether the insulin resistance resulted from failure of insulin to suppress the glucose output from the liver or from failure of insulin to stimulate glucose utilization in peripheral tissues could not be assessed from this study design.

Unfortunately we lack data for insulin action at the level of target tissues in patients with diabetic ketosis. Studies on insulin binding to monocytes and erythrocytes in ketotic, newly diagnosed IDDM patients have shown a heterogenous receptor response, with the most ketotic diabetics having a depressed insulin-binding ability when compared with healthy subjects (Fantus *et al*., 1981; Pederson *et al*., 1982c; Yasuda *et al*., 1982).
Pathogenetic reflections: Theoretically, the impaired action of insulin may be related to a series of factors that operate during ketoacidosis (e.g., acidosis, hypoinsulinemia, hyperosmolarity, increased plasma concentrations of counterregulatory hormones, and intermediary metabolite and electrolyte imbalances).

Chronic metabolic acidosis in healthy volunteers induced by ammonium chloride administration brings about an impairment of in vivo glucose metabolism under experimental conditions of hyperglycemia and hyperinsulinemia (DeFronzo & Beckles, 1979). Similarly, studies in diabetic ketoacidotic rats with intravenous insulin infusion have shown that both maximal insulin responsiveness and insulin sensitivity with respect to the rate of decline in plasma glucose levels are directly proportional to pH (Cuthbert & Alberti, 1978). In the same animal model, experimental diabetic ketoacidosis caused a pronounced fall in insulin binding to fat cells (Whittaker et al., 1981). In human adipocytes, it has also been demonstrated that insulin binding and action is strongly dependent on pH. Thus, at pH 7.0 insulin binding is decreased 50% and accompanied by a rightward shift of the dose response curve for insulin-stimulated glucose transport (Pederson & Gliemann, 1981).

Hypoinsulinemia, with accompanying catabolism, is associated with attenuated responsiveness of glucose metabolism to insulin. Another factor that potentially might add to the insulin resistance of metabolically decompensated diabetics is hyperglycemia causing hyperosmolarity. Experimental hyperosmolarity in normal humans induced by mannitol infusion (Bratusch-Marrain & DeFronzo, 1983) causes a decrease in glucose clearance. Likewise, study of muscle tissue from rodents indicate that hyperosmolar impair insulin-stimulated glucose transport (Forsayet & Gould, 1981).

Diabetic ketoacidosis is characterized by elevated please concentrations of growth hormone, cortisol catecholamines, and glucagon (Unger, 1965; Chelatensen, 1974; Waldbausal et al., 1979; Muller et al., 1983). In vivo experiments have demonstrated that these first three may express their insulin-antagonizing effects through an inhibition of glucose disposal is peripheral tissues (Deibert & DeFronzo, 1980;
Bratusch-Marrain et al., 1982; Rizza et al., 1982a, 1982b; Sacca et al., 1982; Bessey et al., 1983). In vitro studies in human fat is have partly elucidated the mechanisms behind these effects. Thus, epinephrine inhibits the glucose-transport step (Kashiwagi et al., 1983a), where as both growth hormone (Nyberg et al., 1980) and cortisol (Cigolini & Smith, 1979) suppress cellular glucose metabolism. All the counterregulatory hormones, including glucagon, also contribute to glucose intolerance in this state by facilitating hepatic glucose release.

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The increased plasma levels of FFA and ketone bodies may also have pathophysiologic impact on insulin action during ketoacidosis. In vivo studies in humans have shown that elevated plasma FFA concentrations cause insulin resistance. Both in hepatic and peripheral tissues (Randle et al., 1963; Ferrannini et al., 1983). Increase muscle FFA oxidation results in an inhibition of pyruvate dehydrogenase, which controls glucose oxidation (Randle et al., 1964; Hagenfeldt, 1979), and in liver tissue there is some evidence that FFA oxidation may enhance gluconeogenesis (Blumenthal, 1983; Ferrannini et al., 1983). Studies of culture lymphocytes and fibroblast have shown that addition of β hydroxybutyrate to the media may increase insulin binding at subphysiologic pH levels (Merimee et al., 1976, Hidaka et al., 1981). In rat adipocytes, β hydroxybutyrate had little effect on insulin binding. However, the ketone compound potentiated the effect of submaximal concentrations of insulin; i.e. it resulted in a leftward shift in the dose-response curve for glucose transport (Green et al., 1984). Hence, it is possible that ketone bodies may countered to some extent the impairing effects of acidosis on insulin action in ketotic diabetics.

Finally, it cannot be excluded that tissue electrolyte disturbances may aggravate the impaired effects of insulin during ketoacidosis because experimental hypophosphatemia or hypomagnesemia results in a decrease of in vivo glucose utilization of peripheral tissues (DeFronzo & Lang, 1980; Moles & McMullen, 1982; Yajnik et al., 1984).

Taken as a whole, many factors may cause insulin resistance during diabetic ketoacidosis, but their relative roles are currently and peripheral tissues. We do not know if the insulin resistance is
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predominantly associated with the insulin-binding locus or with the many postbinding steps in cellular insulin action, because no data are available on insulin binding and action in hepatocytes or skeletal muscle from ketoacidotic diabetic humans.

**Conclusion**

In IDDM the insulin resistance of peripheral tissues adds to glucose intolerance. The resulting higher plasma glucose levels will by the mass of action increase the glucose uptake in non-insulin-sensitive tissues such as the brain, nerves, and endothelial cells. Together with the peripheral hyperinsulinaemia this phenomenon may have pathogenetic impact on both vascular and neural complication.

It is therefore essential to reduce or eliminate insulin resistance. How can it be done? One possibility is to deliver insulin to the portal circulation by implantation of insulin pumps. This approach will eliminate portal hypoinsulinaemia and peripheral hyperinsulinaemia and consequently, the abnormal metabolism. Until a safe closed-loop system for portal insulin delivery is available, near normalization of glycemia and plasma levels of intermediary metabolites may be believed by intensified subcutaneous insulin administration with insulin pens or pumps. Regular exercise and ingestion of a high-starch, high-fiber, low-fat diet also may have long-term beneficial effects on insulin action.

**3.1.4 Insulin Resistance In Non-insulin-Dependent (type II) Diabetes Mellitus (NIDDM)**

Simple insulin deficiency can not entirely account for the diabetic syndrome in patients with non-insulin-dependent (type II) diabetes mellitus. The evidence for this statement is and comes from the observations that: (1) insulin deficiency does not exist in many type II diabetic patients (Reaven et al., 1976), while (2) In type II diabetic patients who have impaired insulin secretion, insulin resistance can be directly demonstrated (Reaven et al., 1976; DeFronzo et al., 1979a).
Patients with mild diabetes often have normal or even elevated plasma insulin levels following an oral glucose challenge. This combination of glucose intolerance in the face of normo or hyperinsulinaemia clearly indicates an insulin resistant state and these observations have been made where control groups are both age- and weight-matched. When non-obese, type II diabetic patients with significant fasting hyperglycemia are studied, the picture is less clear. These patients may have normal or elevated basal insulin levels but stimulated plasma insulin levels are uniformly high (DeFronzo et al., 1979a). Thus, based solely on simultaneous measurement of concomitant plasma glucose and insulin levels, the presence of insulin resistance in many mild diabetic patients seems straightforward, while the status of insulin effectiveness in the more severe diabetic patient with fasting hyperglycemia is difficult to evaluate. Various studies have reported from a number of different laboratories using a variety of techniques, an attenuated hypoglycemic response to administered exogenous insulin (Kalant et al., 1963; Ginsberg et al., 1975; Reaven et al., 1976; Olefsky & Reaven, 1977; DeFronzo et al., 1979a).

Obesity is a well-known condition that leads to the development of insulin resistance and, since the great majority of adult, type II diabetic patients are overweight, obesity-induced insulin resistance is frequently a contributing factor in the hyperglycemia of these patients. However, obesity cannot account for all of the insulin resistance in this type of diabetic patient since the insulin resistance is greater than that which can be accounted for on the basis of the obesity alone, and furthermore, many non-obese non-insulin-dependent diabetic patients are also insulin-resistant. This subject has been reviewed several times in recent years (Olefsky, 1976; Reaven et al., 1976; Reaven & Olefsky, 1978; Olefsky & Ciaraldi, 1980).

Euglycemic glucose clamp technique was used to provide direct quantitative evidence for insulin resistance in patients with impaired glucose tolerance and overt type II diabetic patients. The patients with impaired glucose tolerance had fasting plasma glucose levels consistently less than 155 mg/dl but had abnormal oral glucose tolerance tests. The
Type II diabetic patients had fasting hyperglycemia (>140 mg/dl) and the mean fasting glucose level in this group of patients was 227 mg/dl. When euglycemic glucose clamp studies were performed at an insulin infusion rate of 40 mU/M^2/min, it was observed that despite comparable steady state insulin levels, the glucose disposal rates decreased in the diabetic groups and the magnitude of this defect was greatest in the most carbohydrate intolerant patients. Thus, in the type II diabetic patients with fasting hyperglycemia, peripheral glucose disposal rates were found to be decreased by 53% indicating marked insulin resistance.

Only in very rare instances can abnormal beta cell secretory products or circulating insulin antagonists lead to insulin resistance in type II diabetic patients (Olefsky & Ciaraldi, 1980). Consequently, the cause of this insulin resistance must reside at the level of the target tissue. To help elucidate this abnormality, when studies of insulin binding to receptors were performed on circulating monocytes from normal and type II diabetic patients with fasting hyperglycemia, it was obvious that the ability of cells from the diabetic patients to bind insulin is greatly reduced and the differences are highly statistically significant at each insulin concentration. This decrease in insulin binding can be due to either a decrease in receptor number, receptor affinity or both. Scatchard plots of these insulin binding data demonstrated that the decreased insulin binding to cells in these diabetic patients is due to a reduced number of cellular insulin receptors. When similar studies were performed on patients with impaired glucose tolerance, analogous results were obtained.

The simple demonstration of decreased insulin receptors in the setting of insulin resistance does not necessarily imply cause and effect, and the actual role of this cellular abnormality in the pathogenesis of the in vivo insulin resistance of diabetes has not been clarified. When steady state plasma glucose levels were plotted as a function of insulin binding in normal subjects and patients with impaired glucose tolerance, a highly significant inverse correlation was found to exist such that the lower level of insulin receptors, the greater the insulin resistance (Shen et al., 1970). On the other hand when this relationship was examined in type II
diabetic patients with fasting hyperglycemia, a correlation was found between the degree of insulin resistance and the levels of insulin binding, with the degree of insulin resistance much greater than in subjects with impaired glucose tolerance. This suggests that insulin resistance in diabetic patients with significant fasting hyperglycemia may be due to defects distal to the insulin binding step.

Tissue resistance to insulin is an important component of the glucose intolerance of type II diabetes mellitus.

3.1.4.1 Hepatic Insulin Resistance

Basal rates of hepatic glucose production in patients with type II diabetes mellitus have been documented as normal or increased (Best et al., 1982; Bogardus et al., 1984a; Kolterman et al., 1984; Revers et al., 1984a; Simonson et al., 1984). As with measurements of insulin secretion, it is important that these production rates be evaluated in the context of the glucose concentration at which they were measured. When this is done, it is apparent that even the normal values are inappropriately elevated for the ambient glucose level. In fact, the degree of the abnormality in hepatic glucose output is positively correlated with the degree of fasting hyperglycemia, suggesting that the rate of hepatic glucose production is an important determinant of the fasting plasma glucose levels.

The increased rate of hepatic glucose production results from an impairment of the effects of insulin and glucose to normally suppress glucose release by the hepatocyte. A shift to the right in the insulin dose-response curve with no reduction in the maximal suppressive response at supraphysiological insulin levels has been demonstrated in diabetic subjects studied at euglycemia (Kolterman et al., 1981). This type of change is compatible with a reduction in hepatic sensitivity to insulin produced by a decrease in insulin receptor number. However, when similar studies are performed in type II diabetics at basal hyperglycemia, maximal suppression of hepatic glucose production occurs at lower insulin levels but the dose-response relation still demonstrates a defect in insulin action when compared to control subjects studied at
normoglycemia (Revers et al., 1984a). Thus, hyperglycemia appears capable of exerting a suppressive effect on hepatic glucose output independent of insulin, but is unable to fully compensate for the reduction in insulin sensitivity found in type II diabetics. This suggests that a defect in the ability of glucose to inhibit its own release from the liver is also contributing to the observed glucose overproduction in the basal state. Glucagon, which is of major importance in the maintenance of postabsorptive hepatic glucose release (Liljenquist et al., 1977; Wahren et al., 1977) has recently been demonstrated to be capable of maintaining more than half of the hepatic glucose production observed in type II diabetics (Baron et al., 1987a). As a result, the abnormal regulation of glucagon secretion in these subjects may help explain the observed hepatic resistance of type II diabetics to the suppressive effects of both insulin and glucose.

During oral intake the liver plays a critical role in the maintenance of glucose homeostasis. The meal-induced alterations in the concentrations of glucose, insulin and glucagon entering the liver changing from its status in the fasted condition as an organ responsible solely for glucose production to one that, during refeeding, restores its glycogen content by increasing uptake and/or synthesis of glucose. Therefore, considering the defects in hepatic sensitivity to glucose and insulin, it is not surprising that following an oral glucose load a delayed reduction in hepatic glucose production can be demonstrated in type II diabetics (Felig et al., 1978). This failure of the liver to adequately suppress its glucose production accounts for a considerable proportion of the observed rise in plasma glucose concentrations following meal ingestion. While a large proportion of this defect in suppression of hepatic glucose release may result from the deficient insulin response, neither the contribution of the increased glucagon response during meals nor the potential for variability in hepatic sensitivity to other neurohormonal responses following oral intake have as yet been identified.
3.1.4.2 Peripheral Insulin Resistance

Using the euglycemic insulin clamp technique it has been conclusively demonstrated that a reduction of more than 55% in the mean glucose disposal rate exists in subjects with type II diabetes (Kolterman et al., 1981). Further analysis of the in vivo dose-response relation suggests that this reduction in insulin responsiveness is the result of two abnormalities. First, the rightward shift in the curve is compatible with a reduction in cellular insulin receptor number. A decrease in receptor number has been reported in in vitro studies using monocytes (Olefsky & Reaven, 1977), erythrocytes (DePirro et al., 1980) and adipocytes (Kolterman et al., 1981). Despite the presence of spare or unoccupied receptors, the marked decrease in the maximal rate of glucose disposal suggests the existence of a second defect in peripheral insulin action, namely a postbinding (intracellular) defect (Kolterman et al., 1981). Insulin-binding studies on isolated adipocytes from individuals with type II diabetes mellitus have shown that the predominant determinant of the severity of the peripheral insulin resistance in untreated patients is this reduction in postbinding insulin action (Kolterman et al., 1981). Further analysis has revealed that part of this defect in intracellular insulin action results from a reduction in the number of glucose transporters (Ciaraldi et al., 1982; Garvey et al., 1988).

These preceding observations of reduced insulin effectiveness were all made under euglycemic conditions and thus do not take into account the ability of glucose, by virtue of mass action, to augment its own disposal into the peripheral tissues (Best et al., 1981; Verdonk et al., 1981). When incremental insulin dose-response studies are performed at the basal level of hyperglycemia in type II diabetics, the relation of insulin’s effect on peripheral glucose disposal is essentially identical to that observed in matched control subjects studied at euglycemia (Revers et al., 1984a). These findings suggest that in the presence of hyperglycemia, any impairment of peripheral insulin action is overcome by a mass action increase of glucose uptake increases by mass action so that a new steady state is created in which the increased glucose levels
are associated with increased glucose utilization despite the impairment of insulin action.

The efficiency of glucose uptake following oral glucose ingestion is also defective in type II diabetics. In the peripheral tissues, ingested glucose normally undergoes oxidative and nonoxidative metabolism, with the rate of these processes being controlled by the enzymes pyruvate dehydrogenase and glycogen synthase, respectively. At low insulin concentrations the major route of peripheral glucose disposal is via glucose oxidation, while at higher levels disposal occurs predominantly by glycogen synthesis (Thiebaud et al., 1982; Mandarino et al., 1987). In type II diabetics the efficiency of glucose uptake by both processes is reduced, with the predominant abnormality being a defect in non-oxidative glucose storage (Felber et al., 1981; Boden et al., 1983). As glycogen synthase activity is stimulated by insulin, the diminished insulin sensitivity compounded by the reduced insulin secretory response to meals leads to a failure to stimulate normal enzyme activity (Wright et al., 1988).

Thus, the mechanisms of insulin resistance in non-insulin-dependent diabetes are heterogeneous and as in obesity, a spectrum of defects exists. In those patients with mild insulin resistance, the receptor defects predominates whereas in those patients with severe insulin resistance, the post-receptor defect predominates.

3.1.5 Unusual Forms Of Insulin Resistance

3.1.5.1 Syndromes Of Insulin Resistance And Acanthosis Nigricans

In a series of well-documented reports Flier, Kahn, Roth and colleagues have described a group of patients with extreme insulin resistance and marked acanthosis nigricans, in the absence of any other diseases associated with insulin resistance such as lipodystrophy, Cushing's disease, acromegaly and so forth (Flier et al., 1975; Kahn et al., 1976, 1977). Patients with these syndromes are classified into two general groups (Kahn et al., 1976). Type A patients tend to be young
females with hirsutism, polycystic ovaries, mild virilism, coarse features, early accelerated growth and of course, acanthosis nigricans. Type B patients tend to be older females (age 37-49), although two males with this entity have now been reported. The syndrome in these patients is suggestive of an autoimmune disease with features such as hypergammaglobulinemia, proteinuria, hypocomplementemic nephritis, leukopenia, arthralgia, alopecia, enlarged salivary glands and positive nuclear and anti-DNA antibodies. Patients with either of these subtypes have carbohydrate intolerance ranging from mild abnormalities of the oral glucose tolerance test to severe fasting hyperglycemia requiring enormous amounts of exogenous insulin. All of these patients are extremely hyperinsulinaemic and respond poorly to the administration of exogenous insulin.

The mechanisms of this insulin resistance have been well studied and in addition to learning about the cause of insulin resistance in a rare form of diabetes, these studies have provided important insights into overall insulin action. In type B patients, the mechanisms for the insulin resistance are fairly clear cut. These patients have a severe defect in insulin binding to circulating monocytes, which is due primarily to an apparent reduction in binding affinity (Flier et al., 1975; Kahn et al., 1976). More important it has been clearly demonstrated that these patients have circulating antibodies directed against some portion of the insulin receptor which impairs insulin binding to the patient’s cells (Flier et al., 1975). When immunoglobulins from these patients’ sera are isolated and incubated in vitro with various cell types, subsequent measurements of insulin binding are also decreased. Furthermore, when the patients’ freshly isolated circulating monocytes are stripped of adherent immunoglobulins, insulin binding returns to normal. Thus, the role of the anti-receptor antibody to directly interfere with the ability of insulin to bind to receptors in vivo is undisputed in these patients.

The insulin resistance of these patients can be quite severe and one subject received as much as 177,000 U of insulin/day. The metabolic abnormalities in this syndrome can wax and wane and a few patients have been described who have experienced complete remission.
of the insulin-resistant diabetic state with disappearance of anti-receptor antibodies from the plasma. In at least two patients remission occurred during immunosuppressive therapy; however, the documented occurrence of spontaneous remissions makes the potential casual relationship between drug therapy and remission unclear.

In other patients with genetic forms of severe insulin resistance (e.g. Type A patients), various defects in insulin action have been described. Some of these patients show marked decreases in insulin binding affinity, whereas others show reduced levels of receptor expression. Intrinsic defects in receptor kinase activity or post-receptor abnormalities have also been reported. With the recent advances in the techniques of molecular genetics, various mutations leading to structural alterations impairing receptor function will be found. Quite likely some of these patients will display structural or regulatory defects in the insulin receptor gene which, in different subjects, lead to abnormal binding, decreased receptor kinase activity, impaired processing of the receptor polypeptide or abnormal receptor gene transcription. Several such mutations in the insulin receptor gene have already been reported.

3.1.5.2 Generalized Lipodystrophy And Lipoatrophy

Many patients have been described who have generalized loss of subcutaneous and deep adipose tissue, hepatosplenomegaly, hyperlipoproteinemia, hypermetabolism and diabetes mellitus. Frequently these patients also have acanthosis nigricans. The form of diabetes associated with this syndrome is of the insulin-resistant type. Thus, these patients have very elevated circulating plasma insulin levels and respond poorly to the administration of exogenous insulin. Similar findings can be observed in patients partial lipodystrophy in whom adipose tissue loss can occur exclusively above the waist, or less frequently, only below the waist. The mechanism of insulin resistance in the lipodystrophies is poorly understood and only a few, and conflicting, reports exist. For example, Oseid et al. (1977) have found that patients with generalized lipodystrophy have decreased insulin binding to circulating monocytes due to a slight decrease in receptorumber and a
large decrease in receptor affinity. However, upon fasting, the ability of cells from these patients to bind insulin increased normally, suggesting that this defect was secondary to the existing hyperinsulinaemia rather than a primary abnormality. On the other hand, Dorfler et al. (1977) have demonstrated decreased insulin binding to cultured fibroblasts from patients with this syndrome and this suggests that the defect may be genetic and primary. Finally Rosenbloom et al. (1977) have found normal insulin binding to cultured fibroblasts and Wachslich-Rodbard et al. (1981) reported normal insulin binding to circulating monocytes from these patients. Possibly these disparate findings indicate that different defects exist in different patients.

3.1.5.3 Ataxia Telangiectasia

Ataxia telangiectasia is a rare recessive syndrome characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia, recurrent sinopulmonary infections and a number of diverse abnormalities of the immune system. Frequently, this syndrome is also accompanied by glucose intolerance and insulin resistance. Schalch et al. (1970) reported that about 60% of patients with this syndrome displayed glucose tolerance, hyperinsulinaemia and an attenuated hypoglycemic response to exogenously injected insulin. In the patients who have been well studied, secondary causes of insulin resistance such as circulating insulin antibodies, obesity, lipoatrophy and excessive growth hormone or corticosteroid secretion have been ruled out. Thus, the insulin resistance which can occur in ataxia telangiectasia appears to be an intrinsic component of this syndrome.

In general, the degree of glucose intolerance has been mild, rarely requiring insulin therapy. Furthermore, a tendency towards exacerbation and remission of the insulin-resistant state had been noted. Recently, data have become available which may reveal the mechanisms of insulin resistance in this syndrome. Earlier workers (Schalch et al., 1970) recognized the resistance to the hypoglycemic effects of exogenous insulin and suggested an unknown circulating insulin antagonist might be a causative factor. Bar et al. (1978) published detailed studies in two
patients with ataxia telangiectasia and insulin resistance. A marked decrease in the binding affinity of insulin receptors on freshly isolated circulating monocytes was noted, while the insulin receptors of fibroblasts cultured from skin biopsies were perfectly normal. The fact that the insulin receptor abnormality did not persist in cell culture indicated that this defect was not genetic and was likely to be secondary to some aspect of the in vivo environment. In confirmation of this idea, these workers were able to demonstrate a circulating inhibitor of insulin binding, most likely an immunoglobulin, in the plasma of these patients. On the basis of these studies, it would appear that insulin resistance in ataxia telangiectasia is due to the production of an antibody, which inhibits insulin binding. Serial studies in one patient demonstrated the disappearance of this circulating inhibitor, coincident with the remission of the insulin resistance (Bar et al., 1978) and this greatly strengthens the argument for a cause and effect relationship between these variables.

3.1.5.4 Leprechaunism

Leprechaunism is a very rare congenital disease characterized by an unusual facial appearance, hirsutism, cliteromegaly, acanthosis nigricans, sparse subcutaneous fat stores and a number of other somatic abnormalities. This syndrome is almost always fatal and patients have been known to die with hyperglycemia and histologic findings of β-cell hyperplasia. Several children with this syndrome have now been studied (Kobayashi et al., 1978b; D’Ercol et al., 1979; Schilling et al., 1979). In those patients in whom in vivo data are available, hyperinsulinaemia and severe insulin resistance are present (Kobayashi et al., 1978b). The immunoreactive material in the plasma of at least one of these patients has been shown to be genuine insulin of normal biologic activity (Kobayashi et al., 1978b). Circulating insulin antagonists have not been detected and this points to a cellular defect in insulin action (Kobayashi et al., 1978b). A variety of cellular defects have been described in the patients studied to date; thus, some patients exhibit relatively normal insulin binding to receptors, but display a marked postreceptor defect in insulin’s ability to stimulate either glucose transport or thymidine
incorporation into DNA (Kobayashi et al., 1978b; D'ercoli et al., 1979). Other patients with this disorder have displayed a marked reduction in cellular insulin-binding capacity with or without (Schilling et al., 1979) a postreceptor defect in insulin action. From these studies, it seems likely that leprechaunism is a rather general syndrome, representing a common phenotypic expression of a variety of genetic abnormalities. Recently, mutations in the insulin receptor gene have been described in several patients with this disorder (Taylor et al., 1986; Endo et al., 1987; Reddy et al., 1988) and it is likely that more receptor gene mutations will be identified as additional patients are studied. Interestingly, the mutations thus far described have been different, indicating that more than one genotype can lead to a common phenotype. It will be of importance to eventually unravel the relationship between the receptor mutation and the multiple congenital anomalies displayed by these subjects.

3.1.6 Therapeutic Interventions To Reducing Insulin Resistance

3.1.6.1 Diet

High-starch, low-fat dieting improves insulin-mediated glucose metabolism (Himsworth, 1933). However, there are data on the effect of that type of diet on in vivo insulin action in IDDM patients. At the cellular level an increased binding of insulin to monocytes has been shown in IDDM patients given diets high in digestible and indigestible complex carbohydrates and low in fat (Pederson et al., 1982b).

There is little doubt that caloric restriction and weight reduction improve insulin sensitivity, insulin binding and glucose tolerance in obese, non-insulin-dependent diabetics (Olefsky, 1976; Beck-Nielsen, 1978; Beck-Nielsen et al., 1979; Beck-Nielsen et al., 1980). Whether normal weight diabetics would benefit from dietary manipulation and if so, of what kind, is not clear. Several lines of evidence would suggest that an isocaloric redistribution of nutrients with an increase in amount of carbohydrate can favorably affect insulin sensitivity. Thus, glucose tolerance has been reported to be improved in non-obese, mild diabetics
on a high carbohydrate, isocaloric diet (Brunzell et al., 1971). Kolterman et al. (1979) have also shown that a high carbohydrate diet can appreciably enhance in vivo insulin sensitivity in healthy subjects after only 2 weeks, despite a concurrent decline in insulin receptor number. When physiologic hyperinsulinaemia with maintenance of basal glucose levels is sustained for 3-5 hrs in healthy subjects, insulin-mediated glucose uptake increases continuously with time, while insulin binding to circulating monocytes falls significantly (Insel et al., 1980). These observations thus indicate that a high carbohydrate diet can enhance insulin sensitivity by stimulating postreceptor steps of insulin action and that this effect is mediated by the higher level of insulin insulinization resulting from chronic β-cell overstimulation. It is intriguing to consider that, if this was the case then the receptor loss associated with chronic hyperinsulinaemia would possibly restrain but not abolish the stimulating action of insulin on glucose metabolism. Receptor changes would thus mirror the state of insulinization without of themselves causing insulin insensitivity.

However, contrary evidence has also been provided i.e. a low carbohydrate diet can improve oral glucose tolerance and increase tissue sensitivity to insulin in non-insulin-dependent diabetics (Beck-Nielsen et al., 1980). Furthermore, the possibility cannot be discarded that a high carbohydrate diet might "exhaust" the β-cells. One might also expect that the resultant hyperinsulinaemia would stimulate triglyceride synthesis and expose the patient to the long-term consequences of hypertriglyceridaemia. It is clear that the effects of dietary manipulations are nor unequivocally predictable and further studies are necessary before any regimen can be advised as a therapeutic adjunct in the management of diabetes mellitus.

3.1.6.2 Physical Exercise

Trained athletes have normal or supernormal glucose tolerance despite plasma insulin responses that are slightly lower than those of untrained individuals (Lohmann et al., 1978; LeBlanc et al., 1979). Acute submaximal exercise is associated with an increase in insulin binding to
monocytes and erythrocytes from untrained patients with IDDM (Pederson et al., 1980). These two observations suggest that physical activity has a beneficial effect on insulin sensitivity both in normal and diabetic subjects. In keeping with this conclusion as measured with the euglycemic insulin clamp at a single steady state insulin concentration in continuous subcutaneous insulin infusion (CSII) treated IDDM patients who participated in a 6-wk moderate training program, the insulin-stimulated glucose disposal to peripheral tissue has been found to rise by 60% compare with sedentary CSII treated control IDDM patients, whereas, no difference has been observed in the maximal insulin suppressive effect on the liver. A significant (~30%) improvement in insulin-mediated glucose uptake has also been observed after 9-wk of regular physical training in normal weight, sedentary healthy volunteers, on obese subjects and in patients with NIDDM as measured with the insulin clamp technique (DeFronzo & Ferrannini, 1982).

3.1.6.3 Drugs
A. Insulin

The direct effect of insulin on peripheral insulin resistance is controversial. Several studies (Garvey et al., 1985) have shown that intensive insulin treatment for several weeks produced a 72% increase in the maximal rate of insulin-stimulated glucose uptake by peripheral tissues. However, these observations do not define whether some metabolic derangement that occurs secondary to the insulin deficiency or insulin deficiency per se is responsible for the development of insulin resistance since treatment simultaneously corrects both the lack and its resultant metabolic disturbances. Karnieli et al. (1987) in a study described that insulin therapy for 8 days in streptozotocin diabetic rats was associated with a threefold increase in glucose transport activity in the intact adipose cell compared with control and with about a sixfold increase when compared with cells isolated from diabetic rats.

Several groups of investigators have looked at the effects of CSII on in vivo insulin action in IDDM patients. In these reports peripheral tissue sensitivity to insulin has been studied with the euglycemic insulin
clamp at one or more steady-state plasma insulin concentrations (Lager et al., 1983; Beck-Nielsen et al., 1984; Yki-Jarvinen & Koivisto, 1984a; Simonson et al., 1985;). Near normalization of glycemia and normalization of plasma concentration of insulin-antagonistic hormones and FFA with CSII partially corrected but did not restore to normal the insulin-stimulated glucose disposal to peripheral tissues. In one study the prepump basal glucose production in the liver was elevated but showed a significant fall during pump treatment (Yki-Jarvinen & Koivisto, 1984a), whereas in another study the prepump basal hepatic glucose output was normal and showed no change during CSII (Simonson et al., 1985). Insulin binding to adipocytes and blood cells is unaltered and the basal glucose transport and metabolism in adipocytes are further suppressed during CSII (Pederson et al., 1986).

CSII causes a decrease of basal hepatic glucose output. In vivo studies also indicate an increment but not a normalization of peripheral insulin sensitivity. The improved peripheral glucose clearance seems to reflect an increase of glucose utilization in muscle tissue because in vitro studies have failed to demonstrate any beneficial effects of CSII on insulin-mediated adipocyte glucose processing. It is hypothesized that the CSII-induced increase of glucose turnover in skeletal muscles may at least in part be secondary to the lower plasma levels of FFA, which increases the glucose oxidation by enhancing the activity of pyruvate dehydrogenase.

B. Sulphonyl Ureas

It has long been known that sulfonyl ureas have extra-pancreatic actions (Feldman & Lebovitz, 1969). When administered acutely, these agents cause a prompt increase in insulin secretion, which can be demonstrated both in vivo (Feinglos & Lebovitz, 1980; Yalow et al., 1960) and in vitro (Loubatieres et al., 1970; Gotfredsen, 1986). This stimulatory effect is observed in both the presence and absence of glucose and is exerted on both the early and late phases of insulin secretion (Loubatieres et al., 1970; Gotfredsen, 1986).
The effect of glibenclamide treatment on insulin-mediated glucose disposal was studied in C-peptide-negative IDDM patients by applying the euglycemic insulin clamp (Pernet et al., 1985). At a physiological steady-state plasma insulin concentration the glucose disposal rate to peripheral tissues increased by 35%. However, with chronic sulfonyl urea treatment basal and insulin stimulated plasma insulin levels return to normal or below (Sheldon et al., 1966; Reaven & Dray, 1967; Chu et al., 1968; Varsano-Aharon et al., 1970; Duckworth et al., 1972; Barnes et al., 1974; Dunbar & Foa, 1974), yet glucose tolerance remains improved. The study by Duckworth et al. (1972) exemplified the findings of prolonged sulfonyl urea therapy. After 6 month therapy with gliburide in seven patients with NIDDM, the fasting plasma glucose levels decreased to normal without any change in plasma insulin levels and glucose tolerance also improved considerably despite a slight decrease in plasma
insulin response. This improvement in glucose tolerance was maintained at 12 months in the face of even greater (40%) reduction in the plasma insulin response.

The increase in tissue sensitivity to insulin induced by these agents appears to be related to enhanced glucose uptake by peripheral tissue (Feldman & Lebovitz, 1969; Feinglos & Lebovitz, 1980) as well as greater efficacy of insulin in inhibiting hepatic glucose production (Tarding & Schambage, 1958; Kaldor & Pogatsa, 1960; Pogatsa & Kaldor, 1963; Colwell & Lein, 1967). In addition, recent evidence indicates that the improvement in insulin sensitivity may be mediated, at least in part, by an increase in insulin binding to membrane receptors in a variety of tissues, including liver cells (Feinglos & Lebovitz, 1978), human fibroblasts (Prince & Olefsky, 1980) and circulating monocytes from patients with NIDDM (Olefsky et al., 1973; Beck-Nielsen et al., 1979). The precise mechanism of action of sulfonyl ureas on tissue sensitivity to insulin in vivo, however, is not known. It has been suggested that these agents may increase the number of insulin receptors by slowing down receptor degradation.

C. Metformin

Metformin, a biguanide that does not bear risk of lactic acidosis unlike phenformin, ameliorates hyperglycemia by improving peripheral sensitivity to insulin and reducing hepatic glucose production (via gluconeogenesis) as well as by limiting gastrointestinal glucose absorption (Iannello, 2000). Metformin also increases glucose utilization by intestine, primarily via nonoxidative metabolism. This results in extra lactate production, which is taken up by liver and used as a gluconeogenic substrate. Unlike sulfonyl ureas, it does not stimulate insulin secretion, nor does it aggravate hyperinsulinaemia or cause hypoglycemia or weight gain (Iannello, 2000). Its more prominent effect in type II diabetic patients is on postprandial hyperglycemia. It is considered as a first-line agent particularly in obese and/or hyperlipidaemic type II diabetic patients (Lefebvre & Scheen, 1992).
Biguanides have been shown to lower insulin needs in IDDM patients and in a short-term study with the euglycemic clamp at a single steady-state plasma insulin level, an 18% improvement in glucose utilization of peripheral tissues was demonstrated in metformin-treated IDDM (Gin et al., 1985).

D. Thiazolidinediones

Thiazolidinediones (pioglitazone, troglitazone, rosiglitazone) reduce fasting hyperglycemia and insulinaemia by improving insulin sensitivity in skeletal muscles, adipose tissue and hepatocytes, while normalizing a wide range of metabolic abnormalities associated with insulin resistance. They activate the peroxisome proliferator activated receptor gamma (PPAR-γ) which acts in conjunction with the retinoid X receptor by de-repression to increase transcription of certain insulin sensitive genes (Henry, 1997; Spiegelman, 1998). Reported effects include (a) decrease in plasma triglyceride, FFA and LDL cholesterol levels and increase in plasma HDL cholesterol, (b) increased expression of glucose transporters GLUT-1 and GLUT-4, (c) activation of glycolysis in hepatocytes, (d) antagonism towards some of the effects of TNF-α, (e) decrease in blood pressure, (f) inhibition of vascular smooth muscle cell proliferation and hypertrophy, (g) enhanced endothelium-dependent vasodilation and (h) antioxidant action (lannello, 2000). Although, thiazolidinediones so not stimulate insulin secretion, they improve the secretory response to b cells to insulin secretagogues. Unfortunately, rare cases of live failure have been associated with troglitazone therapy (Gitlin et al., 1998; Neuschwander-Tetri et al., 1998; Vella et al., 1998). Though newer compound, rosiglitazone does not appear to be hepatotoxic, it is contraindicated in patients with history or sign/symptoms of liver disease and its use requires monitoring of liver function test. Other issues such as fluid retention, haemodilution, an increase in plasma cholesterol and other effects of PPAR-γ stimulation are under investigation.
E. Immunosuppressive Therapy

Kawanishi et al. (1977) described a patient with severe insulin resistance accompanied by hypergammaglobulinaemia and Sjogren's syndrome. In this patient there was a high titre of anti-insulin receptor antibodies. They considered the possibility that the hypergammaglobulinaemia might be a cause of the peripheral insulin unresponsiveness and subsequent severe insulin resistance. Immunosuppressive therapy was attempted consisting of prednisolone and cyclophosphamide. On this treatment regimen, blood glucose levels decreased in parallel with serum gammaglobulin levels. After immunosuppressive therapy for 8 months, the diabetic syndrome disappeared completely and anti-receptor antibodies in the serum were no longer detectable. Insulin sensitivity returned to normal. However, the patient's glucose tolerance deteriorated after the temporary termination of cyclophosphamide treatment and the lowering of prednisolone dosage.

Steroids are recommended for the treatment of insulin resistance caused by presumed insulin binding to high levels of antibody (Foster, 1983). The way in which steroids enhance the sensitivity to insulin can be explained, at least in part, by the fact (Knuston, 1986) that glucocorticoids can decrease the rate of receptor inactivation, perhaps through direct action on enzyme(s) which inactivate the receptor number at the level of the gene or by allosteric modification) and by increasing the total number of receptors.

F. Other Compounds (Iannello, 2000)

The long acting, non sulphhydryl-containing ACE-inhibitor, trandolapril alone and in combination with the Ca$^{2+}$-channel blocker, verapamil, can significantly improve whole body glucose metabolism by acting on the insulin sensitivity skeletal muscle glucose transport system in obese Zucker rats. Data on the role of TNF-α raise the possibility that pharmacological inhibition of this factor may provide a novel therapeutic target to treat patients with type II diabetes.
3.2 Micronutrient Status in Diabetes Mellitus

The intricate relationship between nutrition and diabetes mellitus was suspected as early as 1674 when Sir Thomas Willis suggested that patients with diabetes mellitus should be advised to have gummy and starchy food. Since then a variety of dietary recommendations have been made by different diabetologists (Ernest et al., 1965; Anderson, 1977; Nuttall, 1983; Nuttall et al., 1984). Despite the large literature on the role of dietary composition in control of diabetes mellitus, there are relatively few studies on the effect of diabetes mellitus on nutrient status of the individual. Malnutrition has been suggested as a cause of diabetes mellitus in certain geographic areas (Golden & Golden, 1983; Oli, 1983). However, the exact pathogenetic role of malnutrition in diabetes mellitus has been disputed.

Over last 20 years, numerous studies have found alterations in micronutrient status of patients with diabetes mellitus and in some studies deficiency of certain minerals or vitamins had been correlated with the presence of diabetic complications. Nutritional management of diabetes mellitus has, until recently. Concentrated on regulation of micronutrient intakes. There is now increasing evidence that micronutrient intake may also be important in promoting optimum health for diabetic patients (Mooradian & Morley, 1987). Recent improvements in trace element methodology have yielded more reliable information on the status of essential trace elements in people with diabetes, which may open up new opportunities for improving nutritional management of diabetes.

Trace elements are those elements with an estimated dietary requirement usually less than 1 μg/g and often less than 50 ng/g diet for laboratory animals (Nielsen, 1993). Because many elements are still under study a complete list of essential elements remains controversial.
As of 1994 these may be classified as follows:

(1) Elements accepted as essential in animals or humans
   (a) Defined biochemical functions and signs of deficiency in humans known: iron, zinc, copper, cobalt (as in vit B<sub>12</sub>), iodine, molybdenum, selenium
   (b) Signs of deficiency described in humans but biochemical functions not defined conclusively: chromium and boron
   (c) Essential functions described but unequivocal signs of deficiency in humans not identified: manganese
   (d) Likely to be essential in humans based on animal findings: nickel, silicon, vanadium and arsenic

(2) Elements suggested to be essential — but with controversial or limited data: bromine, cadmium, lead, strontium, tin

In a tertiary referral centre such as the Prince of Wales Hospital, trace element deficiencies will be encountered quite commonly. Protein-energy malnutrition (PEM) frequently accompanies severe or chronic disease or trauma (e.g. burns) and trace element deficiencies readily develop under these circumstances, especially when nitrogen and caloric replacement is being given artificially. In some cases (e.g. for copper) nutritional status prior to disease may already be marginal. Furthermore subclinical deficiencies of trace elements may have important public health implications, increasing the burden of disease in the community for such conditions as malignancy and heart disease (due to lack of protective antioxidant activity) as well as for osteoporosis and diabetes mellitus.

Altered trace element metabolism in type I and type II diabetes mellitus has been well established in both human and experimental animal studies (Mooradian & Morley, 1987; Rossetti et al., 1990). Both deficiencies and excesses of metals have been observed (Mateo et al., 1978; Mather et al., 1979; Failla & Kiser, 1981; Johnson & Evans, 1984). Induced dietary deficiencies of individual trace metals in experimental animals have resulted in impaired insulin release, insulin resistance and glucose intolerance (Brown et al., 1975; Failla, 1983; Baly et al., 1984; Asayama et al., 1986). Conversely, supplementation of the deficient
nutrient has been shown to improve glucose homeostasis in a number of studies (Rabinowitz et al., 1983b; Urberg & Zemmel, 1987). A few studies have also addressed the possibility of trace element supplementation as an adjunct therapy in human diabetic subjects, with mixed results (Niewoehner et al., 1986; Anderson et al., 1987; Clausen, 1988; Walter et al., 1991).

3.2.1 Vanadium

Vanadium (V), a group Va transition element was discovered in 1813 by the mineralogist Del Rio who gave it the name “panchromium” because of its colour changes as a function of its oxidation state. It was rediscovered in 1831 by the Swedish chemist Nils Gabriel Sefstrom and named it after “Freya Vanadis” the Scandinavian goddess of beauty, youth and lustre who was inspired by the palette of colours that is generated by this metal in solution. Subsequently the mineral was called vanadinite (Hudson, 1964).

Vanadium is widely distributed in the world. The average concentration in the earth’s crust is 100ppm (Bertrand, 1950) which puts it ahead of copper, lead, zinc and tin in concentration in the earth’s crust. It has long been known that vanadium is present in fossilized organic matter, such as petroleum and coal and is a common constituent of living plants and animals (Meisch & Beilig, 1980). Due to its wide use in industrial processes as a catalyst, the concentration of vanadium is increasing in the atmosphere (Hudson, 1964). In seawater, vanadium ranks thirty-fourth in abundance, with an average concentration of only 2 ppb. In mammals, it is an ultratrace element widely distributed in tissues with total body pool of about 100 µg on a daily intake of 10-60 µg. According to Simonoff et al. (1984), the serum concentration is 0.26 – 1.30 ng/ml (~ 10^-6 M).

Physiological effects of vanadium were studied long before its current renaissance. It 1876 John Priestly of Manchester reported on the physiological action of vanadium in frogs, pigeons, guinea pigs, rabbits, dogs and cats. Some of his conclusions such as the poisonous effect on introduction of soluble salt (vanadate) into system by the stomach or
direct injection into veins, are still valid today. This report was followed by work concerning the toxicity of various known vanadium forms (Larmuth, 1877). Vanadium was widely used for therapeutic purposes at the turn of the century in France for treatment of anemia, tuberculosis, chronic rheumatism and diabetes (Lyonnett, 1899). In order to increase appetite, strength and weight up to 5 mg/day of sodium metavanadate was given orally (Hudson, 1964).

3.2.1.1 Vanadium Chemistry

Metallic vanadium does not occur in nature. It can exist in variety of oxidation states ranging from -1 to +5 and can be found in a multitude of polymeric forms. The oxidation states of biological interest are +3, +4 (vanadyl) and +5 (vanadate). It can readily change its oxidation state and can take an anionic or cationic form.

The first, $V^{3+}$ [$V(III)$], is stable only in acidic solution ($<\text{pH } 2$) and in the absence of oxygen. On a priori grounds, therefore, it might not be expected to occur in vivo, yet not only is $V^{3+}$ found in tunicate blood cells, but it is the predominant state of the metal therein (Willsky, 1990).

Vanadyl, $V(IV)$ is stable only in acidic solution, being rapidly oxidized by dissolved oxygen at physiological pH. Vanadate [$V(V)$] can easily be reduced to vanadyl by glutathione, catechols and other cellular constituents. Five and six co-ordinate complexes are formed in which the short V-O bond length of 0.16 nm is preserved. Complexes usually approach one of the three idealized geometries (i) square bipyramidal, (ii) square pyramidal or (iii) trigonal bipyramidal (Chasteen, 1981).

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure6.png}
\caption{Chemical structures of the most widely used vanadium compounds which exhibit insulin-like properties}
\end{figure}
The trigonal bipyramidal geometry for vanadyl species is usually found in protein complexes. As vanadyl, VO$^{2+}$, vanadium ion behaves as a simple divalent ion and may compete favorably with Ca$^{2+}$, Mn$^{2+}$ and Zn$^{2+}$ etc for ligand binding sites. The ability to bind in different binding sites is greatly aided by its flexible co-ordination geometry. Below pH 3, vanadium exists as the blue vanadyl cation, VO$^{2+}$. The vanadyl cation is also present in tunicate blood cells. Since the metal in this state has an unpaired electron, it is paramagnetic and hence can be electron spin resonance spectroscopy (ESR). The ESR signal changes dramatically when a solution containing the vanadyl cation is frozen or if the ion binds to a macromolecule. Since the signal is also sensitive to the chemical environment, VO$^{2+}$ has proved a useful probe of metal binding sites in several proteins (DeKoch, 1974). Its only disadvantage in such studies is its susceptibility to hydrolysis. Above pH 3, VO(OH)$^+$ begins to appear followed by the dimer (VOOH)$^{2+}$ and then above pH 4.5 by the formation of the precipitate of VO(OH)$_2$. Above pH 2.3 vanadyl (IV) ion tends to undergo air oxidation to form vanadates. Hydrolyzed species of VO$^{2+}$ are very prone towards air oxidation. However, when the VO$^{2+}$ is chelated, oxidation is considerably retarded. VO$^{2+}$ forms extremely strong complexes with proteins, also complexes with ligands such as citrate, ATP, pyrophosphate, catechols and free amino acids. It is unlikely that VO$^{2+}$ exists free in biological systems at physiological pH except perhaps in acidic vehicles due to its tendency to bind to other compounds and oxidize to vanadate in the free form (Willsky, 1990).

The $+5$ oxidation state i.e. vanadate is rather more complicated, since although in acid one finds a dioxovanadium cation, VO$^{2+}$, vanadates that appear at physiological pH are anion, i.e. [HVO$_4^{-2}$] and tend to aggregate into polynuclear complexes. The form of vanadate, which most closely mimics phosphate, VO$_4^{-3}$ is present at extremely high pH values at which biological systems are mostly inactive. At concentrations of vanadate below 10 mM which is the physiological range, the monomeric form of vanadate can be found, with the tetravalently co-ordinated H$_2$VO$_4^+$ and HVO$_4^{-2}$ being the predominant species at pH values between 6 and 8. Decavanadate is the major
oxovanadate species present at pH 5.5, which is the pH of many vesicles in eucaryotic cells, at concentrations above 0.5 mM monovanadium units. The vanadate geometries varies if the vanadate is complexed with other structures. Vanadate is known to bind to EDTA, citrate, succinate, glycols and catechols. Vanadate solutions are usually colourless; except for the polymerized decavanadate which forms an orange-yellow colour (Willsky, 1990).

The effect of vanadate on phosphohydralases, which have a stable phosphoprotein intermediate in their catalytic cycle, believed to occur due to their similarities between the chemistries of vanadates [V(V)] and the phosphates. Vanadate can readily acquire a stable five co-ordinate trigonal bipyramidal geometry resembling the transition state of phosphate. V-O bond lengths in vanadates are around 0.17 nm compared to 0.152 for the P-O bond in orthophosphate. Vanadic acid $\text{H}_3\text{VO}_3$ ($\text{pK}_a = 3.5, 7.8, 12.5$) is a weaker acid than phosphoric acid $\text{H}_3\text{PO}_4$ ($\text{pK}_a = 1.7, 6.5, 12.1$).

In the body fluids at pH 4-8, the predominant species would be $\text{VO}_3^-$ (+5 oxidation state), vanadate (metavanadate). $\text{VO}_3^-$ may enter certain cells by an anion transport system and be reduced by glutathione to $\text{VO}_4^{2+}$ (+ 4 oxidation state). By way of speculation, the oxidation-reduction reaction may be as follows:

$$\text{H}^+ + \text{VO}_3^- + 2 \text{GSH} \rightarrow \text{VO}_4^{2+} + 2 \text{S}_2\text{O}_3^- + \text{OH}^- + e^- + \text{H}_2\text{O}$$

Extensive binding to extra and intracellular ligands may be expected. Since phosphate and Mg$^{2+}$ are ubiquitous in biological processes, VO$^3-$ as the analogue of phosphate and VO$^{2+}$ which resembles the size of Mg$^{2+}$ (respective ionic radius 0.60 and 0.65 Å), potentially have many biochemical and cellular sites of action. For example, vanadium compounds inhibit ATP phosphohydralases, ribonucleases, adenylate kinase, phosphofructokinase, sequalene synthase, glyceraldehyde-3-phosphate dehydrogenase (Máca, 1980), glucose-6-phosphatase (Singh et al., 1981) and phosphotyrosyl-protein phosphatase (Swarup et al., 1982). The recent finding is that VO$^3-$ is one of the most potent known inhibitors of Na$^+\text{-K}^+$ ATPase (Cantley et al., 1977; Nechay & Saunders, 1978; Quist & Hokin, 1978) and there has
been a suggestion that vanadium may be a physiologic regulator of the Na+ pump (Cantley et al., 1978).

3.2.1.2 Distribution Of Naturally Occurring Vanadium In Biologic Materials

The knowledge of vanadium content in biologic materials is far from complete. In addition to natural variability, inappropriate sensitivity of analytical methods, interference by other elements, non-availability of standard reference materials in the past and easy contamination of sample have contributed to a wide spread of vanadium values reported in the literature (Byrne & Kosta, 1978; Versieck & Cornelis, 1980). The most sensitive and commonly used methods for measuring vanadium are the techniques of neutron activation analysis (NAA) (Byrne & Kosta, 1978) and flameless atomic absorption spectroscopy (AAS) (Myron et al., 1977; Stroop et al., 1982), detection limits are well under 1ng of vanadium. The values given below are in ng/ml or g wet weight (5.1 = 10^-7 M).

A. Humans

Byrne and Kosta (1978), Verseick & Cornelis (1980) and Cornelis et al. (1981) scrutinized published information on vanadium concentration in the blood plasma or serum (ranging from 0.016-570) and whole blood (ranging from 0.5-1500) of healthy individuals and concluded that much of the disparity in these figures was methodological. The lowest ever reported vanadium concentration reported by NAA ranged from 0.016-0.139 in 37 women and from 0.024-0.939 in 37 men (Cornelis et al., 1981). No correlation was found between vanadium content and age or serum concentration of cholesterol, lipoproteins and triglycerides. The lowest mean vanadium concentration determined by NAA in whole blood was 0.5 (Byrne & Kosta, 1978). Values recently obtained by AAS were 3 for serum (Stroop et al., 1982), 8 for plasma (Dick et al., 1982), and 6 for whole blood (Post et al., 1979). According to only a few measurements, erythrocytes appear to have a vanadium content similar to that of plasma (Post et al., 1979; Cornelis et al., 1981). By a photometric method, <1 to 11 (<1 to 24 in chimney sweepers) was found in whole blood
(Kelm & Schaller, 1978). Byrne & Costa (1978, 1979), Byrne & Vrbic (1979), Byrne et al. (1983) reported (and compared with published values) the following vanadium concentrations (by NAA) in tissues and body fluids:

Bone and teeth, <1-8; liver, 5-19; kidney, 3-7; heart, 1; skeletal muscle, 1-7; spleen and thyroid, 3-4; pancreas, 14; brain, fat, urine, <1; lung, 130-140; hair, 12-87; bile, <1-2; dry feces, 141-2210; dry milk and colostrum, <1-1.

Other workers reported:

Liver, 3-13 (NAA) (Cornelis et al., 1979); kidney, 67-194 (AAS) (Corder, 1983); placenta, 3 (photometry) (Thurauf et al., 1978). The US Environment Protection Agency (1979) listed vanadium ranges of 4-140 for hair and 4-625 for nails. Mean vanadium content in neonates' hair was 50 (NAA) (Gibson & DeWolfe, 1979). Vanadium concentrations in hair appear to be sensitive to environmental exposure (Creason et al., 1975). US city dwellers showed increased lung concentrations in the fifth and particularly sixth decade of life (Tipton & Shafer, 1964).

A recent estimate of the total body pool of vanadium in the "reference man" was 100-200μg (Byrne & Kosta, 1978), in contrast to Schroeder's earlier calculation of 22 mg (Schroeder, 1974). The daily dietary intake of vanadium was estimated as 10-60 μg (Tipton et al., 1969; Byrne & Kosta, 1978, 1979; Myron et al., 1978), with excretion mainly in the feces and urine (Tipton et al., 1969). The mean urinary output per 24 hours was 10μg (Stroop et al., 1982). Intake via air and water may be significant (Waters, 1977; Byrne & Kosta, 1978).

B. Animals

Other mammals (Soremark, 1967; Myron et al., 1977; Byrne & Kosta, 1978; Post et al., 1979; Higashino et al., 1983):

Bone, 20-40 (pig, sheep); bone marrow, <1 (pig); liver, 2-10 (beef, pig, rat); kidney, 9-34 (rat, pig, dog rabbit); heart, < 1-9 (pig, rat, rabbit); skeletal muscle, <1-14 (beef, pig, rabbit, horse); brain, <1-3 (rat, cow); lung, 5-25 (rabbit, beef); fat, <1-2 (pork); butter, 1; milk, <1-3; plasma, 2-5 (rat); gelatin, 9-43; whole mouse, 66.
Chicken (Myron et al., 1977; Byrne & Kosta, 1978; Phillips et al., 1982)

Dry bone, 370-760; (turkey bone, 86); liver, 38; kidney, 18 (107 on 3.5μ vanadium/g diet); heart, 5-9; light muscle, 2-22; dark muscle, 12; egg white, <1-2; egg yolk, 2-21.

Aquatic Animals (Soremark, 1967; Myron et al., 1977; Byrne & Kosta, 1978; Blotcky et al., 1979; La Touche et al., 1981)

Salt water fish, 3-28 (cod, mackerel, sardines, tuna); fish bone, 125-2000 (mackerel, tuna); fresh water trout, 0.4; lobster, 5-43; scallop, 22; dry blue crab, mussels, oysters, white shrimp, 455-1840. Vanadium content in shrimp and oysters was higher in specimens taken from industrialized section around Galveston Island, Texas (Blotcky et al., 1979). An extensive list of vanadium concentrations in other biological system has been published (Biggs & Swinehart, 1976).

C. Plants (Soremark, 1967; Myron et al., 1977; Byrne & Kosta, 1978; Post et al., 1979):

Numerous fruits, vegetables, nuts, oils, <1-5; lettuce, radish, spinach, 21-52; dill, 140; dill seed, 431; parsley, 790; cereals, grains, beans, flour, bread, <1-93; dry wild mushrooms, 50-2000 (26 species); dry Amanita muscarina, 51,000 (as amavadin) (Biggs & Swinehart, 1976); cocoa powder, 610; dry tea leaf, 150; dry black pepper, 204–987; wine, 4-32; beer, 8; tobacco, 1000-8000 (10 types); drinking water <1-2.

3.2.1.3 Distribution Of Experimentally Administered Vanadium

The most complete picture of vanadium distribution emerges from autoradiographic studies of sagittal sections of whole mice prepared five minutes to seven days after an intravenous injection of $^{48}$V$_2$O$_5$. These studies included pregnant mice (Soremark & Ullberg, 1962). Half-life of $^{48}$V in blood was <1 hour. Bones and teeth had the highest persistent concentrations of vanadium, which reached a peak 1-2 days after the injection; $^{48}$V accumulated to the greatest extent in the zone of ossification. In a similar study on 7-9 day-old rats, the greatest $^{48}$V uptake was found in the parts of the teeth and bones, where rapid
mineralization was taking place (Soremark et al., 1962). In soft tissues, $^{48}$V rose rapidly after the injection and declines faster than in bone. The highest concentrations were found in kidney cortex, the liver with a spotty distribution, and the lung parenchyma, with no radioactivity in the bronchi. Medium levels of radioactivity were found in the skin and salivary glands, with low concentrations in skeletal and heart muscles cartilage, spleen and brain. Intestinal and urinary bladder contents were high in vanadium. In the pregnant mice, high concentrations of vanadium were visible in the placenta, especially the visceral yolk sac epithelium, the fetal skeleton, and the mammary gland. Other investigators (Oberg et al., 1978; Parker & Sharma, 1978; Bawden et al., 1980; Parker et al., 1980; Peabody et al., 1980; Bogden et al., 1982; Conklin et al., 1982; Hansen et al., 1982; Wiegmann et al., 1982) found similar distribution in selected organs of mice or rats after giving various vanadium compounds by different routes.

Some Accumulation in testes was also noted. A similar organ and subcellular distribution of vanadium after intravenous injections in rats of labeled cationic and anionic compounds with different vanadium oxidation states suggests an in vivo conversion of dissimilar vanadium compounds to common vanadium species (Sabbioni & Marafante, 1978; Sabbioni et al., 1978). In blood, less than 5% of radioactivity was associated with erythrocytes and over 95% was in plasma; some vanadium was bound to transferrin (liver and spleen ferritins also bind vanadium) (Sabbioni & Marafante, 1981; Chasteen & Theil, 1982). In fractions of liver and kidney homogenates, nuclei contained the highest quantity of vanadium, followed in descending ordered by mitochondria, cytosol, lysosomes, and microsomes.

$\text{VO}^{2+}$ is used in electron microscopy; it stains cytoplasmic organelles, collagen fibrils, glycogen granules, secretion granules, and ribosome (Hayat, 1975).

An autoradiographic study on fish of $^{48}$V uptake from water showed an accumulation in skin, fins, intestines, liver, and bones. Vanadium content was low in the brain, eye, and muscle (Soremark, 1967).
3.2.1.4 **Nutritional Importance**

Vanadium is an essential nutrient for chick and the rat (Hopkins, 1974; Mertz, 1974; Underwood, 1977; Davies, 1981; Golden & Golden, 1981). Deficiencies have been included in chicks and rats raised in vanadium-free isolator systems on diets containing <30 or <100 ng/g respectively. The requirement for growth probably lies between 50 and 5000 ng/g. These figures appear high in view of the much lower vanadium content of most feeds. However, in poultry farming too much vanadium is of concern because some rock phosphorus feed additives may contain high concentrations of vanadium; acceptable commercial chicken diets contain 1400-5400 ng V/g (Kubena & Phillips, 1983).

3.2.1.5 **Vanadium in Biological Systems**

**Cellfree Systems**

The most straightforward experiments to interpret concerning the effects of oxovanadium compounds on biological systems are carried out in cell-free systems. In these systems cellular interconversion of oxovanadium compounds in not as serious a problem as it is in whole cell experiments.

1. **Inhibition**

   a) **Na⁺K⁺ ATPase**

   Vanadium has been known since 1965 to inhibit Na⁺ K⁺ ATPase (Rifkin, 1965). However, it was shown only recently by three independent laboratories that certain vanadium compounds are among the most potent known inhibitors of this enzyme system (Cantley et al., 1977; Nechay & Saunders, 1978). The inhibitory effects vanadium on the Na⁺K⁺ ATPase *in vitro* first reawakened interest in interactions of vanadium with metabolic processes. The mechanism of vanadate inhibition for most of the enzyme systems is well understood for enzymes having a phosphoprotein intermediate in their catalytic cycle. These enzymes appear to have a phosphate linked to the protein through an aspartate residue. The vanadate, which has trigonal bipyramidal geometry, binds to
the active site of the enzyme and acts as a transition state analogue. However, it is not clear that inhibition of the Na⁺ K⁺ ATPase by vanadyl (IV) (North & Post, 1980) is much weaker than that found by vanadate, V(V).

(b) Ca²⁺ Mg²⁺ ATPase

Studies have been done mainly on enzymes from sarcoplasmic reticulum of mammalian skeletal muscle and heart and from human erythrocytes. The red cell Ca²⁺ Mg²⁺ ATPases is several times more resistant to VO₃⁻ than Na⁺ K⁺ ATPase (Bond & Hudgins, 1980). Ca²⁺ Mg²⁺ ATPase of sarcoplasmic reticulum requires at least 10 times higher concentration of VO₃⁻ for 50% inhibition than that of erythrocytes (Wang et al., 1979; Hangenmeyer et al.; 1980; Pick 1982).

In reconstituted ghosts, intracellular VO₃⁻ (5 X 10⁻⁵ M) inhibits active Ca²⁺ efflux; this inhibition is promoted by intracellular Mg²⁺ and K⁺ and is antagonized by extra cellular Ca²⁺ (Rossi et al., 1981). The sensitivity of Ca²⁺ pump to VO₃⁻ in vesicles made purified red cell Ca²⁺ Mg²⁺ ATPase is similar to that observed in whole erythrocytes ghosts (Niggli et al., 1981). In the intact red cell, external VO₃⁻ (5 X 10⁻⁵ M) does not inhibit the Ca²⁺ pump (Szasz et al., 1981). When exposed to 5 X 10⁻⁴ M VO₃⁻, fresh erythrocytes become highly labeled with externally added Ca²⁺, which suggests some penetration of VO₃⁻ into the cells as well as inhibition of the outwardly directed Ca²⁺ pumping ATPase (Varecka & Carafoli, 1982). The VO₃⁻ induced accumulation of Ca²⁺ by red cells cause a massive efflux of K⁺, suggesting either an activation of the Ca²⁺-sensitive K⁺ channel in the erythrocyte membrane (Varecka & Carafoli, 1982) or that the intracellular VO₂⁺ metabolite, similarly to Ca²⁺, Mg²⁺ and Pb²⁺ can open the K⁺ channel (Siemon et al., 1982).

(c) K⁺ H⁺ ATPase

VO₃⁻ inhibits microsomal gastric mucosa K⁺ H⁺ ATPase, which is an expression of a part the gastric H⁺ pump; proton transport by gastric microsomal vesicles and acid secretion by gastric glands are also reduced (O'Neal et al., 1979; Faller et al., 1981; Faller et al., 1982). VO₃⁻ also inhibits urinary acidification by the turtle bladder; the mechanism has
not been determined (Arruda et al., 1981). A bacterial membrane-bound proton-translocating ATPase was found to be sensitive to VO$_3^-$.

(d) **DYNEIN ATPase**

Dynein is the collective name for either Ca$^{2+}$- or Mg$^{2+}$- requiring high molecular weight ATPase associated with microtubules. They function in the transduction of the chemical energy provide by ATP hydrolysis into mechanical work such as ciliary and flagellar motility and may have roles in chromosome movement, exoplasmic transport, and the intracellular movement of membrane-bound vesicles (Gibbons, 1982; Johnson & Porter, 1982). The sources of dyneins studied in detail have been the flagella and cilia of the *Tetrahymena* and the sea urchin.

VO$_3^-$ at concentrations on the order of 10$^{-5}$–10$^{-7}$ irreversibly inhibits both the isolated dynein ATPase activity and the motility of demembranated sea urchin of porcine sperm flagella and sea urchin embryo cilia (Gibbons et al., 1978; Kobayashi et al., 1978a). Mg$^{2+}$-activated dynein system is over 30 times more sensitive to VO$_3^-$ than the Ca$^{2+}$- activated one (Gibbons et al., 1978; Shimizu, 1982). The inhibition of Mg$^{2+}$-stimulated emyme activity is noncompetitive with ATP (Gibbons et al., 1978; Shimizu, 1981; Anderson & Purich, 1982), as is the reduction of flagellar beat frequency by VO$_3^-$ (Gibbons et al., 1978). The intact sea urchin spermatozoa are not inactivated by 10$^{-2}$M VO$_3^-$, and those of the pig require 10$^{-3}$ M VO$_3^-$ for complete paralysis (Kobayashi et al., 1978a). Other observations extend and amplify these findings (Majumdar, 1981; Rikmenspoel et al., 1981; Satir et al., 1981; Goodenough & Heuser, 1982; Penningroth, 1982; Penningroth et al., 1982; Warner & McIlvain, 1982).

Myosin and actinomyosin ATPase are not inhibited by VO$_3^-$ concentrations below 5 X 10$^{-4}$ M (Cantely et al., 1977; Gibbons et al., 1978). Other studies, however, have demonstrated an irreversible, slow-onset inhibition of myosin and actomyosin ATPases by millimolar concentrations of VO$_3^-$; the mechanism is the formation of a stable myosin-ADP-vanadium complex (Goodno, 1979; Goodno & Taylor, 1982; Kawamura & Tawada, 1982). The difference in kinetics and sensitivity to VO$_3^-$ offers an opportunity for distinguishing between the actions of
dynein and myosin in different forms of cell motility (Gibbons et al., 1978; Yamin & Tamm, 1982).

$\text{VO}_3^-$ at $\sim 10^{-5}$ M has been shown to inhibit mitotic spindle in lysed cells (Cande & Wolniak, 1978) and translocation of pigment granules in permeabilized erythrophores (Stearns & Ochs, 1982) or when injected into the cell (Beckerle & Porter, 1982).

2. **Stimulation**

The mechanism by which vanadate stimulates biological processes is much less well understood than the mechanism of inhibition by vanadate. One problem has been the lack of data demonstrating which oxovanadium form of vanadate is the active species. At the current time the chemical mechanisms of stimulatory actions of oxovanadium compounds remain elusive.

(a) **Adenyl Cyclase**

This enzyme is one of the firsts to have been shown to be stimulated by vanadate in the +5 oxidation state. Cyclic AMP is formed by catalytic action of adenyl cyclase and is inactivated by phosphodiesterase. $\text{VO}_3^-$ ($>10^{-5}$ M), along with fluoride, catecholamines, vasopressin, prostaglandin E1, parathyroid hormone, and glucagons, stimulates isolated adenyl cyclase activity from a variety of sources (Grupp et al., 1979; Hackbarth et al., 1980; Krawietz et al., 1980; Pertseva et al., 1982; Schmitz et al., 1980). The action of VO$_3^-$ is not shared by V$^{4+}$ and V$^{3+}$ compounds (Schmitz et al., 1982). It is independent of hormones and inhibition of phosphodiesterases by theophylline (Schwabe et al., 1979) and differs from that of fluoride (Lichstein et al., 1982; Johnson, 1982; Krawietz et al., 1982). The postulated mechanism involves formation of an enzyme complex with VO$_3^-$ via guanine nucleotide regulatory protein (Krawietz et al., 1982). Concentrations of VO$_3^-$ in excess of 5mM inhibit adenyl cyclase (Lichtstein et al., 1982).
(b) **Vanadium-Dependent NADH oxidation**

Vanadate-stimulates NADH oxidation activity in the absence of added protein. Stimulation by protein of vanadate-dependent NADH oxidation by plasma membranes was reported by Ramasarma et al. (1981). This plasma membrane activity has been studied in plants (Briskin et al., 1985), the yeast *S. Cerevisiae*, cardiac cells (Erdman et al., 1979), erythrocytes (Vijaya et al., 1984) and rat liver (Coulombe et al., 1987). The plasma membrane-mediated, vanadate stimulated by paraquat, a superoxide dismutase, a superoxide radical scavenger, and is stridulated by paraquat, a superoxide producer. The reaction does not occur anaerobically.

The purpose of plasma membrane-stimulated vanadate-requiring NADH oxidation activity remains elusive. The function of the membrane protein of the reaction appears to be the production of superoxide, which is potentially toxic to the cell, while the continuation of the chain causes the production of hydrogen peroxide, another toxic compound. It has been suggested that the reaction is involved in the generation of heat and/or vanadium toxicity. However, it has been shown that the facultative anaerobic yeast, *S. Cerevisiae*, is equally sensitive to vanadate when grown aerobically or anaerobically, which implies that no oxidative processes of any type are involved in vanadium toxicity.

(c) **Protein Phosphorylation**

The effect of vanadium compounds is not well understood and only a small fraction of the experiments in this field have been done in cell free systems. In isolated cell membranes from A-431 cells it has been reported that µmolar concentration of vanadate inhibit the membrane-dependent dephosphorylation of histones containing phosphotyrosine, but not those containing phosphoserine or phosphothreonine (Swarup et al., 1982). Vanadate (in µmolar quantities) also increased tyrosine phosphorylation in a membrane fraction from a human lymphoblastoic cell (Earp et al., 1983). In experiments using insulin-receptor enriched fractions obtained from microsomal membranes of human placenta and rat liver, vanadate activation of the autophosphorylation of the insulin
receptor was observed. The autophosphorylation occurred in a dose dependent manner, showing a half-maximal response at 30µM (Gherzi et al., 1988). In experiments using cellular fractions from rat brain cortex vanadate and vanadyl ions stimulated total phosphorylation of proteins from synaptic membranes and to a lesser extent from mitochondria (Krivanek, 1988). Vanadate has been shown to rapidly esterify the hydroxyl groups of aromatic rings to yield a phenyl vanadate. The vanadate esterification proceeds with an equilibrium constant much larger than that of the corresponding phosphate esterification reactions (Tracy & Grasser, 1986).

3.2.1.6 Organs Affected By Exogenous Vanadium

Exogenously added oxovanadium compounds have been shown to have many physiological effects on various organ systems. At the current time it is not possible to differentiate primary from secondary effects of vanadium. In addition the form of oxovanadium which is affecting each system has not yet been determined due to the pH, concentration and oxidation-reduction changes that can occur as the oxovanadium compounds move through the body. Throughout the body the most consistent response is one of vasoconstriction. There have been some studies concerning the effect of vanadate on eye, ear, liver and brain (Nechay, 1984; Nechay et al., 1986). However, the bulk of the work consists of studies utilizing the kidney and the heart.

(a) Heart

since inhibition of Na⁺K⁺ ATPase has been implicated in the positive inotropic action of digitalis, there is much interest in the cardiac effect of VO₃⁻. The addition of oxovanadium compounds to cardiac muscle has been shown to have both positive and negative inotropic effects depending upon the animal species studied and the amount of drug used in the study (Inciarte et al., 1980; Takeda et al., 1980). Alterations in the cardiac function by vanadate have not been linked to changes in K⁺ transport in the heart implying that interaction with Na⁺ K⁺ATPase is not the primary action of oxovanadium in the heart. VO₃⁻
stimulates adenyl cyclase and so can increase the concentration of cyclic AMP in cardiac muscle; this effect also seems to be unrelated to its inotropic actions. $\text{VO}_3^-$ increases the force of contraction of isolated rat atrial muscle by increasing the $\text{Mn}^{2+}$ sensitive superficial $\text{Ca}^{2+}$ pool, which is related to the beat-to-beat control of force of contraction; on the other hand, $\text{VO}_3^-$ lowers the force of contraction in guinea pig atrial muscle by inhibiting slow $\text{Ca}^{2+}$ channels (Stemmer et al., 1983). Compounds of vanadium in +4 and +3 oxidation states do not share with $\text{VO}_3^-$ the positive isotropic effect on isolated cat papillary muscles and stimulation of adenyl cyclase (Schmitz et al., 1982).

High concentrations of $\text{VO}_3^-$ ($>10^{-4}$ M) may inhibit (like ouabain) or stimulate (like insulin) the uptake of $\text{K}^+$ by heart muscle cells from various species; both types of effect may be associated with the positive inotropic effect (Werdan et al., 1982). It was shown previously for other tissues that $\text{VO}_3^-$ mummies the stimulating effect of insulin on glucose oxidation (Degani et al., 1981) and transport, which appears to be associated with or mediated by rise in cytoplasmic $\text{Ca}^{2+}$ concentration (Clausen et al., 1981). Another suggestion is that the stimulation by $\text{VO}_3^-$ of rat heart protein kinase C, which promotes the phosphorylation of the membranes of the sarcoplasmic reticulum, may play a role in strengthening myocardial contraction by increasing sarcoplasmic reticulum stores of $\text{Ca}^{2+}$ (Catalan, 1982).

The addition of calcium channel blockers, such as verapamil, attenuate increases in pressure and diminish increases in cardiac output caused by vanadate (Herzig et al., 1981).

(b) Kidney

Renal effects of vanadium include a mixture of haemodynamic and parenchymal actions and, like cardiac effects, are characterized by unexplained profound species differences. Vanadate accumulates to greatest levels in the kidneys and has a strong consistent vasoconstriction effect in that organ (Nechay, 1984). Vanadate causes strong naturesis in various animals. It produces large diuresis in the rat but not in dog (Inciarte et al., 1980; Lopez-Novoa et al., 1982a, 1982b) and cat (Larsen & Thomson, 1980a, 1980b). Vasoconstriction, lowering of
renal blood flow and glomerular filtration rate (GFR) are prominent. The vasoconstriction produced in rat (Day et al., 1980) is much less intense than that in the more sensitive dog. In the rat, GFR may rise simultaneously with increased renal peripheral resistance, suggesting a post capillary vasoconstrictor effect (Kumar & Corder, 1980); in the dog and cat only a fall in GFR was seen. The effect of vanadate and other diuretics are additive. In isolated systems, vanadate is more potent when present in lumen compared to the peritubular bath of isolated perfused renal tubules (Day et al., 1980). Vanadate also inhibits transtubular secretion p-aminohypuric acid (Edwards & Grantham, 1983) and inhibits the action of vaspressin in the collecting tubule (Steffen et al., 1981). The possible inhibitory interactions of vanadate on Na⁺K⁺ ATPase have been considered as a mechanism of action of vanadate in kidney, since K⁺ and Na⁺ fluxes are affected by vanadate (Higashino et al., 1983; Churchill & Churchill, 1980). Vanadate also reduces renal rennin secretion in rat kidney slices (Churchill & Churchill, 1980).

(c) Eye And Ear

Topical application of VO₃⁻ lowers intraocular pressure in the rabbit and monkey, presumably by inhibition of Na⁺K⁺ ATPase in the ciliary body and consequently reduction of intraocular by inhibition of Na⁺K⁺ ATPase in the ciliary body and consequently reduction of intraocular fluid formation (Becker, 1980; Krupin et al., 1980). It proves only marginally effective in human ocular hypertension (Krupin et al., 1980). It proves only marginally effective in human ocular hypertension (Krupin et al., 1983). VO₃⁻ (>10⁻⁴ M) also inhibits active Na⁺ and Cl⁻ transport in the isolated frog cornea (Candia & Podos, 1981). An ATPase from toad retinal rod outer segments that may have a dynein function involved in light-controlled structural changes is sensitive to VO₃⁻ (Thacher, 1981).

Ototoxicity of locally applied VO₃⁻ was studied in guinea pigs. Both the endocochlear and microphoncprentials are inhibited by oubain. Although VO₃⁻ inhibits Na⁺K⁺ ATPase of stria vascularis in vitro, it causes an increase in the cochlear potential followed by a gradual decrease and depression of microphonic potential when applied perilymphatically.
These results were interpreted to suggest that VO$_3^-$ acts by depolarizing the hair cells of the organ of Corti (Marcus et al., 1981, 1982).

(d) Brain

Whole brain microsomal Na$^+$K$^+$ ATPase is several times less sensitive to VO$_3^-$ than is the kidney or heart enzyme in four mammalian species (Nechay & Saunders, 1978); it is not known whether it is related to the presence of two types of Na$^+$K$^+$ ATPase in the brain (Sweedner, 1979) or to other factors. Consistent with inhibition of Na$^+$K$^+$ ATPase, VO$_3^-$ interferes with the uptake of $^3$H-norepinephrine by rat cerebral cortex slices; the high concentrations ($<$10$^{-4}$ M) required suggest poor intracellular penetration of VO$_3^-$. Vanadium intoxication in rats causes changes in brain catecholamine levels (Witkowska & Brzezinski, 1979). VO$_3^-$ (10$^{-3}$ M) also diminishes muscarinic binding sites in homogenates of rat corpus striatum (Danielsson et al., 1983).

The signs of vanadium toxicity in man include tremor and central nervous system depression (Done, 1979). It has been suggested that manic-depressive disorders may be associated with increased vanadium levels and a genetically defective Na$^+$ pump hypersensitive to inhibitors (Naylor & Smith, 1981a, 1981b; Naylor, 1983). Plasma vanadium concentrations in manic-depressed subjects were twice that of normal controls and declined after recovery (Dick et al., 1982). Encouraging results were obtained in the therapy of manic depressive psychosis with a low vanadium diet and therapy with EDTA or reducing agent, ascorbic acid, and methylene blue (Narsapur & Naylor, 1983). Antidepressants such as imipramine and indalpine may also reduce VO$_3^-$ to VO$_{2+}$. Other investigators extended these observations to several ATPase in erythrocytes of patients with effective disorders and found the best correlation between mood and Ca$^{2+}$ ATPase activity (MacDonald et al., 1982).
3.2.1.7 Physiological Effects Of Vanadium

Effects of Vanadium on cellular processes

Exogenous vanadate addition has been shown to alter various cellular processes. Vanadate inhibited the ATP-dependent degradation of proteins in reticulocytes (Tanaka et al., 1984). Vanadate has also been shown to affect lipid metabolism. Administration of vanadate intraperitoneally to rats has been shown to increase brain cholesterol levels and the ratio of cholesterol/phospholipid. Vanadate addition had a similar, though reduced, effect on lipids in in-vivo experiments with cultured cells (Catalan, 1987). Vanadate has also been reported to inhibit the rate limiting step in cholesterol biosynthesis, the synthesis of mevalonate, which is catalysed by HMG CoA (3-hydroxy-3-methylglutaratyl coenzyme A) reductase (Menon et al., 1980).

(a) Cell proliferation, Differentiation and Phosphorylation

The stimulatory actions of vanadium on protein phosphorylation may be related to its hormone-mimetic activities, its potential role in the regulation of cell proliferation and differentiation and its anticarcinogenic properties.

(i) Cell Proliferation and Differentiation

Vanadate addition to the culture medium has produced numerous stimulatory effects on cell proliferation and differentiation. Quiescent human fibroblasts treated with 4µm vanadate were stimulated to divide and DNA synthesis increased. However, at 210mM vanadate in the culture medium no stimulation of DNA synthesis was found (Carpenter, 1981). Vanadate was also found to replace interleukin 3 as a growth factor in a mouse cell line (Tojo et al., 1987). In friend erythroleukemia cells vanadate blocked differentiation without affecting cell viability (English et al., 1983). In human lymphocytes vanadate had an inhibitory effect during the first three days of cell culture, when both differentiation and proliferation take place, but in enhanced DNA synthesis, acting as a co-mitogen in subsequent days of culture (Marini et al., 1987), vanadate added earlier to the culture medium had been found to be mitogenic for a subpopulation of thymus cells, but not for splenocytes and vanadate has
been shown to stimulate bone cell proliferation and collagen synthesis at 5-15 mM (Lau et al., 1988). Vanadate, in vitro, potentiated the estrogen stimulation of proliferation of a Mouse Leyding cell line (Sala et al., 1987).

(ii) Phosphorylation / Dephosphorylation

The effects of vanadate treatment of phosphoprotein levels have been examined in various systems. A stimulatory effect of vanadate treatment on protein tyrosine phosphorylation in fibroblasts has been reported (Klarlund et al., 1988). Biochemical assays for phosphotyrosine residues were used to show that vanadate treatment stimulated phosphotyrosine production in mouse lens cell (Gentleman et al., 1987). The occurrence of protein phosphorylation has been studied in conjunction with the insulin like activity of vanadate. Phosphorylation of insulin receptor in vanadate treated adipocytes (Tamura et al., 1984a) was reported. However, other workers using an antiphosphotyrosine antibody were unable to see an increase in tyrosine protein phosphorylation in the adipocyte system (Mooney et al., 1989). Most recently the effect on oncogenes on incubation with vanadate has been reported. Vanadate stimulated phosphorylation of fms (Tamura et al., 1986) and src (Ryder & Gordon, 1987; Collett & Belzer, 1987) proteins. Those oncogenes are believed to be phosphorylated by protein kinase C whose stimulation by the degradation of membrane phosphoinositol depends on intracellular calcium mobilization. Vanadate has also been shown to induce inositol phosphate formation and inhibit inositol phosphate degradation in CCL39 cell lines (Paris & Poussegur, 1987).

(b) Cellular and Intracellular Motility

The motility of sperm, cilia and chromosomes have been reported to be inhibited by vanadate (Ramasarma & Crane, 1981). This effect may be related to the inhibition of dynein and muscle ATPases by vanadate (Nechay, 1984; Nechay et al., 1986). Intracellular movement of vesicles may be stimulated by vanadate. It has been demonstrated that vanadate addition to yeast growth medium stimulated the accumulation of the fluorescent dye Lucifer yellow in yeast, which is used as a measure of endocytosis.
(c) Transport Across The Plasma Membrane

The effects of vanadate-treatment on transport into cells has been extensively studied in a few cell types. Vanadate-treated rat adipocytes have been used to study transport in many experiments designed to characterize the insulin-mimetic activity of vanadate. The effect if vanadate treatment on glucose and cation transport in isolated adipose tissue and skeletal muscle has been studied (Clausen et al., 1981). In fact pads 0.5 to 5 mM vanadate stimulated glucose uptake and efflux of 3-0 methylglucose and increased calcium wash out from preloaded fat pads. In muscle 3-0 methylglucose and increased calcium wash out from preloaded fat pads. In muscle 3-0 methylglucose efflux and calcium wash out were stimulated, while in other muscle cells vanadate treatment increased K⁺ while decreasing Na⁺. The stimulatory effect of vanadate on glucose transport in this case appears to be associated with or mediated by rise in cytoplasmic calcium level.

Vanadate has been shown to stimulate ion movement across various portions of the gastrointestinal tract. In rabbit colonic epithelia μM amounts of vanadate on the serosal side caused the stimulation of chloride secretion, without affecting sodium transport. In rat jejunum vanadate appears to have a concentration dependent affect on transport. AT 0.1 mM it increased influx and efflux of alanine across the mucosal border, but at higher concentrations, (1 to 10mM) it decreased mucosal to serosal flux and influx of alanine concurrently with reducing Na⁺ K⁺ ATPase activity in the basolateral membranes (Hajjar et al., 1987). It has been suggested that a low concentration effect result from vanadyl stimulation of adenyl cyclase.

Vanadate affects on transport in red blood cells have also been monitored. Calcium transport into red cells was stimulated by 0.5mM vanadate, accompanied by a large efflux of K⁺. It is hypothesized that in these cells vanadate moves inside the cells and the increased calcium accumulation is due to decreased activity of the Ca²⁺ ATPase which pumps calcium out of the cell (Varecka & Carafoli, 1982).
(d) **Intracellular Ca\(^{2+}\) Movement**

Vanadate interference with intracellular Ca\(^{2+}\) movement would link the hormonal affects of vanadate to interactions of protein kinase C. ATP dependent calcium uptake by microsomal fractions of rat salivary gland was inhibited by vanadate (Kangasuntheran & Theo, 1982). Alterations in calcium movement have been associated with the inotropic effects of vanadate (Akera et al., 1983). The increased vanadate induced force of contraction of isolated rat atrial muscle is associated with increases of the Mn sensitive calcium pool, while vanadate lowering of the force of contraction of guinea pig atrial muscle has been associated with inhibition of the closing of calcium channels, vanadate stimulation of glucose metabolism in adipocytes and skeletal muscle is associated with a rise in cytoplasmic concentration (Erdmann, 1980).

(e) **Oxidation-Reduction Processes**

From vanadate chemistry it is expected that vanadium ions would affect oxidation-reduction processes in cells. However, there has not been extensive work directed at identifying redox processes in cells that may be affected by vanadate. Whether vanadate dependent NADH oxidation activity is related to any cellular phenomena is not known. The ability of *S. Cerevisiae* to reduce vanadate to vanadyl has been shown to be increased in vanadate resistant mutants, implying that this pathway may aid in lessening the toxic effects of vanadate (Willisky et al., 1985). Vanadate has been taken up by cells and rapidly reduced to vanadyl (Degani, 1981; Willsky et al., 1984). This reduction is medicated by glutathione and cellular catechols (Macara et al., 1980; Cantley & Aisen, 1979). The formation of an intermediate vanadate-thioester has been proposed as a part of a preequilibrium step in glutathione reduction of vanadate to vanadyl (Legrum, 1986). Administration of vanadate to mice depresses the oxidation rate of formation to CO\(_2\), While *in vitro* in cytosolic liver fractions vanadate inhibited the enzymatic transfer of format to tetrahydrofolic acid (Bruch et al., 1987). In mice vanadate inhibited the oxidation demethylation of substrate of the cytochrome P-450 dependent monooxygenase system. The effect was reduced by pretreatment of the animals with ascorbic acid and vanadyl sulphate did
not produce as marked an effect as orthovanadate (Heide et al., 1983). It appears that in this system vanadate is the more potent inhibitor and reduction to vanadyl by ascorbate lessens the effect of vanadate. Effects of vanadate on one carbon metabolism and cytochrome P-450 dependent processes certainly implicate vanadate as a modulator of oxidation-reduction reactions in the cell.

3.2.1.8 Toxicological Effects

Until recently interest in vanadium was confined primarily to its toxicological properties in certain species. In humans, the major side effects of vanadate are eczema, dermatitis, conjunctivitis, irritation of upper and lower respiratory tract, the later resulting into atrophic rhinitis, pharyngitis, chronic bronchitis and diffuse pulmonary fibrosis. Central nervous system manifestations are characterized by tremors, headaches, tinnitus and changes in mental status.

Vanadium is toxic after acute parenteral administration, but much less so after oral administration because it is only poorly absorbed by the gastrointestinal tract (Llobet & Domingo, 1984). In diabetic animals chronically treated with oral vanadium, the serum concentration of the element averages 20μM (Brichard et al., 1991), which is about 1000-fold greater than in untreated controls. Even higher concentrations may be reached in some tissues (for example, bone, kidney), in which vanadium accumulates (Dai et al., 1994). The most obvious side effect vanadium treatment is the decrease in body weight gain. Diarrhoea can occur but can be resolved with tapering dosage. Although there are exceptions, long-term (up to one year) oral administration of vanadyl to diabetic rats has usually been found not to affect haematological indices and not to alter hepatic or renal function, as shown by morphology and by the lack of increases in liver cytolytic enzymes or in creatinine (Dai et al., 1994). Despite the known contractile effects of vanadium in smooth muscle, oral treatment with the element did not increase blood pressure of normal or diabetic rats. In contrast, vanadium was even reported to lower both blood pressure and elevated plasma insulin levels of spontaneously hypertensive rats (Bhanot et al., 1994).
Little is known about the toxic systemic effects of vanadium in humans. Most investigators have been concerned by occupational exposure to the element, which may result in toxicity by the respiratory route (Goldfine et al., 1994). From the few controlled studies or therapeutic trials in which vanadium was given orally, it emerges that the most common side effect is a mild gastrointestinal intolerance (International Programme on Chemical Safety, 1988; Goldfine et al., 1994; Cohen et al., 1995), no marked perturbations of biochemical parameters were detected on the screening laboratory profiles (Dimond et al., 1963; Goldfine et al., 1994; Cohen et al., 1995). Although the doses of vanadium were substantially lower than in animals studies, the concentrations reached in plasma (1-5μM) were only five to twenty fold lower, i.e., two orders of magnitude higher than in untreated subjects. However, these studies were limited in time. The long term repercussions of a marked elevation of plasma vanadium levels in humans remain unknown. In this respect, the effects of vanadium on tyrosine kinases, which are involved in cell growth and differentiation, deserve particular attention because they raise the problem of the potential carcinogenicity of vanadium. Transformation of different cell lines can be induced by vanadate (Klarlund, 1985). However, no increase in the incidence of cancer was observed in the few studies which addressed this question in whole animals.

Since vanadyl compounds seem to be less toxic than vanadate when given acutely (Llobet & Domingo, 1984) and could be the active form in target tissues, the use of the reduced form has been suggested for therapeutic purposes (Pederson et al., 1989; Brichard et al., 1991). However, a recent study failed to demonstrate any advantage of vanadyl over vanadate salts, with respect to tolerance and efficacy in diabetic rats (Becker et al., 1994). Nevertheless, attempts are being made to develop vanadium derivatives with high potency and lower toxicity. A synthetic organic compound Bis(maltolato)oxovanadium (IV), with high lipophilicity appears hopeful (Yuen et al., 1993a). Peroxovanadium derivatives are also interesting because of their very high efficacy to mimic the effects of insulin in vitro (Posner et al., 1994). These compounds have been shown
to lower blood glucose levels immediately after injection to diabetic rats (Shisheva et al., 1994) but it is unclear whether they will remain active and non-toxic during chronic oral treatment.

3.2.1.9 Effect Of Vanadium On Disease Processes

A. Diabetes

(I) Vanadium Compounds As Insulin Mimetics In Vitro

In 1899, vanadium salts were reported to reduce glycosuria in diabetic patients. Interest in the use of vanadium derivatives for the treatment of diabetes was rekindled when in 1979, vanadium compounds were shown to increase glucose transport and oxidation in adipocytes, to stimulate glycogen synthesis in liver and diaphragm and to inhibit gluconeogenesis in hepatocytes (Tolman et al., 1979). Exogenously added metavanadate mimicked insulin in stimulating hexose uptake (Dubyak & Kleinzeller, 1980), glucose oxidation (Shechter & Karlish, 1980), lipogenesis (Schechter & Ron, 1986) and the inhibition of lipolysis (Degani et al., 1981). These insulin like effects of vanadium were rapidly confirmed and dissociated from its ability to inhibit Na⁺K⁺ ATPase. The half maximally effective concentrations of \( \text{VO}_2^{3-} \) or \( \text{VO}_4^{3-} \) for mediating the various insulin-like effects range between 0.05 and 0.2 mM. These effects do not seem to be secondary to the inhibition of Na⁺K⁺ ATPase activity. This is because \( \text{VO}_3^- \) over a concentration range of 0.5-1 mM does not inhibit Na⁺K⁺ ATPase activity at all intact adipocytes (Dubyak & Kleinzeller, 1980; Shechter & Karlish, 1980).

Since then, vanadium salts have been found to mimic most biological effects of insulin in various cell types.

In isolated adipocytes, vanadium compounds stimulate glucose uptake and its oxidation (Tolman et al., 1979). They also activate lipogenesis, inhibit lipolysis and increase lipoprotein lipase activity from rat adipose tissue (Ueki et al., 1989). They also activate glycogen synthase in adipose tissue. They exert Ca²⁺ influx in adipose tissue (Clausen et al., 1981) and inhibit Ca²⁺Mg²⁺ ATPase in rat adipocytic plasma membranes (Delfert & McDonald, 1985). Recently it was
observed that vanadate was more potent than insulin in stimulating glucose oxidation (\(^{14}\text{CO}_2\) production) from (1-\(^{14}\text{C})\text{glucose in rat adipocytes} (Duckworth et al., 1988). Glucose oxidation from (6-\(^{14}\text{C})\text{glucose was identically stimulated by either insulin or vanadate.} \(^{14}\text{CO}_2\) production from (1-\(^{14}\text{C})\text{glucose is a measurement of pentose phosphate shunt activity, whereas} \(^{14}\text{CO}_2\) production from (6-\(^{14}\text{C})\text{glucose reflects glycolytic flux. Therefore, vanadate may have a larger effect than insulin on the cellular pentose phosphate shunt activity} (Duckworth et al., 1988).

Vanadate inhibited glucose output by 60\% from perfused rat liver (Bruck et al., 1991) and increased glycolysis by affecting a variety of enzymes involved in the glycolytic pathway towards increased glucose breakdown (Rodriguez-Oil et al., 1991). Vanadate inhibited glucose-6-phosphatase, stimulated 2,3-bisphosphoglycerate phosphatase and counteracted glucagon effects on 6-phosphofructo-2-kinase activity in rat hepatocytes and adipocytes. Vanadate accelerated glycolysis by inducing L-type pyruvate kinase gene (Miralpeix et al., 1991). It also lowered mRNA levels of phosphoenolpyruvate carboxykinase in hepatoma cells (Bosch et al., 1990). It also activated glycogen synthase and inactivated phosphorylase in hepatocytes (Jackson et al., 1988). It was found to suppress the secretion of apolipoprotein B from rat hepatocytes. It was generally found to promote glycogen deposition in hepatocytes.

In muscles, in vitro effects of vanadium were not comparable to insulin's metabolic actions and might be tissue dependent. Vanadate enhanced glucose uptake, glycogen synthesis and glycolysis to a lesser extent compared to insulin but caused a greater stimulation of lactate and glucose oxidation (Clark et al., 1985). However, unlike insulin, had no effect on protein synthesis or degradation (Clark et al., 1985). Amino acid transport was reported to be enhanced in rat skeletal muscle (Henriksen, 1991; Munoz et al., 1992). Improved glucose transport in peripheral tissue, a major effect of vanadate demonstrated in rat adipocytes (Dubyak & Kleinzeller, 1980), mouse brain ([Amir et al., 1987), rat skeletal muscle (Okumura & Shimazu, 1992) could be attributed to an enhanced translocation of insulin-regulatable transporter (GLUT4) to the plasma membrane. Vanadate increased glucose transporter
expression in vitro in NIH 3T3 mouse fibroblasts (Mountjoy & Flier, 1990) and in vivo in rat skeletal muscle (Strout et al., 1990).

Unlike the effects of insulin in certain cells, the mitogenic related delayed events of insulin (or EGF) are augmented in the presence of vanadate but are not stimulated alone in the sense that insulin must be constantly bound to its cellular receptors for many hours (Kadota et al., 1986). Hence, intracellular signal(s) for DNA synthesis are transitory and disappear on removal of insulin. Vanadate added to the cells stabilizes the messenger(s) in such a way that the signal does not decrease (Reid & Reid, 1987).

Further studies have indicated that insulin and vanadate share several common features. Vanadate at sufficiently high concentrations, maximally stimulates hexose uptake, glucose oxidation and lipogenesis. Furthermore, no increment in stimulation could be achieved by the addition of insulin to vanadate-stimulated cells, and vice versa. In addition, both agents show the same concentration dependency on extracellular glucose. Agents and other conditions which suppress the effects of insulin, such as anti-calmodulin drugs, polymyxin B, bicarbonate-depleted buffers and exogenously-added ATP are equipotent in suppressing vanadate-mediated effects. Also, similar rates in the termination of lipogenesis were observed after removing either insulin or vanadate from stimulated adipocytes (reviewed by Schechter et al., 1988).

(II) Vanadium Compounds As Insulin Mimics In Vivo

The properties of vanadium described above prompted in vivo investigations of these compounds in animal models type I and type II diabetes. Orally administered vanadium has been shown to lower blood glucose in both chemically induced and spontaneously diabetic rats and mice (Heyliger et al., 1985; Ramanadham et al., 1989a; Heffetz et al., 1990; Ramanadham et al., 1990; Shechter, 1990; Shechter et al., 1990).

(a) Animals models resembling type I diabetes mellitus

The first report of insulin mimetic and anti-diabetic potential of vanadium in vivo was published by Heyliger et al. (1985) wherein normalization of hyperglycemia and improvement of cardiac depressed
function without increased in plasma insulin levels of streptozotocin (STZ)-diabetic rats (insulin deficient rats resembling type I diabetes) with chronic sodium orthovanadate treatment was reported. This observation demonstrated the ability of vanadium to improve insulin sensitivity. Hyperinsulinaemic clamp studies later confirmed a decreased insulin resistance following vanadium treatment. Since vanadyl sulfate was reported to be 6-10 times less toxic than vanadate, this form of vanadium was extensively investigated for its insulin like effects. Moderate to good diabetic control had been obtained with several vanadium compounds in the STZ-diabetic rats at oral doses between 0.1 and 0.7 mM/Kg/day (Meyerovitch et al., 1987; Pederson et al., 1989; Blondel et al., 1990; Sekar et al., 1990; McNeill et al., 1992; Thompson et al., 1993). The dose of vanadium required to achieve good diabetic control varies with the initial diabetic state of the animal (Thompson et al., 1993), the particular vanadium compound given (Cam et al., 1993b; Yuen et al., 1993a), addition of other trace elements (Srivastava et al., 1993) and as yet undetermined individual factors (Bendayan & Gingras, 1989; Cam et al., 1993b). More severely diabetic rats required much higher doses of vanadyl sulphate. Some diabetic rats which were not rendered normoglycemic by low dose treatment with vanadium (<0.5mM/Kg/day) nonetheless had significantly lower plasma triglyceride and cholesterol levels and improved glucose tolerance compared to untreated diabetic animals (Yuen et al., 1993a).

Oral administration of vanadate or vanadyl causes a fall in blood glucose levels within 2-5 days. The efficacy of the treatment appears to be inversely related to the severity of diabetes and persists for upto one year. The treatment also reversed some or all of the following symptoms in treated diabetic animals: hyperphagia, polydipsia (Pugazhenthi & Khandelwal, 1990; Venkatasan et al., 1991), hyperlipidaemia (Mongold et al., 1990) and hypothyroidemia (Ramanadham et al., 1989a). Plasma insulin levels were either not affected or were lower than controls in treated diabetic and non-diabetic rats (Cam et al., 1993b; Yuen et al., 1993a). The improvement in glucose homeostasis could not be ascribed to an accelerated glucose excretion as glucosuria was also decreased or
to a reduction in intestinal glucose absorption (Madsen et al., 1995) as effects of vanadium were detectable during intravenous and oral glucose tolerance tests as well as in the fasting state.

Chronic treatment of diabetic rats with vanadium restored the ability of insulin to inhibit hepatic glucose production in vivo (Blondel et al., 1990) by correcting the abnormal expression of genes coding for the key enzymes of glucose metabolism, thus shifting the gluconeogenic flux to glycolytic flux (Valera et al., 1990; Brichard et al., 1993). Impaired glycogen synthase activity and glycogen reserve were returned to normal (Gil et al., 1988; Bollen et al., 1990). Vanadate also restored the impaired glucose disposal by muscles through correction both of decreased expression of glucose transporter and decreased activity of glycogen synthase (Rossetti & Launghlin, 1989; Blondel et al., 1990; Venkatasan et al., 1991). Vanadate also decreased the high rate of lipolysis (Cam et al., 1993) and promoted lipogenesis, however, the blunted expression and activity of lipogenic enzymes were not modified (Brichard et al., 1994).

Oral vanadyl sulphate, 0.5 g/L reduced sorbitol accumulation (Saxena et al., 1992) and at 1 g/L effectively prevented cataract development in STZ-diabetic rats (Thompson & McNeill, 1993). Vanadyl sulphate treatment, upto 1.25 g/L in the drinking water, resulted in near normal organ/body weight ratios of lung, heart, liver, kidney and adrenal glands as opposed to the significantly elevated ratios determined in untreated diabetic rats (Dai et al., 1994). Histopathological tests showed no acceleration in morphological abnormalities in a variety of tissues, while overall mortality was 19% in the treated diabetic rats compared to 60% in the untreated diabetic animals. Tissue vanadium levels varied from 6.5-15.1 μg/g in bone, from 3.6-7.3 μg/g in kidney and from 0.2-0.5 μg/g in plasma (Dai et al., 1994).

Cam et al. (1993b) tested the putative prophylactic action of vanadium against the cytotoxic destruction of pancreatic β cells by STZ in STZ-diabetic rats. Irrespective of the delay separating diabetes induction and beginning of the treatment, parameters such as glucose tolerance, adipose tissue function were normalized indicating that insulin
mimetic action of vanadium is not due to its protective effect on pancreatic β cell against deleterious effects of STZ.

Concentration dependent effects and the in vivo interaction of vanadyl with insulin was studied by Battell et al. (1992) and Ramanadham et al. (1990). Vanadyl sulfate reduced the dose of insulin required by diabetic BB rats, a spontaneous model of type I diabetes that does not produce insulin, in a dose dependent manner, but could not totally replace insulin, thus demonstrating insulin-sparing effect of vanadium compounds in vivo (Battell et al., 1992).

(b) Animal models of type II diabetes mellitus

Anti-diabetic effects of vanadium have also been investigated in chemically induced and genetic models of type II diabetes. Vanadium treatment corrected basal and stimulated hepatic glucose production and peripheral glucose utilization in neonatal-STZ diabetic rats. Vanadium treatment attenuated hyperglycemia, improved glucose tolerance and hepatic glycogen content in ob/ob (Brichard et al., 1989) and db/db (Pugazhenthi et al., 1991) mice in which a marked insulin resistance leads to overt diabetes despite very high plasma insulin levels (Brichard et al., 1990; Meyerovitch et al., 1991). It also prevented pancreatic exhaustion of insulin. In genetically obese and mildly glucose intolerant fa/fa rats, oral vanadate treatment attenuated hyperinsulinaemia and impaired glucose tolerance (Brichard et al., 1989). Vanadium decreased food and fluid intake, reduced weight gain, attenuated hyperglycemia, hyperinsulinaemia and hyperlipidaemia in Zucker diabetic fatty rats. It restored glucose tolerance and decreased pancreatic insulin depletion. This improvement resulted from a correction of poor sensitivity to insulin of peripheral tissue, particularly muscles (Brichard et al., 1992). Neither the number or affinity of insulin receptor, nor the tyrosine phosphorylation of the receptor itself or of its substrate was affected by vanadate treatment (Lyonnet et al., 1899). It is, therefore, likely that vanadium acts at site(s) distal to the receptor in these models of insulin resistance. It was found to produce the beneficial effects on glucose homeostasis through functional improvement of GLUT 4 transporter (Brichard et al., 1992).
(III) Vanadium Coordination Complexes In Vivo

Coordination complexes of vanadium which have been tested to date are summarized below.

- Bis(maltolato)oxovanadium (IV) (BMOV)

- Bis(cysteinemethylester)oxovanadium (IV)

- Bis(cysteine,amideN-octyl)oxovanadium (IV) (Naglivan)

Figure 7. Coordination complexes of vanadium (V) and vanadyl (IV) recently reported to be insulin mimetics.
Ligands have been chosen to impart specific features to the resulting V complexes: improved lipophilicity - vanadyl cysteine methyl ester (Naglivan) (Sakurai et al., 1990), improved oral absorption by passive diffusion - Bis(maltolato)oxovanadium (BMOV) (McNeill et al., 1992), potentiation of in vitro insulin-mimetic effects - peroxovanadate (Kadota et al., 1987; Shisheva & Shechter, 1993b; Posner et al., 1994), or facilitation of transmembranal ion uptake - RL-252 and RL-262 (Shechter et al., 1992).

Naglivan, bis(N-octylcysteineamide)oxovanadium (IV), is insoluble in water. When administered in a suspension of 3% acacia gum by oral gavage at doses of 0.1-0.3 mM/Kg/day, Naglivan was effective in lowering blood glucose of STZ-diabetic rats to near-normal levels but had a very slow onset of action compared to treatment with inorganic vanadium. The advantage over vanadate or vanadyl treatment included lack of weight loss or reduction in food and fluid intake in control animals administered Naglivan (Cam et al., 1993a).

A series of bis(ligand)oxovanadium (IV) complexes were studied in STZ-diabetic rats (Sakurai et al., 1990). The ligands were salicylates, oxalate, malonate, tartrate and cysteine methyl ester. The bis(cysteine methyl ester)vanadyl complex, at a dose of 10 mgV/Kg body weight (0.2mM/Kg), was slightly more effective than the others in lowering blood glucose in diabetic rats when administered intraperitoneally at doses up to 0.2 mM/Kg/day, approximately the same as for intraperitoneal treatment with vanadyl sulphate (Sakurai et al., 1990). Oral toxicity tests demonstrated no toxicity at 10 mg/Kg/day vanadium (0.2 mM/Kg/day); however, at 100 mg/Kg/day vanadium (2 mM/Kg/day), all test animals died of diarrhoea within 4 days (Sakurai et al., 1990).

BMOV [Bis(maltolato)oxovanadium] (IV), was prepared as a vanadyl complex which would be water soluble, charge neutral and readily available for gastrointestinal absorption by passive diffusion from drinking water solutions (McNeill et al., 1992). BMOV, at a dose of 0.4 mM/kg/day, was effective in reducing blood glucose and lipid levels to near-normal with no diarrhoea and no mortality during the six month study (Dai et al., 1993; Yuen et al., 1993a). The increased absorption of
vanadium from BMOV administration was reflected in higher tissue concentrations of vanadium for a similar course of treatment with vanadyl sulfate (Yuen et al., 1993b). An advantage of the apparent increased absorption is the more rapid onset of action of BMOV compared to other vanadium compounds tested so far.

A series of dihydroxamic acid chelators has been designed as hydrophobic carriers for the vanadyl ion (Shechter et al., 1992). In assays of lipogenic stimulation in rat fat cells, RL-252 and RL-262 were maximally effective at molar ratios of 10:1 vanadyl sulfate/chelator suggesting a shuttle effect. Compounds were electrically neutral; lipid soluble and optionally chiral and release bound metal ion when treated with aqueous glutathione solutions.

Potentiation of vanadate complexes by addition of hydrogen peroxide was a serendipitous discovery (Kadota et al., 1986; Kadota et al., 1987). New complexes, termed pervanadates, were found to stimulate lipogenesis, inhibit lipolysis and promotes protein synthesis in rat adipocytes at micromolar concentrations (Fantus et al., 1989). Addition of catalase to vanadate solution prior to addition of peroxide abolished the synergism of vanadate with hydrogen peroxide; however, addition of catalase 15 mins after the mixing of vanadate and peroxide preserved the synergism (Kodata et al., 1987), suggesting that complexation of the peroxide to the vanadium has taken place (Posner et al., 1990). Subsequently, a variety of new monoperoxovanadate and diperoxovanadate compounds have been synthesized as insulin mimetics (Posner et al., 1994), including monoperoxopicolinatovanadate and monoperoxopyridyne 2,6-dicarboxylato)vanadate which activated insulin receptor kinase activity and inhibited protein phosphotyrosine phosphatase (PPTPase) in rat liver endosomes at 5-80 mM concentrations and also lowered plasma glucose at doses in the mM/kg body weight range. The stimulation of insulin receptor kinase by pervanadate complexes presumably represents the different mechanism of insulin mimesis from vanadate or vanadyl, which appear to have post-IR (or cytoplasmic receptor kinase) stimulatory effects.
(IV) **Acute Studies And Glucose Clamp Investigations**

Vanadium's insulin-mimetic effects *in vivo* have been confirmed by acute and short term studies demonstrating increasing percentage achievement of normoglycemia with increasing doses of vanadium for variety of compounds (Yuen *et al.*, 1993a). At doses ranging from 16 to 280 μM/kg body weight by intraperitoneal injection, plasma glucose was lowered to less than 8.3 mM within eight hour in progressively more rats (all initial diabetic) as the dose was increased (Yuen *et al.*, 1993a). Tissue levels of vanadium ranged from 0.3-59 μg/gm wet weight for kidney and 0.1-48 μg/ml of plasma.

In short term euglycemic clamp studies indwelling venous and arterial catheters permit maintenance of plasma glucose levels in response to exogenous insulin by infusion of tritiated glucose. Using this technique, sodium metavanadate, 0.2 g/L (Blöndel *et al.*, 1989) as well as vanadyl sulfate, 0.5 g/L (Venkatesan *et al.*, 1991) has been shown to suppress hepatic glucose production and improved glucose utilization induced by submaximal or maximal insulin levels, compared to control rats. In partially pancreatectomized Sprague-Dawley rats sodium metavanadate, 0.2 g/L (36-39 mM/kg/day) lowered fed. and fasted plasma glucose levels without altering plasma insulin levels or affecting body weight gain (Cordera *et al.*, 1990).

Vanadium has also been combined with lithium (Rossetti *et al.*, 1990; Srivastava *et al.*, 1993) and zinc, lithium as well as magnesium in combination (Rossetti *et al.*, 1990) to potentiate the effect of vanadium alone in improving glucose tolerance. Pancreatectomized rats given sodium vanadate, 0.05 g/L and lithium carbonate 0.3 g/L in the drinking water for 2-3 weeks prior to euglycemic clamp investigation showed improved glucose uptake and skeletal muscle glycogenic rate, compared to untreated pancreatectomized rats (Rossetti *et al.*, 1990). Addition of magnesium sulphate 0.3 g/L and zinc sulphate 0.3 g/L to some of the treated rats resulted in further slight but significant improvement in tissue glucose uptake (Rossetti *et al.*, 1990). In a separate experiment, diabetic rats given sodium vanadate 0.05g/L and lithium carbonate 0.3g/L in the drinking water were rendered normoglycemic within four
days and had partially restored liver and kidney superoxide dismutase activities following 16 days of treatment (Srivastava et al., 1993), demonstrating an ameliorative effect on antioxidant status in lithium and vanadate treated diabetic rats.

(V) Human Trials

Recently, limited clinical trials of vanadium compounds have been initiated on human type I (IDDM) and type II (NIDDM) diabetic subjects. Sodium metavanadate administered for 2 weeks at 125 mg daily in divided doses resulted in significant increases in mean rates of glucose metabolism in 2 out of 5 subjects with type I diabetic and 5 out of 5 subjects with type II diabetes (Goldfine et al., 1995). In type I diabetic patients, vanadium lowered insulin requirements without an effect on C-peptide levels suggesting the absence of an influence on insulin release (Goldfine et al., 1995). Treatment lowered serum cholesterol levels in all subjects. In type II diabetic patients, improved insulin sensitivity, enhancement of non-oxidative glucose disposal rates and higher basal MAP and S6 kinases activity in monocytes were recorded. Hepatic glucose production was unchanged. Diarrhoea was the main side effect observed (Goldfine et al., 1995). Treatment with vanadyl sulfate (100 mg/day) for 3 weeks caused improved insulin sensitivity, reduction in hepatic glucose production and increased rate of glucose disposal, all of which were sustained for 2 weeks after treatment was withdrawn (Cohen et al., 1995). This finding is in agreement with some animal studies (Pederson et al., 1989; Becker et al., 1994). In both the studies, incidence of mild gastrointestinal intolerance was observed. Vanadyl therapy for 6 weeks at 100 mg/day also showed similar results i.e. reduced fasting plasma glucose and HbA1c were recorded without an effect on plasma insulin levels. In a more recent study, 25 mg/day vanadyl sulfate, there was no change in glucose and lipid metabolic parameters (Goldfine et al., 1998). Doubling the dose of vanadyl sulfate improved insulin sensitivity without any significant change in plasma glucose levels (Goldfine et al., 1998). There was no increase in thiobarbituric acid reactive substances at these doses. Though those studies are encouraging, further evaluation
of the long term effectiveness of vanadium salts, particularly in type II diabetic patients is warranted.

**[VI] Mechanism Of Action**

The mechanism of action of vanadium in producing its anti-diabetic effects in vivo is poorly understood and is currently the subject of much investigation. In vitro and in vivo data demonstrate that vanadium affects various aspects of insulin signaling pathway. Vanadium stimulates autophosphorylation of insulin receptor (Ueno et al., 1987; Gherzi et al., 1988) either by activation of tyrosine-kinase (Smith & Sale, 1988) present in the β sub-unit of the receptor itself or by inhibition of phosphotyrosine phosphatase that dephosphorylates the receptor (Swarup et al., 1982; Tracy & Gresser, 1986). These insulin mimetic effects of vanadium could be by virtue of it behaving as a phosphate analog. Other studies however demonstrated that vanadium was equally effective in stimulating glucose metabolism in rat fat cells when half the insulin receptors had been inactivated by insulin over-stimulation (Green, 1993). Furthermore, glucose-lowering effects with oral vanadium treatment were observed while no insulin receptor kinase activity change could be recorded (Mooney et al., 1989) indicating potential post-receptor effects of vanadium, further downstream in the insulin-signaling cascade (Shisheva & Shechter, 1993a; Shechter et al., 1982). Vanadium activated a cytosolic (non-receptor) insulin insensitive protein tyrosine-kinase (Cyt PTK) distinct from IR tyrosine-kinase. This activation was linked to glucose oxidation and lipid synthesis but dissociated from glucose uptake and inhibition of lipolysis. Cyt PTK would be highly selective for vanadium since neither insulin nor isoproterenol, dibutyryl cAMP, okadaic acid, hydrogen peroxide or phorbol ester TPA did affect Cyt PTK activity.

In addition to vanadium, other PTPase inhibitors have also been shown to activate Cyt PTK in adipocytes. It should be noted that insulin mimetic effects of vanadium on hexose uptake and inhibition of lipolysis are not blocked by staurosporine (a blocker of Cyt PTK) indicating that
this pathway is definitely not the only means by which vanadium influences cellular physiology (Sekar et al., 1996).

**Figure 8.** Putative sites of vanadium action in the insulin signal transduction pathway

PY – tyrosine kinase residues, IRS-1 – insulin receptor substrate

Insulin signal transduction is mediated intracellularly through a complex network of cascades of reversible protein phosphorylations and dephosphorylations. The phosphorylation of the insulin receptor by vanadate is weak, even when the element exerts antilipolytic action similar to that of insulin. Vanadium also stimulates MAP and S6 kinases that have been demonstrated to be defective in both the basal insulin stimulated state in STZ-diabetic rats (Hei et al., 1994, 1995). Thus insulin resistance associated with long term diabetes may be linked with altered signaling through these kinases and vanadium could rectify the
observed defects. Taken together, these finding strongly suggest that vanadium salts by-pass the early events of the phosphorylation-dephosphorylation cascade triggered by insulin. This raises the possibility that the element might be able to influence glucose metabolism even when the insulin transduction pathway is not functioning correctly.

When long term effects of vanadium treatment were studied, a persistent hypoglycemic state following vanadium treatment with only minor improvements in pancreatic secretory function was observed. The vanadium-treated rats could sustain an increased sensitivity to circulating insulin even after the treatment was stopped. A possibility could be that vanadium is released from potential tissue storage sites producing the anti-hyperglycemic effects although this seems highly unlikely. Alternatively, Cam et al. (1997) suggested that vanadium-induced amelioration of the diabetic state may be partially due to preservation of a functional portion of pancreatic beta cells in the STZ animals. This study showed that a modest increase in β-cell content was crucial to the long-term effect of vanadium even though the total insulin content was still much less than normal. The absence of normal plasma insulin levels strongly suggests the presence of additional actions of vanadium, perhaps at the levels of insulin sensitive tissues (Cam et al., 1997).

**B. Hypertension**

Oxovanadium compounds cause vasoconstriction and increased arterial blood pressure. Oral treatment of rats with vanadate also increased rat blood pressure (Steffan et al., 1981). However, whether this is due to direct effects of vanadate on smooth muscle remains to be determined. Vanadate infusion in dog or cat caused arterial hypertension, increased peripheral resistance and caused a marked reduction of coronary and renal blood flow. Larger arteries (femoral and carotid) were not constricted (Borchard et al., 1981, Larsen & Thomsen, 1980a).
C. Carcinogenesis

In mice there has been shown to be a 50% reduction in the ability to induce mammary carcinomas with N-methyl nitroso urea for animals fed vanadyl sulfate (Thompson et al., 1984). The interactions of vanadium with phosphorylation/dephosphorylation processes, especially involving known oncogene products will be related to its effects on carcinogenesis.

D. Depressive Illness

The levels of vanadium in depressive illness have been examined (Naylor et al., 1987). Vanadium concentrations in hair, whole blood and serum were greater in depressed patients compared to vanadate levels after recovery in the same patients. Renal clearance was lower and mean serum vanadium concentration higher using two different populations of depressed patients compared to controls. Patients taking lithium for depressive illness were found to have lower serum vanadium and cobalt, with elevated serum aluminum (Campbell et al., 1988).
3.2.2 Chromium

Chromium (Cr), a first-series transition element from group VI B, is present ubiquitously in water, soil and living matter (Schroeder et al., 1963). The chromium concentration within the mammalian organism is generally within the range of 10 to several hundred parts per billion (ppb). It is somewhat lower in vegetable products. On the other hand, concentrations of approximately 100 parts per million (ppm) on a dry-weight basis have been found in an ascidian (Levine, 1961) and up to 800 ppm in a ribonucleoprotein fraction from beef liver (Wacker & Vallee, 1959). The physiological significance of these high concentrations is not clear at this time.

3.2.2.1 Chromium Chemistry

The chemical state in which chromium is present, is of great importance for the biological response (Rollinson, 1966). Of the three common valence states II, III and VI, only the trivalent form has strong co-ordinating tendencies.

The chromous ion is a strong reducing agent and, unless protected by co-ordination to strong ligands, is unstable because easily oxidized to the trivalent form. It is unlikely that it exists in the organism, except perhaps as an unstable part of a redox system, in conjunction with the trivalent form (Mertz, 1967).

The trivalent state is the most stable and biologically the most important one. It is characterized by a strong tendency for co-ordination. Its coordination number is six, the resulting complexes are octahedral. Of the transition elements with a known biological function, chromium (III) is outstanding by its strong tendency to hydrolyze in aqueous solution and to form polynuclear, olated complexes of the type

![Figure 9. Polynuclear olate complexes of chromium (III)](image-url)
In a suitable milieu, this process can lead to the formation of macromolecule with resulting participation of chronic hydroxides. Heat, time and alkaline conditions promote this reaction, the reaction products are inert and biologically little effective. Olation is repressed by ligands, which can compete for coordination with hydroxyl groups at physiological pH. Indeed, apparently insoluble hydroxides can be solubilized and deolated in vitro by reaction with certain ligands. There is evidence suggesting that small changes in the degree of olation are of importance for biological activity (Mertz & Rollinson, unpublished data), but an exact definition has not been accomplished as yet.

Biological activity also depends on the stability of chromium complexes. Very stable, unreactive compounds exhibit little or no biological activity, when given by stomach tube (Schwarz & Mertz, 1959). In the hexavalent state, chromium has acidic properties and does not form coordination compounds. Although it is a strong oxidizing agent and is easily reduced to the trivalent form, it has been found in natural materials. Of particular interest is its affinity to red blood cells, upon in vitro equilibration or when injected intravenously (Gray & Sterling, 1950). In contrast to the trivalent form, it penetrates the erythrocyte membrane easily and becomes firmly bound to the haemoglobin. The observation that trivalent chromium has a greater affinity that the higher oxidation state when allowed to react directly with haemoglobin suggests that the chromate ion, after having penetrated the cell membrane, may be reduced to the trivalent form with subsequent complex or chelate formation.

The metabolism of chromium-hemoglobin has been investigated (Gabrieli et al., 1963). There is evidence that the chromium in red cells also reacts with other compounds of lower molecular weight; the formation of chromium-glutathione complex has been suggested (Prins, 1962). Stimulation of growth rates in long-term experiment by hexavalent chromium has been reported (Byerrum, 1961). In short term studies this form has no effect on glucose tolerance.

The chemical nature of naturally occurring chromium complexes or chelates is not exactly defined. In glucose-tolerance factor (GTF),
chromium is present in the form of a water soluble complex with cationic properties (Schwarz & Mertz, 1957). A naturally occurring low molecular weight (400-600 daltons) organic compound is a water soluble complex with cationic properties. It is stable against wet heat, acid and alkali treatments. The exact chemical structure is still unknown, but it appears to be a complex of nicotinic acid, amino acid components of this compound. Several synthetic GTFs have been found to have chemical and biological properties similar to naturally occurring GTF. Richest sources of GTF include brewer's yeast, liver and kidney.

3.2.2.2 Reactions In vitro

Of the many reactions of the chromium with biological materials, chromium tanning of skins was probably the first known and used. The chemistry of this process is not completely understood as yet; it probably involves complex formation between polynuclear chromium species and the carboxyl groups of protein (Stinsny, 1931). The reaction of chromium with living skin is well known (Fregert, 1964); reduction to the trivalent form and subsequent complex formation may play a role. The selective affinity of hexavalent chromium for red blood cells and of the trivalent form for the plasma proteins in vitro and in vivo is being used as an invaluable tool for the study of mass and turn over of erythrocytes and plasma protein (Gray & Sterling, 1950).

Several enzymes systems have been found to respond to chromium in-vitro with increased activity. In a succinate-cytochrome dehydrogenase system, addition of 20 μg per flask stimulated enzyme activity twice as much as did and equal amount of aluminum (Horecker et al., 1939). Other metals had lesser activity. Phosphoglucomutase which requires magnesium and a second metal, responded to chromium as the second metal with higher activity than to Al, Pb, Fe, etc; in the absence of magnesium, only chromium increased enzyme activity (Strickland, 1949). Rat liver responded to chromium in vitro and in vivo with an increase of fatty acid and cholesterol synthesis from acetate (Curran, 1954). Chromium has been reported as a constituent of proteolytic enzymes,
essential for function; the activity of trypsin, inactivated by dialysis was reconstituted by addition of the element (Langenbeck et al., 1961). Stimulation of growth and biotin synthesis by chromium in *Aerobactor agrogenes* was observed by Perlman (1945). The reactions of the element with various compounds of biological importance have been investigated. These findings present clear proof that chromium does react with biochemical systems but an interpretation in nutritional sense is difficult because of the small specificity of most of the observed effects. This is not surprising since transition elements are known to substitute for one another in many *in vitro* systems. The experiments were not superimposed on a deficiency state except, perhaps, for one (Curran, 1954) in which a diet relatively low in chromium was used.

### 3.2.2.3 Nutritional Considerations

The nutritional significance of the disturbances of carbohydrate metabolism described so far is difficult to assess since the observed symptoms are mild. The absence of fasting hyperglycemia and of glycosuria suggests that the changes are compensated by the organism. Thus, it is not surprising that under these conditions no depression of growth rates or of other vital functions was observed. Evidence for more severe symptoms of low-chromium state came from a laboratory especially equipped to minimize extraneous metal contamination. The use of this laboratory and observation of animals throughout their life or even for several generations have produced very valuable results, not only for chromium, but also for a variety of other trace elements. On a whole rye, skin milk diet with adequate supplementation of vitamins and Zn, Cu, Mn, Co and Mo, male mice (Schroeder et al., 1964) and rats (Schroeder et al., 1963) receiving 2-5 ppm of chromium (III) in water grew significantly better than their controls. This stimulation of growth was associated with decreased mortality. In these experiments, but not in others, the effect of chromium was less pronounced in females. The basal diet contained 0.1 ppm of the element, a concentration apparently not sufficient for optimal growth and survival. Chromium was little
accumulated in organs, had no effect on the incidence of tumors, but appeared to protect female rats against lung infection. Of particular interest is the observation that the bodies of young rats mostly contained the element, whereas the livers of their mothers had little or no detectable amounts (Schroeder et al., 1964). It appears that the embryo is supplied with a considerable amount of chromium and that repeated pregnancies can deplete the tissue stores of the mother on a low-chromium diet. As could be expected, glucose tolerance and other aspects of glucose metabolism in these rats were more impaired than those of 'conventional' rats and were significantly improved by chromium in vivo and in vitro (Mertz & Roginski, 1963). A very severe impairment of intravenous glucose tolerance was found in old breeder rats. In some instances, blood glucose levels did not decline at all during 1 hour after glucose load. Chromium supplementation gradually improved these conditions, but not to normal, within the experimental period of 11 days (Mertz & Roginski, 1963). The absence of net blood glucose changes during the tolerance test and increased water consumption of the low-chromium rats suggested the possibility of a diabetes-like state. However, the fact that fasting glucose concentrations were not elevated and the absence of glycosuria excluded this diagnosis. The disturbance of glucose tolerance appeared to be still compensated by other mechanisms. That this compensation can be exhausted by more severe dietary restrictions was demonstrated in rats raised in the strictly controlled environment on a Torula-yeast ration. In addition to the depressed growth rates and increased mortality discussed before, the animals on the low-chromium ration exhibited significantly higher fasting serum glucose levels than chromium-supplemented rats (Schroeder, 1966). The reported values of 137-139 mg/100ml must be considered abnormal, in view of specific glucose oxidase method used.

Diabetes in man is associated with increased incidence of vascular lesions (Goldenberg & Blumenthal, 1964). In the rat, the impairment of glucose tolerance induced by a low-chromium diet is equally followed by an increase in the incidence of plaques of stainable lipids and of fluorescent material in the aorta (Schroeder & Balassa, 1965). Of 47 low-
chromium rats which spontaneously after more than 1 year, six exhibited aortic plaques (13%) but only one of 43 of the chromium supplemented rats (2%). Chromium also lowered serum cholesterol levels in male rats, but this effect was not specific since it was also observed in animals receiving lead and cadmium.

The sequence of events developing in a low-chromium state can be described as an initial, slight impairment of the response of peripheral tissue to insulin. With more pronounced deficiency, growth rates become depressed, these is increased mortality and increased incidence of atheromatous lesions in the aorta, and glucose metabolism can be disturbed to approach a diabetes-like state. On the basis of this data, chromium must be considered an essential for health of rats.

**Role of chromium in Human nutrition**

The question whether low-chromium states exist in man is of considerable theoretical and practical interest. There are a number of syndromes related to impaired glucose metabolism with unknown etiology. The most common one is the gradual decline of glucose tolerance with increasing age which has been observed in samples of United States population (St Rat, 1948). Another example is maturity-onset diabetes, which is not caused by lack of insulin, but has been attributed by different investigators to widely varying causes (Berson & Yalow, 1965). A severe impairment of glucose metabolism in protein malnutrition in many areas is also well known (Baig & Edozien, 1965) and a high incidence of abnormal glucose tolerance tests in obesity (Vajda et al., 1964) and atherosclerosis (Wahlberg, 1962) has been described. The first suggestion of the existence of low-chromium states in parts of the United States population came from an analytical survey of tissue-chromium levels (Tipton, 1960). A marked decline of chromium concentrations with age was found in samples of kidney, liver, aorta, heart, and spleen. An interpretation of these findings is difficult because all samples of the two youngest age groups with high-chromium content came from one area of the country, and values for adult tissues from this location were not reported. However, a trend toward decreasing
chromium concentrations is still apparent in the remaining age groups for kidney, liver and aorta (Schroeder et al., 1964). Marked differences also appeared when tissues from various geographic areas within the United States were compared. Chromium was found in only 70% of the live samples from Denver, Colorado as compared to 100% in those from New York and Chicago. The average in the livers, which contained the element, differed by a factor of 9. The low average chromium content in liver samples from Nigeria is approximately one third of the average of United State population. In general, mean tissue chromium levels in man are considerably lower than those of experimental or wild animals, perhaps reflecting a lower dietary intake in refined food. An institutional diet provided approximately 80 µg of chromium per day and person, contained mainly in one meal (Schroeder et al., 1963).

The question whether a low chromium state is associated with impairment of glucose tolerance in man has been under investigation for several years. It appears that plasma-chromium levels in the fasting state have little correlation to glucose tolerance but that the absence of a rise in plasma chromium following a glucose load may suggest a deficiency of available tissue-chromium stores (Glinsmann et al., 1966). Not enough data are available as yet to allow a correlation of this phenomenon with the efficiency of glucose utilization. However, it has been clearly established that chromium supplementation with 150 to 1000 µg per day does improve some cases of impaired glucose tolerance in man. Normal tests in young, healthy subjects were not influenced by chromium, just as normal tests in chromium sufficient rats were not further improved and effects were observed only in people with impaired glucose utilization.
3.2.2.4 Chromium Requirements and Intake

The Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for chromium is shown in Table 1.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Recommended chromium intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children 7 yrs to adults</td>
<td>50-200 μg</td>
</tr>
<tr>
<td>Ages 4 to 6 yrs</td>
<td>30-120 μg</td>
</tr>
<tr>
<td>Children 1 to 3 yrs</td>
<td>20-80 μg</td>
</tr>
<tr>
<td>Infants 0.5 months to 1 yr</td>
<td>20-60 μg</td>
</tr>
<tr>
<td>Infants aged ≤ 6 months</td>
<td>10-40 μg</td>
</tr>
</tbody>
</table>

The ESADDI is similar to an RDA (Recommended Dietary Allowance) and is usually established prior to the RDA. Normal dietary intake of chromium for adults is suboptimal based upon the recommended intakes and also studies showing beneficial effects of supplemental chromium.

In 1979, an American Medical Association Panel (American Medical Association Department of Foods and Nutrition, 1979) recommended the daily administration of 10-15 μg of chromium for adult TPN patients and 0.14 μg/kg to 0.20 μg/kg for pediatric patients. Fleming et al. (1989) recommended 10 μg to 20 μg daily for adults and Green et al. (1988) proposed 0.2 μg/kg/day for infants and children. However, the chromium content of adult TPN solutions may not be adequate for severely stressed patients. By contrast, TPN solutions may be too high for infants and children (Bougle et al., 1993), leading to negative effects that include reduced growth (Moukarzel et al., 1992). The basal chromium content of TPN solutions varies widely and should be monitored (Anderson, 1995b).

Normal dietary intake of people in US is approximately 50 to 60% of the minimum suggested daily intake of 50 μg. In the study of Anderson and Kozlovsky (1985) involving the 7-day intake of 32 subjects, determined using the duplicate diet technique, none of the subjects averaged even the minimum suggested intake and none of the individuals diet provided even half of the upper limit of the ESADDI. Similar intakes
have been reported for people in US and other Westernized countries (Cauwenbergh et al., 1996).

Not only is the total dietary intake of chromium important, but also the total diet consumed. For example, increased intakes of simple sugars lead to increased losses of supplemental chromium (Kozlovsky et al., 1986). This becomes a double-edged sword since high sugar foods are often also low in chromium. Diets high in simple sugars lead to elevated levels of circulating insulin and once insulin increases, chromium is mobilized. Chromium does not appear to be reabsorbed by the kidney and is lost in the urine.

3.2.2.5 Chromium Transport, Distribution Metabolism And Excretion

With the use of trace techniques, a considerable amount of knowledge on fate and distribution of chromium in organism has been accumulated (Schroeder et al., 1963; Hopkins, 1965; Mertz et al., 1965). The physiological amount of trivalent chromium given by oral route or are absorbed from gastrointestinal tract only at the amount of a few percent, regardless of dietary chromium status and the dose applied (Mertz et al., 1965). It is not known how chromium is transferred across the intestinal wall. Chromium absorption is inversely related to dietary intake (Byerrum, 1961). At daily dietary intakes of 10 µg, chromium absorption is approximately 2% and at intakes of 40 µg is 0.5%. This leads to an absorption of approximately 0.2 µg per day which appears to be a minimal basal level. At dietary intakes above 50 µg chromium per day, chromium absorption is approximately 0.4% and chromium from chromium picolinate approximately 1.2% at intakes of approximately 1000 µg per day (Feldman et al., 1966). In addition to form, oxidation state and route of administration, ascorbic acid, carbohydrates, phytate, oxalate, aspirin, antacids and indomethacin also alter chromium absorption. Ascorbic acid was shown to significantly increase chromium absorption in humans (Gabrieli et al., 1963) with similar results in rats (Glinsmann et al., 1966). Using radiolabeled chromium chloride, animals fed starch were shown to have higher chromium absorption than those
fed sucrose, fructose or glucose (Glinsmann & Mertz, 1966). Phytate has been reported to have either no effect on chromium absorption (Goldenberg & Blumenthal, 1964) or an inhibitory effect (Gray & Sterling, 1950). Oxalate also inhibits chromium absorption (Gray & Sterling, 1950). Prostaglandin inhibitors such as aspirin and indomethacin enhance chromium absorption and antacids inhibit chromium absorption.

Whole-body distribution studies indicate that the highest concentrations of chromium were found in the kidney followed by liver, spleen, heart, lungs and gastrocnemius muscle. The subcellular distribution of chromium in the liver and kidneys results in 45% to 50% of the total cell burden locating to the nuclear fraction, with lesser amounts retained by cytoplasmic, mitochondrial and microsomal fractions (Curran, 1954). Chromium appears in the blood firmly bound to siderophilin, the iron-carrying protein. Tissue uptake is quite rapid and the blood is cleared of a dose of chromium 51 within a few days (Hopkins, 1965). Chromium incorporation into rat tissues was shown to vary widely depending upon form (Fregert, 1964). Whole-body radioactivity, on the other hand, disappears much more slowly, indicating concentration by some organs. The loss of chromium 51 from the organism can be expressed by at least three components with half-lives of 0.5, 6 and 83 days, respectively. It is not influenced by the nutritional state with regard to chromium, dose range or by superimposing additional doses of unlabeled chromium.

Following clearance from the blood, chromium is excreted principally in the urine by way of the kidney (Schroeder et al., 1963). Secondary excretion of small portion of oral chromium which is not absorbed occurs via bile and feces. A minor route of excretion is through the skin in hair, perspiration. Various stresses including high sugar intake, exercise, infection, pregnancy, lactation and physical trauma (Hopkins, 1965). The more serious the stress, the greater the losses of chromium.
3.2.2.6 Toxicological Effects

The toxicity of hexavalent chromium is well established. Chronic exposure to chromate dust has been correlated with increased incidence of lung cancer (Brinton et al., 1952). Oral administration of excessive levels (50 ppm) was associated with depression of growth and damage in liver and kidney of experimental animals (Mackenzie et al., 1958). Lower, more nearly physiological doses, on the other hand (from 0.45 to 77 ppm in the drinking water), resulted in decreased mortality of dogs, as compared with controls. No pathological changes were observed in liver and kidney, even though these organs had a relatively high chromium content (Byerrum, 1961).

Compared with the hexavalent form, trivalent chromium, the form of chromium found in foods and nutrient supplement, is much less toxic owing to the low permeability in intact cells and a smaller redox potential (E° = -0.4 V) than the strongly oxidizing hexavalent form (E°= +1.41V; +3e-) (Mackenzie et al., 1958). Some cases of hypersensitivity to chrome-tanned leather in man have been described (Fregert, 1963). Elevation of blood glucose levels has been reported in mice on a bread and milk ration to which trivalent chromium had been added. With lower concentrations of the element, blood sugar levels were lowered and ascorbic acid levels in blood and liver increased (Wachrusheva, 1960). Acute toxicity studies in rats with intravenously injected chromium (III) established the lethal dose for 50% of the animals at approximately 10 mg element /100 g body wt. This dose, compared with amounts required to correct the impairment of glucose metabolism in vivo (0.05-0.1 μg /100 g), demonstrates a wide margin of safety (Mertz et al., 1965). The reference dose established by the US Environmental Protection Agency for chromium is 350 times the upper limit of the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) of 200 μg /day. The reference dose is defined as “an estimate of a daily exposure to the human population, including sensitive sub-groups, that is likely to be without an appreciable risk of deleterious effects over a life time” (Mertz et al., 1994). This conservative estimate of safe intake has a much larger safety factor for trivalent chromium than
for almost any other nutrient. The ratio of the reference dose to the Recommended Dietary Allowance (RDA) is 350 for chromium, compared to less than 2 for zinc, roughly 2 for manganese and 5 to 7 for selenium (Mertz et al., 1994). Anderson et al. (1997b) demonstrated a lack of toxicity of chromium chloride and chromium picolinate in rats at levels several thousand times the upper limit of the ESADDI for humans. Trivalent chromium is one of the least toxic nutrients and there have been no documented negative side effects, nor have there been any documented toxic effects in any of the human studies involving supplemental chromium. While toxic effects of chromium are limited to a small percentage of the population primarily exposed to chromium in occupational settings, the effects of marginal chromium nutrition appear widespread and may affect a large percentage of the general population. Hexavalent chromium can be reduced to trivalent, but there is no conversion of trivalent to hexavalent in living organisms.

3.2.2.7 Chromium And Lean Body Mass In Humans

Chromium has been reported to increase lean body mass in both male (Evans, 1989) and female subjects (Hasten et al., 1992). Beginning weight training college students and college football players displayed an increase in lean body mass while consuming chromium as chromium picolinate (Evans, 1989). Lean body mass of students supplemented with chromium, 200 µg per day as chromium picolinate, increased 1.6kg, while that of students on placebo increased only 0.04 kg during the 40-day weight lifting study. In a separate study, lean body mass of football players increased 2.6 kg in athletes taking chromium and only 1.8 kg in those on placebo during a 42-day weight lifting study. Percent body fat of athletes on chromium also decreased significantly, while decreases in percent body fat of football players on placebo were insignificant (Evans, 1989).

Follow-up studies to confirm these results have met with limited success (Anderson, 1993b). Hasten et al. (1992) reported increased lean body mass in females on a weight training program consuming 200 µg daily of supplemental chromium as chromium picolinate, but not in
males. It was postulated that 200 μg of supplemental chromium may not be enough for the male subjects. Clansy et al. (1992) did not observe any significant changes in lean body mass, percent body fat, or muscle girth following supplementation of 200 μg per day of chromium as chromium picolinate, compared to the placebo group in football players on a weight regime for 9 weeks. Lefavi et al. (1993) reported no significant effects of supplemental chromium on lean body mass or percent body fat, but did not report improvements in blood cholesterol in weight training subjects consuming chromium as chromium nicotinate.

Hallmark et al. (1996) completed a study involving 16 untrained males (ages 23 ± 4 yrs) on a 12-week resistive exercise training program. The 16 subjects were pair-matched on initial strength levels and placed in either the chromium-supplemented (200 μg/day as chromium picolinate) or the placebo group. Increases in lean body mass in the chromium group were double that of the placebo group, but were not significant based on the Bonferonni statistical treatment of the data. However, by paired t-test, there was a significant effect of chromium on lean body mass at the p<0.01 level.

Associated with the exercise-induced changes in glucose, insulin and strength parameters, weight training exercise also leads to improved chromium absorption (Rubin et al., 1998). Chromium absorption (based on urinary excretion) of a stable isotope of chromium increased significantly following a single bout of exercise. Strength training (16 weeks) also increased absorption (Rubin et al., 1998).

The mechanisms for improvements in glucose and insulin metabolism following exercise are unclear and likely to involve several factors. One possible mechanism for these improvements may involve chromium. Chromium regulates blood glucose by potentiating insulin activity, leading to increased insulin sensitivity (Anderson, 1993a). Exercise training alters chromium distribution and excretion (Anderson et al., 1982, 1984, 1988, 1991a; Vallerand et al., 1984), leading to more efficient utilization of glucose. The increased insulin sensitivity associated with supplemental chromium may also be improved by the
compensatory mechanisms to conserve chromium, as observed in trained vs. untrained athletes (Anderson et al., 1988).

### 3.2.2.8 Chromium And Immune Function

Stress alters chromium metabolism, but chromium also counteracts the effects of stress (Anderson, 1994). The mechanism of how chromium counteracts stress may involve the effects of chromium on immune response. During stress, glucose metabolism increases, cortisol increases and chromium mobilization and excretion also increase (Anderson, 1991a, 1994). Blood cortisol is correlated with urinary chromium losses (Anderson, 1991a). Cortisol acts antagonistically to insulin by preventing entry of glucose into peripheral tissue such as muscle and fat to spare it for tissues of higher demand such as brain and liver (Burton, 1995). Stress and glucocorticoids like cortisol have suppressive effects on the immune system (Kahansari, 1990). Therefore, the immune system may be compromised when it is needed. In humans, as well as farm animals, immunoenhancing compounds in times of stress may be needed. This would be particularly true in stressed and/or immunocompromised farm animals in which the response to even the best vaccines is poor (Butron, 1995).

Like humans, farm animals display signs of chromium deficiency. Chang and Mowat (1992) reported that chromium in the form of high chromium yeast increased average daily gain by 30% and feed efficiency by 27% in steer calves following the stress of shipping. Chromium had no significant effects on the non-stressed animals. Supplemental chromium decreased serum cortisol and increased immunoglobulin M and total immunoglobulins in the stressed animals. These effects were also diet-dependent (Chang & Mowat, 1992), suggesting that form of dietary chromium or dietary chromium interactions alter the beneficial effects of supplemental chromium on cellular immunity in stressed animals. Humoral immune responses of periparturient and early-lactating dairy cows were also improved by supplemental chromium (Burton et al., 1993). Moonsie-Shageer and Mowat (1993) reported that average daily weight gain increased 27% for the chromium supplemented
stressed feedlot calves compared to the placebo animals. Chromium supplementation in the form of high chromium yeast also caused increased immunoglobulin levels and peak primary antibody responses. Serum cortisol was also reduced by supplemental chromium and morbidity and rectal temperatures tended to be lower. These results were confirmed in a follow-up study utilizing chromium in the form of a high chromium yeast or a chromium amino acid chelate (Mowat et al., 1993). In that study, beneficial effects were reported on average daily gain, blood cholesterol, glucose, morbidity, immunocompetence and disease resistance.

The mechanism of the beneficial effects of chromium on immune function likely involves increased insulin sensitivity, decreased cortisol (which is antagonistic to insulin), and increased cyclic adenosine monophosphate (cAMP) phosphodiesterase. Striffler et al. (1995) reported that chromium deficiency led to significant decreases in cAMP-dependent phosphodiesterase activity. This would lead to increased levels of cAMP, since this enzyme is involved in the degradation of cAMP. Therefore, improved chromium nutrition would lead to decreased cortisol and increased cAMP-dependent phosphodiesterase and improved immune function.

This series of studies not only demonstrates the beneficial effects of chromium on immune function, but also document that farm animals like humans are often chromium-deficient. The signs of chromium deficiency are overt in the stressed animals. There have not been any reported studies on the effects of chromium on the immune function of humans. This is an important research area, and well controlled studies are urgently needed.

3.2.2.9 Chromium And Diabetes Mellitus

Chromium was shown to be an essential nutrient more than three decades ago when it was shown that rats fed a Torula yeast-based diet developed impaired glucose tolerance that was reversed by an insulin potentiating factor whose active component was shown to be trivalent chromium (Mertz & Schwarz, 1959; Schwarz & Mertz, 1959). Chromium
has subsequently been shown to be an essential element for fish, mice, squirrel monkeys, guinea pigs, pigs, cattle and humans (Table 2).

Table 2. Signs and symptoms of chromium deficiency

<table>
<thead>
<tr>
<th>Function</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impaired glucose tolerance</td>
<td>Human, rat, mouse, squirrel monkeys, guinea pigs, cattle</td>
</tr>
<tr>
<td>Elevated circulating insulin</td>
<td>Human, rat, pig, cattle</td>
</tr>
<tr>
<td>Glucosuria</td>
<td>Human, rat</td>
</tr>
<tr>
<td>Fasting hyperglycemia</td>
<td>Human, rat, mouse</td>
</tr>
<tr>
<td>Impaired growth</td>
<td>Human, rat, mouse, turkey</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>Human</td>
</tr>
<tr>
<td>Elevated serum cholesterol and triglycerides</td>
<td>Human, rat, mouse, cattle, pig</td>
</tr>
<tr>
<td>Increased incidence of aortic plaques</td>
<td>Rabbit, rat, mouse</td>
</tr>
<tr>
<td>Increased aortic intimal plaque area</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Nerve disorders</td>
<td>Human</td>
</tr>
<tr>
<td>Brain disorders</td>
<td>Human</td>
</tr>
<tr>
<td>Cornea lesions</td>
<td>Rat, squirrel monkey</td>
</tr>
<tr>
<td>Ocular eye pressure</td>
<td>Human</td>
</tr>
<tr>
<td>Decreased fertility &amp; sperm count</td>
<td>Rat</td>
</tr>
<tr>
<td>Decreased longevity</td>
<td>Rat, mouse</td>
</tr>
<tr>
<td>Decreased insulin binding</td>
<td>Human</td>
</tr>
<tr>
<td>Decreased insulin receptor number</td>
<td>Human</td>
</tr>
<tr>
<td>Decreased lean body mass</td>
<td>Human, pig, rat</td>
</tr>
<tr>
<td>Elevated percentage of body fat</td>
<td>Human, pig</td>
</tr>
<tr>
<td>Impaired humoral immune response</td>
<td>Cattle</td>
</tr>
<tr>
<td>Increased morbidity</td>
<td>Cattle</td>
</tr>
</tbody>
</table>

Chromium was shown to be an essential element in humans during the seventies when a patient on Total Parenteral Nutrition (TPN) developed severe signs of diabetes including weight loss, glucose intolerance and peripheral neuropathy that were refractory to insulin (Jeejeebhoy et al., 1977). Since conventional treatments for diabetes, including 200 units of
insulin per day, were unsuccessful, the patient was given supplemental chromium based on previous animal studies and preliminary human studies. Following two weeks of supplemental chromium (200µg daily as chromium chloride), signs and symptoms of diabetes were reversed and exogenous insulin requirements dropped from 200 units per day to zero. The patient has been maintained on 20µg of chromium daily for more than 20 years with a control of signs and symptoms of diabetes and no adverse effects. Beneficial effects of chromium on patients on TPN have been confirmed on numerous occasions and documented in the scientific literature from three separate laboratories (Freund et al., 1979; Brown et al., 1986; Anderson, 1989). In addition, diabetes and neuropathy of a patient on home parenteral nutrition were alleviated upon addition of supplemental chromium to TPN solution (Verhage et al., 1996). Chromium is now routinely added to TPN solution (Anderson, 1995b).

Chromium presently added to TPN solution may not be adequate for some patients. Peripheral neuropathy and glucose intolerance of a patient receiving recommended levels of chromium in his TPN solution (Total parenteral intake approximately 15µg daily) were alleviated by an additional 250µg daily of chromium as chromium chloride (Verhage et al., 1996). Peripheral neuropathy was improved significantly within four days of additional chromium and normalization of nerve conduction within three weeks. Glucose intolerance was also normalized within three weeks of supplemental chromium.

Beneficial effects of chromium are not limited to patients on TPN. Children, the elderly, people with type I and II diabetes mellitus as well as those with low blood sugar have all been shown to display positive effects in response to supplemental chromium (Anderson, 1998). In addition to humans, beneficial effects of supplemental chromium have been observed in rats, mice, squirrel, monkeys, guinea pigs, rabbits, pigs, cattle and horses. In last five years chromium has been shown to play a role in type 2 diabetes mellitus (Anderson et al., 1997a), gestational diabetes (Jovanovic et al., 1999), steroid induced diabetes (Ravina et al., 1999) and glucose tolerance (Cefalu et al., 1999).
In a study conducted in 180 type II diabetic patients in China supplemental chromium as chromium as chromium picolinate for 4 months improved the blood glucose, insulin, cholesterol and haemoglobin A1c in a dose dependent manner (Anderson et al., 1997a). Follow-up studies, involving more than 800 people with type II diabetes and lasting for more than 1 yr, have confirmed these findings (Cheng et al., 1999).

Supplemental chromium also decreased the blood glucose of women with gestational diabetes in a dose dependent manner (Jovanovic et al., 1999). Thirty women with gestational diabetes were given 8 week treatment with 4 and 8 μg/kg chromium as chromium picolinate daily. Chromium-supplemented groups had significantly lower glucose and insulin levels compared to their baseline values and with those of placebo group. The group receiving 8μg of chromium per kg body weight had significantly lower postprandial glucose levels than the 4μg/kg group.

Blood glucose levels of 38 of 41 subjects with steroid-induced diabetes decreased from greater than 8.3 mM/L (250 mg/dl) to less than 8.3 mM/L (150 mg/dl) following treatment with 200μg of chromium as chromium picolinate three times per day (Ravina et al., 1999).

The amount of insulin or oral hypoglycemic agents was also reduced in a study involving 162 patients with diabetes (48 with type I diabetes and 114 with type II diabetes). A 200μg supplement of chromium as chromium picolinate reduced insulin and sulfonylurea or metformin requirement by 50% in 118 of 162 patients (Ravina et al., 1995). The results were similar for both types of diabetes. Insulin action of people with glucose intolerance is also improved by supplemental chromium. Moderately obese subjects with glucose intolerance were divided into two groups and given either placebo or 1000μg of chromium as chromium picolinate daily for 8 months (Cefalu et al., 1999). There was significant increase in insulin sensitivity in the chromium group and no effect in the placebo group.

In a study involving free-living subjects free of diabetes, chromium supplementation was found to decrease 90-minute glucose levels of the subjects with 90-minute glucose greater than 5.56mM/L (Anderson et al., 1983a). Blood glucose of subjects with good glucose tolerance (defined as
90-minute glucose less than 5.56 mM/L but greater than fasting) was unchanged by supplemental chromium. The blood glucose of subjects who tended to have low blood sugar (90-minute glucose less than fasting) increased after chromium supplementation. A follow-up study confirmed that subjects with low blood sugar respond to supplemental chromium (Anderson et al., 1987).

Further documentation that the chromium requirement is related to the degree of glucose intolerance was reported by Anderson et al. (1991b) in a study in which subjects consumed low chromium diets. Consumption of diets comprised of normal food containing less than 20 µg of chromium daily resulted in no significant changes in the glucose and insulin variables of subjects with good glucose tolerance, but consumption of these same diets by people with 90-minute glucose values greater than 5.56 mM/L resulted in increased blood glucose insulin levels that were reversed by supplemental chromium (200 µg/day as chromium chloride).

Human trials of supplementation of either chromium chloride or chromium-rich brewers yeast have yielded equivocal results. All subjected responded to chromium supplementation with improved glucose tolerance in some studies (Offenbacher & Pi-Sunyer, 1980; Riales & Albrink, 1981; Mossop, 1983; Saner et al., 1983), while others found improvements in of 40-60% of the patients evaluated (Glinsmann & Mertz, 1966; Levine et al., 1968; Anderson et al., 1983a), no response (Sherman et al., 1968; Rabinowitz et al., 1983a; Uusitupa et al., 1983; Hunt et al., 1985; Offenbacher et al., 1985) or even deterioration of glucose control (Wise, 1978). The varying results chromium supplementation could be due to the diverse genetic and nutritional background as well as the diet, duration of study, amount and type of supplemental chromium.

In addition to improvements in blood glucose and insulin, studies have reported improvements in blood lipids following chromium supplementation. Such improvements are usually greatest in subjects with the highest blood lipids but significant changes may take several months to appear (Anderson, 1995a). In a study with 250 µg chromium as...
chromium chloride, increased HDL cholesterol and decreased triglyceride did not appear until 6-16 months (Abraham et al., 1992). Several studies have reported beneficial effects of chromium on blood lipids in 3 months or less (Riales & Albrink, 1981; Bourn et al., 1986 Wang et al., 1989; Press et al., 1990, Lefavi et al., 1993). The variable responses to chromium in blood lipids is likely similar to responses in blood glucose.

Mechanism of action

A proposed mode of action of chromium in regulation of insulin is shown in figure 10. Chromium increases insulin binding to cells due to increase insulin receptor number (Anderson et al., 1987). The insulin receptor is present in essentially all cells, but its concentration varies from approximately 40 receptors per cell per erythrocytes to more than 200,000 receptors for adipocytes and hepatocytes (Saad, 1994). The insulin receptor is composed of two extracellular α sub-units with a molecular weight of 135,000 that contained the insulin binding site and two transmembrane β sub-units with a molecular weight of 95,000 (Kahn, 1985).

Wortmanin is an anti-fungal agent that inhibits phosphotidylinositol 3'-kinase, which in turn also inhibits many effects of insulin stimulation in insulin dependent cell (Okaka et al., 1994; Kanai et al., 1993). Wortmanin also inhibits chromium potentiation of insulin activity (Imparl- Radosevich et al., unpublished observations). This suggests that chromium, like insulin, affects protein phosphorylation-dephosphorylation reactions. Once insulin binds to the α sub-unit of the insulin receptor, a specific phosphorylation of the β sub-unit occurs through a cascade of intermolecular phosphorylation reaction (Saad, 1994; Kahn, 1985; Roth et al., 1994). The enzyme partly responsible for phosphorylation, which leads to increased insulin sensitivity, is insulin receptor tyrosine kinase which is activated by chromium (Davis & Vincent, 1997). A low molecular weight chromium binding compound dose not affect the protein kinase activity of rat adipocytes in the absence of insulin but stimulates kinase activity 8-fold in the presence of insulin. Removal of chromium from the low molecular weight chromium binding
compound results in the loss of kinase potentiating activity (Davis & Vincent, 1997). Chromium also inhibits phosphotyrosine phosphatase (PTP-1), a rat homolog of tyrosine phosphatase (PTP-1B) that inactivates the insulin receptor (Imparl-Radosevich et al., unpublished observations). The specific inhibition of insulin receptor phosphotyrosine phosphatase activity needs to be studied more closely since a low molecular weight chromium binding substance has also been shown to activate a membrane phosphotyrosine phosphatase (Davis et al., 1996). The activation by chromium of insulin receptor kinase activity and the inhibition of insulin receptor tyrosine phosphatase would lead to increased insulin sensitivity (Saad, 1994; Kahn, 1985; Roth et al., 1994). Increase glucose utilization and β cells sensitivity have also been demonstrated using the hyperglycemic clamp technique (Potter et al., 1985).

Figure 10. Mode of action of chromium in potentiation of insulin.

PI 3-kinase – phosphatidylinositol 3'-kinase