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VANADIUM

Diabetogenic action of streptozotocin was reported for the first time by Rakieten et al. (1963) who observed frank diabetes on intravenous administration of STZ in dogs and rats. Further, it was found that STZ produces diabetogenic effects in rats ranging from mild to moderate diabetes to severe ketotic stage at higher doses (Hoffeizer & Carpenter, 1973). STZ treatment produces hyperglycemia by its selective β-cell cytotoxic effect. Fragmentation of β-cell DNA is considered as the crucial event, caused by accumulation of superoxide and OH radicals and/or DNA alkylation. This stimulates poly(ADP-ribose)synthase and NAD depletion leading to cell necrosis, thus causing deterioration in insulin synthesis and secretion (Okamoto et al., 1988). In the present investigation, a single intravenous injection of 40 mg/kg in adult rats resulted in the development of cardinal signs of type I diabetes i.e. loss of body weight, polydipsia, polyphagia, hyperglycemia and insulinopenia. This is consistent with the earlier reports (Rodrigues et al., 1986; Goyal et al., 1987; Kawashima, 1987). This model resembles type I diabetes mellitus in humans (Kasuga et al., 1978; Kobayashi & Olefsky, 1979). Different laboratories have used different doses of STZ ranging from 30-80 mg/kg. In our laboratory, a dose of 45 mg/kg produced a high mortality (80%) and hence, a dose of 40 mg/kg that produced significant hyperglycemia associated with hypoinsulinaemia and glycosuria (>2%) was used.

Previous studies have reported that neonatal administration of STZ results in the development of non-insulin dependent diabetes mellitus in the adult state which closely resembles type II diabetes in humans (Portha et al., 1974; Bonner-Weir et al., 1981; Weir et al., 1981). It is reported that neonatal rats treated with STZ (80-100 mg/kg) at birth or within the first 5 days following birth experience severe pancreatic β-cell destruction, accompanied by a decrease in pancreatic insulin store and a rise in plasma glucose levels (Weir et al., 1981; Blondel et al., 1989). However, in contrast to adult rats treated with STZ, the β-cells of the treated neonates partially regenerate (Wang et al., 1996). Following the initial spike in plasma glucose, the STZ-treated neonatal rats become
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normoglycemic and remain so until 6 weeks of age, whereupon frank chronic hyperglycemia develops with plasma glucose concentrations usually ranging between 200-350 mg/dl (Bonner-Weir et al., 1981). Though an active regeneration of β-cells occurs in the neonatal STZ-treated rats, the plasma insulin values in these rats were lower compared to control rats and insulin secretion was extremely insensitive to glucose (Bonner-Weir et al., 1981; Weir et al., 1981). In the present investigation, a single intraperitoneal injection of 90 mg/kg STZ in 2 day old rat pups resulted in the development of NIDDM at 12 weeks of age as evident from mild hyperglycemia and severe glucose intolerance. However as opposed to earlier reports (Bonner-Weir et al., 1981; Weir et al., 1981), the basal insulin levels in the NIDDM rats in the present investigation were in the normal range.

In the present investigation, BMOV at the dose of 0.23 mM/kg/day effectively decreased elevated fasting serum glucose levels without any significant change in serum insulin levels of type I STZ-diabetic rats. These results agree with those reported by McNeill et al. (1992) and Yuen et al. (1993a). Improvement in glucose homeostasis without any significant change in serum insulin levels was also observed in type II neonatal STZ diabetic rats treated chronically with BMOV (0.23 mM/kg/day). Earlier study by Yuen et al. (1999) has reported similar findings with chronic BMOV treatment in rodent model of type II diabetes mellitus, however, of genetic origin i.e. zucker fa/fa rats.

The newly synthesized oxovanadium complex VUR1 at the dose of 0.22 mM/kg/day significantly decreased elevated glucose levels in both type I and type II diabetic rats without any significant change in serum insulin levels indicating improvement in glucose homeostasis. The glucose-lowering effect of VUR1 was found to be comparable to that of BMOV.

Treatment with vanadium complexes did not produce any significant effect on serum glucose and insulin levels of control rats. These findings are contradictory to those of several earlier studies reporting vanadium-induced reduction in circulating plasma insulin
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levels in control rats (Heyliger et al., 1985; Pugazhenthi & Khandelwal, 1990; Cam et al., 1995).

At the end of treatment schedule the animals were subjected to oral glucose tolerance test which directly measures the action of endogenous insulin in response to a glucose stimulus (Alford et al., 1971). However, this method does not allow a separate evaluation of β islet cells or peripheral dysfunction because of the interrelationship between β islet cells and peripheral insulin sensitive tissues. In this method, AUCglucose (mg/dl.120min) indicated insulin stimulated glucose disposal, whereas, AUCinsulin (μU/ml.120min) indicated insulin response to glucose stimulus. In the present investigation, AUCinsulin of type I diabetic rats was significantly lower whereas AUCglucose was significantly higher as compared to those of control rats indicating impaired glucose tolerance. Chronic treatment with BMOV and VUR1 significantly decreased AUCglucose of diabetic rats without any significant change in AUCinsulin indicating an increase in cellular sensitivity to insulin as a result of vanadium therapy. Improvement in glucose tolerance of type I diabetic rats with chronic BMOV treatment is consistent with the findings of Yuen et al. (1993a). Though the improvement in glucose tolerance with VUR1 was greater as compared to BMOV, the difference was not statistically significant.

The AUCinsulin in response to a oral glucose challenge in type II diabetic rats was not significantly different from that of control rats. Despite normal basal serum insulin levels and normal insulin response to oral glucose load, the NIDDM animals exhibited mild hyperglycemia and significantly higher AUCg as compared to control rats. This indicates that these animals are insulin resistant. Earlier Serradas et al. (1990) have reported that chronic treatment with vanadate does not produce any improvement in β cell response to glucose despite appearance of normoglycemia in neonatal-STZ diabetic rats. As opposed to these reports, in the present investigation, we found a significant reduction in AUCg of neonatal STZ-diabetic (type II) rats with chronic treatment with BMOV and VUR1, the effect being more pronounced in case of VUR1
indicating improvement in glucose tolerance. The improvement in glucose response was not associated with any change in insulin response to oral glucose load in type II diabetic rats. It seems more likely that the effects of oral vanadium complexes in STZ-induced type I and Type II diabetic rats involve direct effects on the peripheral target tissue at the level of insulin receptor or post receptor events which are not dependent upon normalized β-cell function.

The glucose levels and amount of insulin released in response to oral glucose load in control and control rats treated with BMOV and VUR1 were not significantly different from each other. These findings contradict those of Yuen et al. (1993a) who have reported a significant reduction in basal as well as glucose stimulated insulin response in control rats during OGTT.

While OGTT provides an indirect measurement of insulin sensitivity, insulin tolerance test provides direct measurement of the metabolic response to exogenous insulin. KIT, the insulin sensitivity index assesses the peripheral insulin resistance (Alford et al., 1971) which is a net result of resistance to insulin action at different sites including hepatic levels or target tissue levels i.e. adipose tissue and skeletal muscle which consists of receptor and post-receptor defects or combination of both processes (Kahn, 1978). KIT represents percentage decline in plasma glucose concentration per min. Insulin resistance is important characteristic features of both type I and type II diabetes mellitus. In IDDM, chronic insulin deficiency renders the target organs resistant to the acute actions of insulin (Taylor et al., 1994). Several studies in IDDM diabetic ketoacidotic patients with insulin tolerance test have indicated impaired whole body insulin effectiveness (Walker et al., 1963; Alford et al., 1971). Also under steady state conditions of plasma insulin and glucose, a significant insulin resistance of peripheral glucose disposal in type I diabetic patients with ketoacidosis has been observed (Ginsberg, 1977b). Insulin resistance is an important pathogenic factor in NIDDM characterized by resistance to the effects of insulin in peripheral tissues which is manifested as impaired uptake and utilization
of glucose in insulin sensitive target organs (adipocytes and skeletal muscle), impaired inhibition of hepatic glucose output and reduced insulin secretion from pancreatic β cells (DeFronzo, 1988). Using the insulin tolerance test, investigators have documented a blunted decline in plasma glucose concentration in type II diabetic patients with fasting hyperglycemia (Alford et al., 1971; Beck-Nielsen et al., 1980; Bonora et al., 1989). In our investigation also we found similar results. Both type I and type II diabetic rats exhibited significant reduction in the glucose disposal in response to exogenously administered insulin compared to the controls as evident from the lower Kirr values of diabetic rats indicating presence of insulin resistance in these animals. Chronic treatment with BMOV and VUR1 significantly increased Kirr values of diabetic rats, the effect being more pronounced in VUR1 treated animals, indicating improvement in insulin sensitivity in these animals. As the test assesses peripheral insulin sensitivity, improvement in the insulin sensitivity of diabetic animals with vanadium therapy could be due to increase in the cellular sensitivity at the hepatic level or at the level of target organs like adipose tissue and skeletal muscle. Previous studies have presented evidence that supports the notion of a mechanism of vanadium in increasing tissue sensitivity to exogenously administered insulin. Using hyperinsulinaemic-euglycemic clamps, it was demonstrated that vanadium enhanced hepatic and muscle sensitivity in STZ-diabetic rats (Blondel et al., 1989). In addition, vanadium administered to control (Challiss et al., 1987) and STZ diabetic (Ramanadham et al., 1990) animals increased the hypoglycemic response to exogenous insulin. Furthermore, in spontaneously diabetic (BB) rats, vanadium reduced the amount of exogenous insulin required to maintain an aglycosuric state (Battell et al., 1992). Treatment with vanadium compounds, however, was not found to produce any change in the insulin sensitivity of control rats as evident from the unaltered Kirr values. This is contradictory to several earlier studies reporting increase in the insulin sensitivity of control rats as a result of vanadium therapy (Heyliger et al., 1985; Pugazhenthi & Khandelwal, 1990; Yuen et al., 1993a).
Insulin resistance could result from augmented hepatic glucose production, impaired hepatic uptake of glucose or impaired glucose uptake by peripheral tissues (fat and muscle) (DeFronzo, 1988). Skeletal muscle is the chief site of insulin mediated glucose disposal. Thus, the peripheral insulin resistance reflects mainly a reduced uptake by muscle after exposure to exogenous insulin (DeFronzo & Ferrannini, 1982). Although adipocytes are responsible for the disposal of less than 5% of an ingested or infused glucose load, they play an important role in the defect in whole body glucose disposal (Bjornorp et al., 1971; Bjornorp & Sjostrom, 1978; Jansson et al., 1994). Because of the important interaction between free fatty acid and glucose metabolism (Randle, 1993), insulin resistance in adipocytes with respect to free fatty acid metabolism (Groop et al., 1989b; Reaven, 1995) can lead to the development of insulin resistance (DeFronzo, 1988, 1992; Foley, 1992) and impaired insulin secretion (Unger, 1995; Prentki & Corkey, 1996). IDDM patients with long standing diabetes are reported to exhibit reduced glucose disposal to peripheral tissue. In animal models of absolute insulin deficiency, insulin stimulated glucose transport and intracellular glucose metabolism by adipose tissue (Kobayashi & Olefsky, 1978; Kobayashi & Olefsky, 1979) and muscle (Kipnis & Cori, 1959; Morgan et al., 1961) are markedly impaired. Euglycemic insulin clamps (DeFronzo, 1988) and minimal model analysis of frequently sampled intravenous glucose tolerance test (Bergman, 1989) have documented that in type II diabetics all the major target tissues are resistant to the biological actions of insulin (DeFronzo, 1992; Reaven, 1988). However, in type II diabetic individuals with modest fasting hyperglycemia (<160-180 mg/dl) neither a defect in suppression of hepatic glucose production nor a defect in hepatic glucose uptake can account for the major impairment (> 40-50%) in tissue sensitivity to insulin that is observed during the euglycemic clamp study (DeFronzo, 1997) and thus peripheral tissues remain the primary site of insulin resistance (DeFronzo et al., 1985).

In the present investigation, results of oral glucose tolerance test and insulin tolerance test indicate insulin resistance in both type I and type II diabetic rats and improvement in insulin sensitivity of these
animals with vanadium therapy. To assess the effect of these complexes at the insulin target organs, 3T3-L1 adipocyte cell line and C2C12 myoblasts (a skeletal muscle cell line) were used. 3T3-L1 cells are a well established cell line that respond to physiological doses of insulin with increases in glucose uptake, glucose oxidation, glycogen synthesis and lipogenesis under in vitro condition (Knutson et al., 1995; Shepherd et al., 1995; Knutson & Balba, 1997). In the present investigation, it was observed that vanadium complexes, both alone and in combination of insulin significantly increased intracellular triglyceride synthesis which was considered as an index of adipocyte differentiation. Studies carried out by various workers have reported activation of adipogenesis (Schechter & Ron, 1986) in addition to enhancement of glucose transport and oxidation (Dubyak & Kleinzeller, 1980; Shechter & Karlish, 1980) and inhibition of lipolysis (Duckworth et al., 1988) in rat adipocytes. Triglyceride synthesis in response to vanadium treatment alone was not significantly different from that of co-incubation of vanadium complexes with insulin indicating that vanadium complexes did not show synergism of insulin action. This suggests that vanadium acts as an insulin mimik in vitro as opposed to insulin enhancer in vivo. The insulinomimetic effects attributed to vanadium in vitro have been demonstrated to occur at higher concentrations (10^{-4}–10^{-3} M) than used for in vivo demonstration (Mountjoy & Flier, 1990; Okumara & Shimazu, 1992; Paquet et al., 1992; Nakai et al., 1995).

C2C12 mouse myoblasts is a frequently employed skeletal muscle cell line for studying the glucose transport (del Aguila et al., 1999; Shimokawa et al., 2000; Liu et al., 2001). Insulin produced a significant stimulation of glucose uptake by C2C12 myoblast cells. BMHV and VUR1 when incubated alone significantly increased the uptake of glucose by C2C12 myoblasts. However, when co-incubated with insulin did not potentiate the insulin action indicating that at 10\mu M concentration vanadium acts as an insulin mimik rather than insulin enhancer in vitro. These findings are in accordance with the observations of several earlier workers indicating enhancement of glucose transport and oxidation in rat
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skeletal muscle (Clark et al., 1985; Okumara & Shimazu, 1992), correction of the defect in insulin-stimulated muscle glycogen synthesis in pancretectomized rats (Rossetti & Laughlin, 1989). Thus, results of in vitro studies in the present investigation indicate that the insulin sensitizing action of vanadium complexes observed in vivo could be attributed to its insulin like effects at the peripheral target organs.

It has been documented that diabetes mellitus is associated with changes in lipid metabolism. Increase in fatty acid metabolism is typical in diabetes and has been associated with the development of atherosclerosis in diabetic patients and with an increased risk of coronary heart disease (Kannel & McGee, 1979). It has been reported that rats treated with STZ have increased plasma levels of triglycerides, cholesterol, free fatty acids and phospholipids (Rodrigues et al., 1986). In our investigation also we observed significant elevation in cholesterol and triglyceride levels in type I diabetic rats. Type II diabetic rats also showed significantly higher cholesterol but not triglyceride levels compared to control rats. The absolute (type I) or relative (type II) deficiency of insulin may be responsible for the elevation of lipids in diabetic animals as insulin has an inhibitory action on HMG-CoA reductase, a key enzyme that acts as a rate limiting step in the metabolism of cholesterol rich LDL particles. The mechanisms responsible for the development of hypertriglyceridaemia in uncontrolled diabetes in humans (possibly in STZ-diabetic rats) are due to the number of metabolic abnormalities that occur sequentially. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, resulting in increased secretion of VLDL-triglyceride from liver (Balasse et al., 1972). With longer insulin deficiency liver converts free fatty acids into ketone bodies and VLDL-triglyceride secretion diminishes (Basso and Havel, 1970). At the same time, lipoprotein lipase activity decreases (Nikkila et al., 1977) resulting in impaired clearance of VLDL and chylomicrons from plasma (Bagdade et al., 1968). Reaven (1988) proposed that insulin resistance in diabetic subjects is associated with increased LDL and reduced HDL cholesterol. Chronic treatment with both BMOV and VUR1 normalized

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the elevated levels of both cholesterol and triglycerides in type I and type II diabetic rats indicating improvement in lipid homeostasis. These results are consistent with the findings of Cam et al. (1993b) and Yuen et al., (1993a) who have reported normalization of deranged lipid metabolism in STZ type I diabetic rats with chronic vanadyl sulfate and BMOV treatment. However, no improvement in lipid metabolism in genetic type II diabetic i.e. zucker fatty rats with chronic BMOV treatment has been reported by Yuen et al. (1999). Though, both, BMOV and VUR1 normalized lipid profile of diabetic rats, the effect was more pronounced in case of VUR1. The cholesterol levels in VUR1 treated diabetic rats were significantly lower as compared to BMOV treated diabetic rats. Serum triglyceride levels in VUR1 treated diabetic rats were lower as compared to those of BMOV treated diabetic rats, however the difference was not statistically significant. Decrease in total cholesterol could be due to increased HDL-cholesterol or decreased VLDL cholesterol. Triglyceride lowering effect of vanadium is likely to be the result of enhancement of two separate actions of insulin: activation of lipoprotein lipase leading to an enhanced degradation of triglycerides (Sadur & Eckel, 1982) and concurrent suppression of lipolysis resulting in a decreased supply of free fatty acids required for triglyceride biosynthesis [Reaven, 1988]. It has been reported earlier that normalization of lipid parameters was independent of the attainment of euglycemia (Yuen et al., 1993a).

Typical pathological (mesangial thickening) and immunohistochemical changes of diabetic renal disease both in rats with experimentally induced diabetes (Brown et al., 1982) and in rats with spontaneous diabetes (Cohen et al., 1987) have been reported. Such changes are also seen in normal kidneys transplanted in diabetic rats (Lee et al., 1974). Islet cell transplantation into diabetic rats which results in normalization of carbohydrate metabolism, also causes reversal of established renal lesions (mesangial thickening and immunohistochemical changes) (Mauer et al., 1974) and corrects the increased rate of albumin excretion observed in these animals (Mauer et al., 1978b). It has been shown that in rats with either experimental
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(Fox et al., 1977; Rasch, 1979a, 1979b; Rasch, 1980) or spontaneous (Cohen et al., 1987) diabetes, vigorous insulin therapy prevents development of mesangial and glomerular basement membrane thickening. All the functional and structural changes in kidneys resulting from STZ administration in rats can thus be attributed to the altered metabolism in diabetes. Moreover, it has been reported that STZ does not possess any significant nephrotoxic potential, hence, its direct effect on the kidney need not be considered when studying a drug for its effect on renal function and structure in diabetes (Evan and Mong, 1984). Increase in serum creatinine, urea and blood urea nitrogen (BUN) levels has been observed in patients with diabetes (Mulec et al., 1990).

Histologically the major pathology of diabetic kidney is confined to the glomerulus which includes nodular changes in glomerulus (William, 1961). In the present investigation, histological examination of both type I and type II diabetic rats did not show any such changes. However, type I diabetic rats showed multifocal areas of cortical tubular vacuolations with dilatation of tubules especially at the corticomedullary junction. Patches of interstitial mononuclear cell infiltration were observed suggesting presence of moderate degree of chronic inflammatory changes in these animals. Type II diabetic rats showed tubular vacuolations with tubular epithelial hypertrophy. Tubular degeneration evident from the cosinophilic appearance with pyknosis of nuclei was also observed. These morphological abnormalities in type I and type II diabetic rats were associated with a significant elevation in serum creatinine and urea levels indicating impaired renal function of diabetic animals. These observations are consistent with those reported earlier by Jensen et al. (1981), Bleasel & Yong (1982), Cam et al. (1993b) and Dai et al. (1993). Treatment with vanadium complexes produced considerable reduction in the intensity and incidence of these pathological changes. Chronic treatment with BMOV and VUR1 also significantly decreased elevated serum creatinine and urea levels of diabetic animals. Improvement of the impaired renal morphology and function associated with STZ-diabetes with vanadium treatment observed in the present investigation is in agreement with those reported by Mongold et al. (1990) and Dai et al.
Since BMOV and VUR1 have been found to be potent anti-diabetic agents, it is reasonable to believe that improvement of renal morphology and function resulted from the alleviation of the diabetic state of animals.

Vanadium is highly concentrated in kidneys following absorption (Ramanadham et al., 1991; Yuen et al., 1993b). The possible nephrotoxicity of this element was thus considered and investigated in the current investigation. However, results of studies reported by various workers are controversial (Al-Bayati et al., 1989; Mongold et al., 1990; Domingo et al., 1991a). Domingo et al. (1990, 1991a, 1991b) have repeatedly suggested that chronic administration of vanadate or vanadyl in drinking water is not a suitable therapy in STZ diabetic rats because vanadium compounds, at doses which alleviate some symptoms of diabetes, may also induce various important toxic effects such as renal impairment and even death. In the present investigation, histological examination of kidney section of control rats treated with vanadium complexes showed multifocal areas of tubular regeneration indicating degenerative damage due to the treatment. However, this morphological abnormality was not associated with significant changes in serum creatinine and urea levels, indicating that the renal function of these animals was not significantly impaired.

STZ-diabetic rats have been shown to exhibit an elevated plasma ALT level without morphological changes in liver (Domingo et al., 1991a, 1991b; Cam et al., 1993b; Dai et al., 1993). In the present investigation also a significant elevation in serum levels of liver enzymes of both type I and type II diabetic rats was observed. However no appreciable changes were observed in the hepatic morphology of the diabetic rats. The liver sections of type I diabetic rats were comparable to those of control rats. Whereas, type II diabetic rats showed multifocal areas of hepatocellular vacuolations with cellular infiltration. No correlation was observed between the functional and structural changes in the livers of diabetic rats. Further, following administration and absorption, a considerable amount of vanadium is distributed to and retained in liver
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(Ramanadham et al., 1991; Yuen et al., 1993b). The possibility that vanadium may affect hepatic function or cause hepatotoxicity was considered during the present investigation. A study in pigs revealed periportal infiltration of mononuclear leukocytes in the livers after 10 weeks (Van Vleet et al., 1981). Studies in rats and mice also showed that accumulation of vanadium in the liver caused depletion of hepatic glutathione (Bruech et al., 1984; Younes & Strubelt, 1991), inhibition of the cytochrome P-450 dependent oxidative drug metabolism (Bruech et al., 1984), and disturbances in the neutralizing and secretory functions of the liver (Roschin et al., 1980). However, previous studies have shown that vanadium treatment does not cause morphological changes in liver (Al-Bayati et al., 1989; Mongold et al., 1990) and does not increase, but rather reduces, plasma levels of liver enzymes (Al-Bayati et al., 1990; Domingo et al., 1991a, 1991b). Similarly, in the current study we found that vanadium treatment did not cause any change in serum GPT and GOT levels of control rats rather produced a significant decrease in the elevated serum levels of these enzymes in type I and type II diabetic rats. Treatment with vanadium complexes did not produce any changes in the morphology of livers of control as well as diabetic rats. This indicated that improvement of liver function was a consequence of alleviation of diabetic state of animals and that vanadium complexes did not cause hepatotoxicity under the condition of this experiment.

Intravenous injection of STZ in adults rats produce the cardinal signs of type I diabetes i.e. loss of body weight, polydipsia and polyphagia. Chronic treatment with BMOV and VUR1 did not prevent the loss of body weight of diabetic animals, however, the body weight gain in control rats was not affected by chronic treatment with vanadium complexes. A negative effect on weight gain of vanadyl sulfate treatment has been observed previously (Brichard et al., 1991; Mongold et al., 1990; Pugazhenthi & Khandelwal, 1990), but is not a consistent finding (Cam et al., 1993a; Yuen et al., 1999). It has been reported that vanadate induced decrease in body weight in control and diabetic rats may be due to decreased food intakes (Pugazhenthi & Khandelwal, 1990). In contrast
to these findings, in the present investigation, we observed that chronic treatment with vanadium complexes did not produce any significant effect on food and fluid intakes of control rats. However, these compounds significantly decreased the elevated food and water intakes of type I diabetic rats. A correction in diabetic state could have produced a correction in food and fluid intake.

The body weight, food and fluid intake of NIDDM animals was not significantly different from control rats. Treatment did not produce any significant effect on these general features of type II diabetic as well as control rats indicating lack of toxic effects of chronic vanadium treatment. It is also clear that effects observed due to chronic vanadium treatment were independent of the effects on body weight, food and fluid consumption.

Chronic vanadium treatment has been reported to produce deleterious effects on body weight, food and fluid intake (Mongold et al., 1990; Pugazhenthi & Khandelwal, 1990; Brichard et al., 1991). It is reported to produce important side effects like renal impairment and even deaths (Domingo et al., 1990, 1991a, 1991b), however these findings are inconsistent. An important side effect that has been reported consistently has been of diarrhoea (Heyliger et al., 1985; Ramanadham et al., 1989a, 1989b; Domingo et al., 1991a). However, in the present investigation no apparent signs of vanadium toxicity occurred in animals treated with vanadium complexes. BMOV treated animals exhibited diarrhoea, however, no evidence of diarrhoea was observed in VUR1 treated animals. No deaths were observed in any of the vanadium treated groups.

In conclusion, BMOV and the newly synthesized vanadium complex, VUR1 produced a number of beneficial effects in experimental models of diabetes mellitus. Correction of deranged glucose and lipid metabolism of both type I and type II diabetic rats with chronic treatment with vanadium complexes was not associated with any apparent toxic effect. Treatment with vanadium complexes also improved altered kidney
and liver functions of diabetic rats. These effects appear to be the result of *in vivo* insulin sensitizing action of insulin. The site of insulin sensitizing action appears to be peripheral *i.e.* adipocytes and skeletal muscles. Efficiency of the newly synthesized complex, VUR1 was found to be slightly higher than, if not comparable to that of BMOV. Further, it also did not produce one of the major side effects of vanadium therapy *i.e.* diarrhoea which was observed with BMOV treatment. Thus, VUR1 can be considered as a compound superior to BMOV in the treatment diabetes mellitus.

**CHROMIUM**

The first row transition elements from vanadium to zinc are essential for some form of life (Frausto da Silva & Williams, 1991; Kaim & Schwedoski, 1994; Lippard & Berg, 1994). Medical studies have clearly indicated that chromium, the next element in periodic table to vanadium, is required for normal carbohydrate and lipid metabolism (Anderson & Kozlovsky, 1985; Anderson, 1986). Conclusive evidence of the role of trivalent chromium in human nutrition was reported in 1977 (Jeejeebhoy *et al.*, 1977) when the severe diabetic symptoms of a female patient on total parenteral nutrition that were refractory to insulin were alleviated by supplemental chromium, an observation that was further confirmed on several occasions (Freund *et al.*, 1979; Brown *et al.*, 1986). Deficiency of chromium has been implicated as one of the causes of diabetes mellitus. While numerous well controlled studies have reported the beneficial effects of improved chromium nutrition (Offenbacher & Pisunyer, 1980; Riales & Albrink, 1981; Mossop, 1983; Saner *et al.*, 1983; Potter *et al.*, 1985; Bourn *et al.*, 1986; Urberg & Zemmel, 1987; Press *et al.*, 1990; Anderson *et al.*, 1991b; Abraham *et al.*, 1992), there are also few well controlled studies reporting no (Sherman *et al.*, 1968; Rabinowitz *et al.*, 1983a; Uusitupa *et al.*, 1983; Hunt *et al.*, 1985; Offenbacher *et al.*, 1985; Uusitupa *et al.*, 1992; Thomas & Gropper, 1997; Trow *et al.*, 2000) or minimal (Levin *et al.*, 1968; Glinsmann & Mertz, 1966; Anderson *et al.*, 1983a) beneficial effects of chromium. Thus, although the essentiality of
chromium in normal glucose homeostasis has been established, its usefulness as a therapeutic agent for the treatment of diabetes mellitus remains questionable.

Lack of dietary chromium has been reported to lead to the development of abnormal glucose tolerance in rats (Schwartz & Mertz, 1957; Mertz & Schwartz, 1959; Schwartz & Mertz, 1959). Moreover, it is reported that the impairment of glucose tolerance was the earliest recognized symptom of a low chromium state in animals (Glinsmann and Mertz, 1966). However, in the present investigation corresponding non-diabetic control rats of both type I and type II diabetic rats exhibited normal glucose tolerance. It is also reported that chromium deficiency is difficult to produce in animals (Striffler et al., 1995) as it required strict control of dietary (from food and water) as well as environmental (from steel cages and lids, nozzles of drinking water bottles, pipes supplying drinking water) chromium and develops slowly over a period of several months (Anderson, 1987). In the present investigation, intake of chromium by the experimental animals from these sources was not controlled. Though many investigators have reported lower chromium levels in serum (Jeegeebhoy et al., 1977), hair and tissues (Doisy et al., 1976) as compared to those of non-diabetic subjects, it is claimed that chromium concentrations in blood, hair, urine and other body fluids and tissues do not reflect chromium status. To the best of our knowledge, there is no study that gives correlation between chromium status and glucose, insulin and lipid variables before and after chromium supplementation. In the present investigation, STZ induced type I and type II diabetic rats exhibited serum chromium levels that were not significantly different from their respective non-diabetic control rats. These observations indicate that all the observed metabolic derangements in diabetic (IDDM and NIDDM) rats could be attributed to STZ action rather than altered chromium status of these experimental animals. Most of the earlier investigators have clinically studied the impairment due to chromium deficiency and its reversal with chromium supplementation (Riales & Albrink, 1981; Urberg & Zemmel, 1987;
Anderson et al., 1991b; Anderson et al., 1997a). Experimentally also most of the investigators have used whole animal models of induced chromium deficiency (Mertz et al., 1956; Mertz & Schwarz, 1959) or tissues isolated from them (Mertz et al., 1961; Mertz & Roginski, 1963) to assess the effect of chromium supplementation. In contrast to these earlier reports, the observed effects of chromium administration in the present investigation were found to be independent of the existence of chromium deficiency.

As mentioned earlier, STZ induced IDDM and neonatal NIDDM rats exhibited deranged glucose metabolism as evident from the elevated serum glucose levels as well as impaired glucose response during OGTT. Chronic treatment with chromium chloride (3.75 µM/kg) and chromium picolinate (1.91 µM/kg) significantly reduced the elevated glucose levels of non-diabetic and type II diabetic rats, however they failed to do so in STZ-induced type I diabetic rats. Treatment with chromium chloride and chromium picolinate also significantly decreased elevated AUCg of both type I and type II diabetic rats indicating improvement in glucose tolerance of treated rats. Improvement in glucose tolerance observed in the present investigation is consistent with earlier reports indicating that chromium supplementation improved glucose tolerance in rats (Schwartz & Mertz, 1959) and in diabetic humans (Offenbacher & Pi-Sunyer, 1980; Urberg & Zemmel, 1987; Anderson et al., 1991b; Jovanovic et al., 1999). These reports are unequivocal as some studies have reported no improvement even with chromium supplementation (Gurson & Saner, 1971; Rabinowitz et al., 1983b; Offenbacher et al., 1985; Thomas & Gropper, 1997, Uusitupa et al., 1992; Trow et al., 2000). Decrease in the fasting glucose levels or AUCg of diabetic animals was not accompanied by any change in basal insulin levels or glucose stimulated insulin response during OGTT indicating insulin sensitizing action of chromium. Some clinical studies have reported lower plasma insulin levels in response to chromium therapy (Anderson et al., 1991b; Wilson & Gondy, 1995; Anderson et al., 1997a). Improvement in glucose tolerance without any change in basal insulin levels or insulin response during OGTT
suggests that chromium may not have any effect on secretion of insulin from β-cells but possibly acts by enhancing insulin actions. This is further substantiated by the observation that chromium compounds failed to lower elevated glucose level in type I diabetic rats which are insulinopenic as opposed to type II diabetic rats having normal insulin levels. The improvement in glucose tolerance with chromium compounds as evident from the decrease in AUCg was also found to be greater in NIDDM rats (34% with chromium chloride and 46% with chromium picolinate) as compared to that in IDDM rats (21% with chromium chloride and 25% with chromium picolinate). Human trials have also reported that chromium would be less effective or ineffective in those who were relatively insulinopenic or more glucose intolerant at the baseline (Riales & Albrink, 1981). Thus, insulinopenia in addition to severe glucose intolerance could explain failure of chromium picolinate to lower fasting glucose levels in type I diabetic rats despite improvement in glucose tolerance as observed during OGTT.

It is reported that an organic form of chromium ‘GTF’ i.e. glucose tolerance factor unequivocally improved carbohydrate tolerance in genetically diabetic mice, whereas, the administration of inorganic chromium was ineffective (Doisy et al., 1976; Tuman & Doisy, 1977; Tuman et al., 1978). Chromium salts per se were thought to have no biological activity and it was concluded that chromium must be converted into active GTF having high in vivo and in vitro biological activity and that diabetic animals appear to loose the ability to efficiently convert to a usable form (Doisy et al., 1976; Tuman et al., 1978). In the present investigation both the inorganic i.e. chromium chloride and organic form i.e. chromium picolinate improved carbohydrate tolerance of diabetic rats, the effect being more pronounced with chromium picolinate as compared to that of chromium chloride (46 and 25% as opposed to 34 and 21%).

As discussed earlier, the presence of insulin resistance in both type I and type II diabetic rats was clearly established in the present
investigation. Chronic treatment with chromium compounds significantly increased the K\text{rrr} values of diabetic animals during insulin tolerance test indicating increased peripheral insulin sensitivity as a result of chromium therapy. Chromium administration to STZ-induced diabetic rats is reported to enhance insulin responsiveness while the insulin receptor number remained constant (Yoshimoto et al., 1992). Diminished hypoglycemic response of exogenous insulin and improvement upon chromium supplementation has been reported in chromium deficient rats, the deficiency being induced by low-chromium diet (Roginski & Mertz, 1969). Chromium has also been shown to increase glucose utilization and β-cell sensitivity measured in euglycemic clamp studies (Mertz et al., 1974; Potter et al., 1985). Chromium compounds also increased K\text{rrr} values of control rats, however, the effect was statistically significant only in case of chromium picolinate.

From the current study, it appears that the relative efficacy of chromium in whole animal may in fact depend upon the presence of insulin and that differences in degree of severity of diabetic state and relative deficiency in residual circulating insulin can ultimately determine responsiveness to chromium treatment. All these results of in vivo studies indicate insulin sensitizing action of chromium. It was observed that chromium was more effective in type II rats having normal insulin levels as compared to insulinopenic type I diabetic rats. It has been reported that nanogram quantities of chromium were required for the optimal effect of insulin in every insulin-dependent system that has been investigated (Mertz, 1969). A close dependence of biological role of chromium on the presence of insulin has been observed in many in vitro studies of chromium deficient tissues where uncorrected deficiency of chromium necessitated the addition of unphysiologically large concentration of insulin to the media to achieve normal response.

Further, of the first raw-transition elements from vanadium to zinc, chromium is only element for which atleast one metalobiomolecule containing the element had not been well characterized in terms of its mechanism of action (Davis et al., 1996). Hence, it was thought of interest to study the effect of chromium at insulin target organs namely
adipocytes and skeletal muscle to define the site and mechanism of chromium action more closely. In the present investigation, effect of chromium on insulin stimulated triglyceride synthesis was studied in 3T3-L1 adipocytes. When incubated alone chromium compounds did not produce any effect on the intracellular triglyceride synthesis, however, co-incubation with insulin significantly increased the intracellular triglyceride synthesis. Earlier studies have reported increase in the rate of glucose uptake (Mertz et al., 1961) oxidation of glucose to CO$_2$ (Mertz et al., 1965), incorporation of glucose carbon in to fat (Mertz et al., 1961) with addition of chromium to the media of rat epididymal fat pad obtained from chromium deficient animal. The rate of incorporation of acetate in to fat, which is not insulin dependent, was found to be unchanged. It has also been reported that although the synthetic GTF like complex potentiated insulin response in rat epididymal fat pad of chromium deficient rats, inorganic chromium failed to produce any response (Anderson et al., 1978). In all these observations, the observed effects of chromium were found to depend on the existence of a deficiency state.

When effect of chromium compounds on glucose uptake was studied in C2C12 myoblasts, results similar to those observed in 3T3-L1 adipocytes were obtained. Incubation of C2C12 myoblasts with chromium compounds alone did not produce any change in basal glucose uptake. However, when incubated together with insulin, chromium compounds resulted in increased uptake by the cells and the effect was significantly higher than that of insulin alone indicating insulin sensitizing action of chromium in vitro.

These results indicate that chromium compounds per se did not exhibit biological activity in vitro and the effects of chromium administration observed in vivo were mainly due to potentiation of insulin actions. As mentioned earlier, insulin resistance in type I and type II diabetic rats could be mainly due to resistance to insulin action at the peripheral tissue which are the main site of glucose disappearance (Pedersen & Beck-Neilsen, 1987; DeFronzo, 1997). Conversion into fat appears to be a major metabolic pathway for the excess sugar. Thus,
chromium appears to improve glucose homeostasis in diabetic animals by enhancing the action of insulin at adipocytes and skeletal muscle by increasing intracellular triglyceride synthesis and by normalizing the reduced peripheral glucose uptake respectively.

As discussed earlier derange lipid metabolism in diabetes mellitus has been well established both clinically (Kannel & McGee, 1979) as well as experimentally (Rodrigues et al., 1986). In the present investigation also alteration in lipid metabolism in both STZ induced type I and type II diabetic rats were observed. Chronic treatment with chromium compounds significantly decreased elevated cholesterol levels of both type I and type II diabetic rats. Treatment also significantly reduced the triglyceride levels of both type I and type II diabetic rats as well as non-diabetic control rats, indicating improvement in lipid homeostasis. These findings are consistent with the earlier reports indicating decrease in hyperlipidaemia in genetically diabetic mice (Doisy et al., 1976; Tuman et al., 1977; Tuman et al., 1978). Abraham et al. (1991) have reported reduction in the percentage of the aortic intimal surface covered by plaques in chromium treated rabbits. However, chromium supplementation in diabetic patients has been found to produce varied effects on lipid metabolism from no change in elevated lipid levels (Anderson et al., 1983a; Offenbacher et al., 1985) to decrease in triglyceride and cholesterol with increase in HDL levels (Abraham et al., 1992). The improvement in lipid metabolism in the present investigation was independent of attainment of stable euglycemia in IDDM diabetic rats suggesting that fat metabolism was corrected even before carbohydrate metabolism. Decrease in the total cholesterol, observed in the present investigation could be due to increased HDL-cholesterol or decreased VLDL cholesterol. Increase in HDL-cholesterol has been reported both in non-diabetic and diabetic subjects with chronic chromium treatment (Schwarz & Mertz, 1959; Press et al., 1990; Abraham et al., 1992). Triglyceride lowering effect of chromium is likely to be the result of enhancement of two separate actions of insulin: activation of lipoprotein lipase leading to an enhanced degradation of
triglycerides and concurrent suppression of lipolysis (Sadur & Eckel, 1982) resulting in a decreased supply of free fatty acids required for triglyceride biosynthesis (Reaven, 1988).

Alteration in kidney structure and function in clinical as well as experimental diabetes due to the metabolic derangement of diabetes mellitus has been discussed at length in one of the earlier paragraphs. Chromium compounds as observed in the present investigation by improving the derange carbohydrate and lipid metabolism were expected to correct impaired kidney function. However, as the highest concentration of administered chromium is accumulated in kidney and excreted out in urine through kidney, a possible nephrotoxicity of this heavy metal was considered in the present investigation. Chronic treatment with both chromium chloride and chromium picolinate considerably decreased the pathological changes observed in both type I and type II diabetic rats. This improvement in the renal morphology could be correlated with a significant decrease in the elevated serum urea and creatinine levels in STZ-induced type I and type II diabetic rats indicating improvement in kidney function. No change in renal morphology and serum urea and creatinine levels of non-diabetic control rats indicated lack of toxic effects of chromium compounds in the present set up. Oral administration of 0.45-77ppm of trivalent chromium drinking water did not produce any pathological changes in the liver and kidneys of dogs, though these organs had a relatively high chromium content (Byerrum, 1961). However, in one case 1200 to 2400 mg chromium picolinate for 4-5 months was found to produce renal toxicity (Cerulli et al., 1998) and in another case 600 mg of chromium daily for 6 weeks was found to produce acute interstitial nephritis (Wasser et al., 1997)

Both STZ type I and type II diabetic rats exhibited significantly higher serum GPT and GOT levels as compared to control rats, which is consistent with the earlier reports of altered hepatic function in diabetes (Domingo et al., 1991a, 1991b; Cam et al., 1993b). However, no
appreciable changes were observed in the hepatic morphology of the type I diabetic rats. Type II diabetic rats showed multifocal areas of hepatocellular vacuolations with cellular infiltration. No correlation was observed between the functional and structural changes in the livers of diabetic rats. Treatment with chromium compounds significantly decreased elevated GOT and GPT levels without affecting hepatic morphology of diabetic rats. Improvement in altered hepatic function which could possibly be due to improved glucose and lipid homeostasis with chromium therapy.

Further, liver being the next organ to kidney in accumulating chromium, the possibility of nephrotoxicity in addition to the effect on altered hepatic function was studied in the present investigation. Treatment with chromium compounds did not alter serum GPT and GOT levels as well as liver morphology of non-diabetic rats. This indicates lack of toxicity of chromium under the conditions of present investigation.

In the present investigation chronic chromium therapy did not produce any significant change in the body weight as well as food and fluid intakes of both IDDM and NIDDM diabetic rats as compared to their respective diabetic controls. This indicates that the observed beneficial effects of chromium therapy are independent of the effects on the body weight, food and fluid intake. Though several studies have reported reduction in body weight with chromium therapy (Evans, 1989; Hasten et al., 1992; Kaats et al., 1992), the results are controversial (Clansy et al., 1994; Trent & Thieding-Cancel, 1995; Hallmark et al., 1996; Lukaski et al., 1996). No effect on the general features of non-diabetic control rats indicates lack of toxicity of chronic chromium therapy.

Chromium is reported to have high safety margin. In cats injected with 500-1000 mg of chromium complexes daily for 1-3 months did not show any negative effects (Mertz, 1969). Studies in rats have shown no toxicity even at 100mg/kg dose of chromium chloride or chromium picolinate over a period of 24 weeks (Anderson et al., 1997b). In the
Discussion

present investigation also no apparent signs of toxicity were observed. No deaths were found in any of the chromium treated groups.

It is reported that chromium chloride is usually the least available form of the chromium compounds tested and that chromium as chromium picolinate is utilized more effectively. Chromium picolinate is more effective than chromium chloride in human and animal studies. Absorption of chromium from chromium chloride is in the region of 0.4% (Anderson et al., 1983b) and from chromium picolinate is 1.2% at intakes of 1000 µg/day (Campbell et al., 1999). In the present investigation, we found that chromium picolinate was more effective than chromium chloride. Though the improvement in glucose and lipid metabolism or kidney and liver function with chromium chloride and chromium picolinate was not significantly different from each other, the dose at which chromium picolinate (800 µg/kg) produced these effects was lower compared to chromium chloride (1000 µg/kg).

In conclusion, chromium compounds possessed significant anti-diabetic activity in various experimental models of diabetes that were not chromium deficient. The anti-diabetic activity of chromium is mainly due to its insulin enhancing effects particularly at the peripheral tissues including skeletal muscle and adipocytes. It did not exert any toxic effects under the conditions of the present investigation, rather improved impaired kidney and liver functions that were subsequent to diabetes mellitus. Chromium picolinate appears to be more effective in all these actions compared to chromium chloride.