Summary
I. GENERAL

Studies of several investigators have established that vanadium compounds mimic many of the insulin effects. Using in vitro studies vanadate has been found to stimulate glucose oxidation and transport in hepatocytes, adipocytes and skeletal muscle. Recently it was demonstrated that oral administration of sodium orthovanadate to diabetic rats normalized the elevated blood glucose level and could correct some of the diabetic state metabolic disorders in various tissues. However, the mechanism of vanadate for its insulin-mimetic properties is not well understood.

In the present study, 0.6 mg/ml of sodium orthovanadate given in drinking water was found to be the safe, minimal effective dose that brought about a stable normoglycemia within 4 days of treatment. Vanadate treatment also normalized the increased thirst but could not restore the body weight loss and the decreased plasma insulin in the diabetic rats. It was observed that the discontinuation of treatment resulted in recurrence of diabetic signs; hyperglycemia and increase in fluid intake within 48 hours.

II. EFFECT OF VANADATE ON HEXOKINASE ISOZYMES, PYRUVATE KINASE AND MALIC ENZYME IN INSULIN-DEPENDENT AND
-INDEPENDENT TISSUES

The effects of vanadate on hexokinase isozymes, malic enzyme and pyruvate kinase in insulin-dependent and -independent tissues namely liver and kidney respectively, of control and diabetic rats, were examined and compared. During diabetes, it was found, as reported by earlier researchers also that in liver the activities of hexokinase isozymes, pyruvate kinase and malic enzyme are decreased, in kidney these enzyme activities were found to increase except for malic enzyme which was unchanged. The increase in enzyme activities seem to keep pace with kidney growth in diabetes. Vanadate therapy in the dose applied completely normalized the differentially altered enzyme profiles in the liver and kidney of diabetic rats. Normalization of hexokinase by vanadate is of critical importance as the enzyme regulates glucose entry in the tissue and also into alternative metabolic routes.

The restoration of pyruvate kinase by vanadate in the liver and kidney suggests a probable role of vanadate in the channelling of pyruvate and glucose into the tricarboxylic acid cycle. A marked improvement of hepatic malic enzyme clearly indicate that vanadate therapy may also influence the hydrogen shuttle systems and transhydrogenase reactions of lipogenic pathways. The present work strongly confirms that vanadate has potent antidiabetic effects even under conditions of depressed insulin secretion, both in insulin-dependent and
-independent tissues.

III. POLYOL PATHWAY IN DIFFERENT TISSUES: EFFECT OF DIABETES AND INSULIN TREATMENT

The polyol pathway consists of aldose reductase and sorbitol dehydrogenase that catalyze the formation of sorbitol and fructose respectively. Under normal physiological conditions, there is very little glucose flux and in a diabetic hyperglycemic condition, the flux through this pathway increases dramatically in insulin-independent tissues like kidney, erythrocytes and lens, which do not require insulin for glucose uptake and its phosphorylation and could lead to renal hypertrophy, decreased erythrocyte deformability and cataract formation.

The polyol pathway enzymes were studied in the four tissues namely liver, adrenal, kidney, and brain. The activity and role of these enzymes in the insulin-independent tissue like liver is not yet known. Diabetes induced enhancement in both aldose reductase and sorbitol dehydrogenase activities in rat brain and kidney and were found to be partially reversed by insulin treatment. This action of insulin may possibly be due to increased glucose entry into the insulin target tissues and thereby, reducing glucose entry and preventing activation of polyol pathway enzymes in insulin-independent tissues in diabetes.
IV. POLYOL PATHWAY IN WHOLE KIDNEY: EFFECT OF VANADATE AND INSULIN

Diabetes caused an increase in aldose reductase and sorbitol dehydrogenase activities and also in sorbitol and glucose levels in the whole kidney. Vanadate therapy proved to be very effective and completely normalized the increased enzyme activities and metabolite levels in the kidney of diabetic rats that may be due to glucose lowering capacity of vanadate leading to stable normoglycemia, thereby preventing increase in glucose flux and activation of polyol pathway. Insulin, on the other hand could not completely normalize the polyol pathway as it is unable to produce stable normoglycemia in diabetic rats.

Since oral administration of insulin is ineffective and insulin injections cause large variability in blood glucose levels that may lead to various cardiovascular diseases, thus, availability of orally administered potent insulin-mimetic agents like vanadate could be of immense importance for better therapeutic interventions in the treatment of diabetes.

V. EFFECT OF VANADATE ON POLYOL PATHWAY IN KIDNEY CORTEX AND MEDULLA OF DIABETIC RATS

Aldose reductase was found mainly in the renal medulla while sorbitol
dehydrogenase was largely confined in cortex of control rat kidney. Diabetes caused a significant elevation of both enzymes in the two regions except for sorbitol dehydrogenase activity in renal medulla that remained unchanged. Sorbitol accumulated to several folds higher in the medulla than in cortex in diabetic rats. This renal medullary sorbitol accumulation may lead to increase in osmotic pressure causing kidney dysfunction at a later stage of diabetes. The activated polyol pathway during diabetes, and its correlation with other pathways like pentose phosphate pathway and biosynthetic mechanisms may cause increase in kidney growth characterized by hypertrophy. Vanadate treatment to diabetic rats restored the raised enzyme activities and metabolite levels of polyol pathway to almost control values in both renal cortex and medulla. This reversal could be due to the normalization of blood and kidney glucose levels by vanadate. Vanadate therapy, unlike insulin, almost completely reversed the increase in kidney growth.

VI. IN VIVO STUDIES ON ERYTHROCYTE ALDOSE REDUCTASE AND SORBITOL LEVELS IN DIABETES: EFFECT OF VANADATE

The activity of aldose reductase in erythrocytes was found to be very low as compared to that in other insulin-independent tissues like kidney. Diabetes caused a significant elevation in the aldose reductase activity and accumulation of sorbitol in the erythrocytes.
Oral vanadate was found to effectively normalize the highly elevated aldose reductase and sorbitol levels in the erythrocytes of diabetic rats. This reversal action of vanadate seems primarily due to its glucose lowering ability and normalizing the increased glucose flux and activation of aldose reductase in insulin-independent tissues.

VII. IN VITRO STUDIES ON ERYTHROCYTE SORBITOL CONTENT: EFFECTS OF GLUCOSE CONCENTRATIONS, VANADIUM COMPOUNDS, INSULIN AND ALDOSE REDUCTASE INHIBITORS

The in vitro incubations of erythrocytes with the increasing concentrations of glucose caused the sorbitol content to increase linearly and rapidly. Thus, erythrocyte intracellular sorbitol may reflect severity of diabetes. While in vitro insulin could not prevent sorbitol increase in erythrocytes, aldose reductase inhibitors namely tetramethylene glutarate, diphenylhydantoin and quercetin could effectively block the increase in sorbitol formation due to high glucose media. The study confirmed the presence of an active aldose reductase in erythrocytes. Sodium orthovanadate and vanadyl sulfate at 1 mM or above concentration in the incubation medium could reverse the several fold elevated sorbitol level due to high glucose. The study indicated that vanadyl sulfate and sodium orthovanadate were more effective than sodium metavanadate and
vanadium pentaoxide at similar concentrations.

Vanadate after entering the cell, gets reduced to vanadyl by GSH and forms vanadyl-GSH complexes that has been suggested to exert its insulin-like properties. Thus, orthovanadate (V$^{+5}$) or vanadyl sulfate (V$^{+4}$) possibly may form vanadyl ions and these complexes more readily, to induce a more effective and potent response than the other two compounds. Vanadate seems to have an upper hand over insulin since vanadate, unlike insulin, could effectively control the metabolic pathways in insulin-independent tissue, both in vitro and in vivo conditions, possibly by regulating the expression of glucose transporters in a tissue specific manner. The field is wide open to characterize the mechanism of action of vanadate for its insulin-like action.

**VIII. IN VITRO STUDIES ON ERYTHROCYTE GLUCOSE OXIDATION USING U$^{14}$C-LABELLED GLUCOSE: EFFECTS OF VANADIUM COMPOUNDS AND INSULIN**

Further to substantiate that vanadium compounds could control glucose overutilization in erythrocytes, total glucose oxidation using U$^{14}$C-glucose was studied in erythrocytes in the presence of high glucose in the medium, with and without vanadium compounds and insulin to get a direct evidence.
A three fold enhanced total glucose oxidation due to the presence of 20 mM glucose (a diabetes mimicked condition) was observed to be almost completely normalized by vanadium compounds; the reversal efficacy followed the order, vanadyl sulphate > orthovanadate > metavanadate > vanadium pentaoxide. Such reversal effect was, however not observed with insulin incubation.

IX. EFFECT OF VANADATE ON ANTIOXIDANT STATUS OF DIABETIC RAT LIVER

In diabetes, increased production and ineffective scavenging of toxic reactive oxygen species are reported to play a crucial role in determining tissue injury. The present data showed that the levels of glutathione peroxidase, catalase, CuZn-superoxide dismutase, Mn-superoxide dismutase and GSH were significantly decreased in the diabetic rat liver. This decrease in antioxidant enzymes may be due to the inactivation caused by reactive oxygen species or emaciation produced in the diabetic rats.

In this study, it was observed that vanadate therapy could almost completely normalize the glutathione peroxidase and Mn-superoxide dismutase activities but it only partially restored the activity of CuZn-superoxide dismutase. This suggests that the increased oxidative stress during diabetes is controlled to some extent only. Interestingly, the catalase and GSH levels in control and
diabetic rats declined further following vanadate treatment from their counterparts, an observation not expected from an insulin-mimetic agent like vanadate in an insulin-dependent tissue. The decrease in GSH in the vanadate treated rats may be due to its consumption in vanadyl-GSH complexes. Vanadate administration in the dose applied may have some other role in the biological system independent of its insulin-mimetic effects since vanadium is a transition element with variable oxidation states (+3, +4, +5) and theoretically should influence the tissue redox state.

In conclusion, the study clearly indicates that vanadate therapy is only partially effective in controlling the impaired antioxidative system of diabetic rats as, unlike insulin, it fails to normalize catalase and GSH levels. Based on this study it can be suggested that vanadate treatment, if accompanied by some known antioxidant eg. flavanoids to improve the concomitant defence system disturbances, may produce better therapeutic results. More incisive studies are warranted to explore other insulin-like effects of vanadate that may also elucidate the mechanism of vanadate action at the molecular and cellular level.

X. EFFECT OF PRETREATMENT OF VANADATE PRIOR TO DIABETES INDUCTION

Several investigators suggested that the diabetogenic action of alloxan, which exhibits highly selective cytotoxicity against pancreatic beta cells, is
mediated by the generation of toxic oxygen free radicals. Pretreatment of rats prior to diabetes induction with free radical scavengers; such as reduced glutathione, cysteine, ethanol, dimethyl sulfoxide, superoxide dismutase and alpha-tocopherol are shown to prevent alloxan induced diabetes and its toxic manifestations. The present study was designed to evaluate whether the pretreatment of vanadate to rats, like above mentioned agents, could prevent, reduce or delay the diabetogenic action of alloxan. The rats were pretreated with sodium orthovanadate for 5 weeks prior to diabetes induction.

It was observed that vanadate treatment to control rats caused a decrease in their fluid intake which was more significant as the vanadate concentration was increased that may be due to the bitter taste of vanadate. Despite the pretreatment with vanadate, alloxan produced its toxic characteristic effect, appearance of hyperglycemia, following which more fluid intake was expected as seen in diabetic rats due to their altered physiology. Instead, in vanadate pretreated diabetic rats, a decrease in fluid intake from their corresponding control values was observed and signs of dehydration appeared in later hours after alloxan injections. Pretreatment with vanadate, however, offered some resistance against the main toxic manifestation of diabetes namely elevation of blood glucose levels only upto 24 hours after alloxan injection. Similarly, in the vanadate pretreated diabetic rats the lipid peroxide formation (TBARS content) in plasma and pancreas was found to increase slowly upto 24 hours and at 48 hours after
alloxan injection, both the TBARS content in plasma and pancreas and blood glucose level reached the values similar to that in diabetic rats.

The results showed that though administration of vanadate after induction of diabetes causes marked improvement in the diabetic state, however, administration of vanadate prior to induction of diabetes could not prevent the diabetogenic process and its toxic manifestations.

The mechanism of action of vanadate remains unclear at this stage. Further studies are required to explore its other insulin-mimetic properties and antidiabetic effects that would also help in the elucidation of its mechanism of action and would provide better therapeutic interventions in the treatment of diabetes.