STUDIES ON THE ROLE OF SELENIUM IN EXPERIMENTAL DIABETES

Thesis submitted for the degree of
Doctor of Philosophy (Medicine)
of the University of North Bengal

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PREFACE

The work embodied in this thesis entitled "Studies on the role of Selenium in experimental diabetes" is submitted for the degree of Doctor of Philosophy (Medicine) of the University of North Bengal.

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INTRODUCTION

1.0 Introduction :

Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycaemia. Though it is difficult to ascertain the real frequency in population because of the large number of persons affected, the initial asymptomatic nature of disease, different standards of diagnosis used by clinicians but probably in between 1-2% if fasting hyperglycaemia is accepted as the criterion for diagnosis. Fasting venous plasma glucose is at present accepted as the most reliable and convenient test for diagnosis of Diabetes Mellitus and is strongly recommended as a screening test by W.H.O. and National Diabetes Data Group of U.S.A. The metabolic dysregulation associated with D.M. causes pathophysiologic changes in multiple organ systems like eyes, kidneys, nerve and blood vessels. Several distinct clinical syndromes can be described under the heading of Diabetes Mellitus as a result of its heterogeneous nature [1].

Diabetes is one of the leading causes of morbidity worldwide. Major causes of prolonged morbidity in Diabetes include coronary heart disease, glomerulosclerosis, retinopathy, gangrene of lower extremity, stroke and cataract. In fact Diabetes Mellitus is one of the leading causes of end-stage renal disease, non-traumatic lower limb amputations and blindness in adults. With increasing incidence globally, it will likely to continue as a major cause of morbidity and mortality [2].

1.1 Etiological classification of diabetes :

I. Type 1 Diabetes (β cell destruction usually leading to absolute insulin deficiency).

   (a) Immune mediated

   (b) Idiopathic

II. Type 2 Diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance.)

III. Other specific types of Diabetes

(A) Genetic defects of β cell function characterised by mutations in.

   (1) Hepatocyte Nuclear Transcription Factor (HNF) 4α (MODY 1)

   (2) Glucokinase (MODY 2)

   (3) HNF-1α (MODY 3)

   (4) Insulin Promoter Factor (IPF) 1 (MODY 4)

   (5) HNF-1β (MODY 5)

(6) Mitochondrial DNA

(7) Proinsulin or insulin conversion

(B) Genetic defects in insulin action

(i) Type A insulin resistance

(ii) Leprechaunism

(iii) Rabson Mendenhall Syndrome

(iv) Lipoatrophic Diabetes

(C) Disease of the exocrine pancreas : Pancreatitis, Pancreatectomy, Neoplasia, Cystic fibrosis, Haemochromatosis, Fibrocalculous Pancreatopathy.

(D) Endocrinopathies : Acromegaly, Cushing's Syndrome, Glucagonoma, Phaeochromocytoma, Hyperthyroidism, Somatostatinoma, Aldosteronoma.

(E) Drug or chemical induced : Vacor, Pentamidine, Nicotinic acid, Glucocorticoids, Thyroid hormone, Diazoxide, β adrenergic agonists, Thiazides, Phenytoin, α interferon, Protease inhibitors, Clozapine, β blockers.

(F) Infectious : Congenital rubella, Cytomegalo virus, Coxsackie.

(G) Uncommon forms of immune mediated diabetes : "stiff-man" syndrome, Anti insulin receptor antibodies.

(H) Other genetic syndromes sometimes associated with diabetes : Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Wolfram's syndrome, Friedreich's ataxia, Huntington's chorea, Laurence-Moon-Biedl syndrome, Myotonic dystrophy, Porphyria, Prader-Willi syndrome.

IV. Gestational Diabetes Mellitus (GDM) [3].

1.2 Epidemiology :

Worldwide prevalence of Diabetes has risen dramatically over two decades. It is projected that diabetes will increase in frequency in near future. Although the prevalence of both type 1 and Type 2 DM is increasing, the prevalence of Type 2 DM is likely to increase more as a result of increase in obesity and reduction of physical activity level. Considerable geographic variations exist in prevalence and seem to be due to variation in both genetic and environmental factors. Considerable variation in prevalence of DM exists between different ethnic populations within a given country [4].

1.3 Pathogenesis :

The exact etiopathogenesis of both type 1 and type 2 diabetes remain uncertain. Genetic predisposition alongwith environmental factors determine which persons are going to develop the clinical syndrome of diabetes. The pathogenetic pattern differs in type 1 and type 2 diabetes.

1.3.1 Pathogenesis of type 1 DM

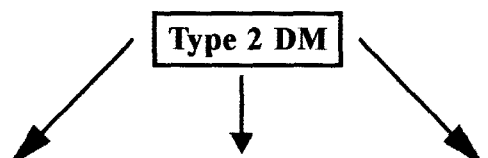
Step	Event	Agent or Response
1.	Genetic Susceptibility	HLA D region genes
	↓	
2.	Environmental Event	Infection or Food
	↓	
3.	Insulitis	Infiltration of activated T lymphocytes in pancreatic islets.
	↓	
4.	Activation of auto immunity	Self-nonself transition
	↓	
5.	Immune destruction of β cells [5]	Islet cell autoantibodies, activated T lymphocytes.
	↓	
6.	β cell death	Tonic effect of cytokines like TNF, α interferon or IL. Apoptosis of β cells, formation of nitric oxide metabolites. at Direct CD8+ T cell Cytotoxicity.
	↓	
7.	Gradual decline in β cell mass at variable rate, differing from one to other.	Progressive impairment of insulin release over a period of months to years.
	↓	
8.	80% or more β cells destroyed.	Diabetes mellitus [6].

1.3.2. Pathogenesis of Type 2 DM :

Type 2 DM is not HLA linked and no evidence of auto immunity or viruses have anything to do with its development. Although it occurs with a strong familial associations, the definition

of genetic abnormalities remain a challenge. Insulin resistance and abnormal insulin secretion remain central to the development of type 2 diabetes. Although controversy exists but most evidence points to insulin resistance precedes insulin secretory defects [7].

Type 2 DM is characterised by 3 pathophysiologic abnormalities (i) impaired insulin secretion, (ii) peripheral insulin resistance [8], (iii) excessive hepatic glucose production. Obesity augments the genetically determined. insulin resistance of type 2 DM. Adipocytes secrete a number of biologic products (leptin, TNFα, FFA) that modulate processes such as insulin secretion, insulin action, body weight and may contribute to insulin resistance. In early stage glucose tolerance remains normal despite insulin resistance as pancreatic β cells compensate by increasing insulin output. An insulin resistance and compensatory hyperinsulinemia progress, impaired glucose tolerance develops. With further decline in insulin secretion and concomittant increase in hepatic glucose release, overt diabetes supervenes. Ultimately β cells may fail to secrete insulin [9].



Insulin Resistance

Decreased ability of insulin to act effectively on peripheral tissues (muscle + liver) [post-receptor abnormality as main cause postulated e.g., polymorphism in IRS1 and PI3 kinase signalling defect with reduced translocation of GLUT4 to plasma membrane. ↑FFA may also contribute to ↓glucose utilization in skeletal muscle, ↑glucose production in liver and ↓β cell function [10].]

Impaired Insulin secretion

Insulin secretion and sensitivity are correlated. Initially insulin secretory defect is mild and selectively involves glucose with sparing of other secretagogues e.g., arginine. Eventually gross deficiency in insulin secretion supervenes and endogenous insulin secretion is grossly inadequate. [Cause is not known with intense genetic investigations exclude mutation of genes as a cause. Islet amyloid polypeptide, amylin is co-secreted with insulin and deposited in islets but significance is unknown. However, chronic hyperglycaemia and elevation of FFA impairs β cell function]

Increased Production of glucose from liver

Liver maintains blood glucose levels during fasting by glycogenolysis and gluconeogenesis. Insulin suppress glycogenolysis and gluconeogenesis. Type 2 DM insulin resistance in liver causes failure of supression of glycogenolysis and gluconeogenesis in post prandial state causing hyperglycaemia.

Risk factors associated with development of type 2 DM includes :

(i) Family history of diabetes (i.e., parent or sibling with type 2 DM).

(ii) Obesity (≥20% desired body weight).

(iii) Age ≥ 45 years.

(iv) Race/Ethnicity (African, Asian especially Indian, Native American).

(v) Previously identified IFG or IGT.

(vi) History of GDM or of delivery of a large baby.

(vii) Hypertension (B.P. ≥ 140/90 mm Hg.).

(viii) HDL cholesterol level ≤ 35 mg/dl and/or triglyceride level ≥ 250 mg/dl.

(ix) Polycystic ovary syndrome.

1.3.3 Mody (Maturity onset Diabetes of young; monogenic form of DM) :

Mody constitutes phenotypically and genetically heterogenous subtypes of DM. Five different forms of MODY have been described owing to mutations in genes encoding Islets cell transcription factors or glucokinase. All are transmitted as autosomal dominant disorder. MODY 2 is the commonest one involving mutation in glucokinase gene MODY1, MODY 3, MODY 5 are results of mutation in hepatocyte nuclear transcription factor HNF-4α, HNF-1α and HNF-1β respectively. MODY 4 is a rare variant caused by mutation in Insulin Promoter Factor Gene (IPF1). Pathogenesis of diabetic states in MODY are not well known [11].

1.4 Clinical Features :

Symptoms of hyperglycaemia includes polyuria, polyphagia and polydipsia.

No specific sign is attributed to DM and a complete assesment of diabetic state, presence of complications and associated disease should be attempted from history and physical examination. Body weight, orthostatic hypotension, hypertension, foot examination, peripheral pulses, retinal examination, examination of deep reflexes and posterior column sensation, etc. are done to assess the type severity and complication of DM. Mental confusion, coma, severe dehydration, acidotic breathing may accompany diabetic ketoacidosis. Severe dehydration, vomiting and altered mental state may be features of non-ketotic hyperosmolar state [12].

Potential Diabetics :

An individual with a first degree relative with DM is a potential diabetes with increased risk to develop DM or IFG in later life.

Latent Diabetics :

They are persons with normal GTT but shows abnormality under conditions like pregnancy, steriod therapy, therapy with other diabetogenic drugs like thiazide diuretic.

1.5 Diagnosis :

Criteria of diagnosis of diabetes mellitus :

(i) Symptoms of diabetes and a random blood glucose > 250 mg/dl

(ii) Fasting plasma glucose ⩾ 126 mg/dl. (Fasting is defined as no calorie intake for 8 hours.)

(iii) 2 hour plasma glucose ⩾ 200 mg/dl during an oral GTT. (The test is performed by using a glucose load containing the equivalent of 75 gm of anhydrous glucose, dissolved in water; not recommended for routine clinical use). In the absence of unequivocal hyperglycaemia and acute metabolic decompensation, these criteria should be confirmed on a repeat testing on a different day (Adopted from American Diabetes Association 2000)

1.6 Management :

The goal of therapy in diabetes includes :

(i) Fredom from symptom of hyperglycaemia

(ii) Reduction or elimination of complications of diabetes.

(iii) To achieve as normal life-style as possible

Ways of achievement of goal

(1) Identification of target level of glycaemic control in each patient	(2) Provision of educational, non-pharmacological and pharmacological resources to establish the glycaemic control	(3) Monitor, Treatment of complications

Comprehensive diabetic case means more than establishment of glycaemic control though it is central to optimal management of diabetes. Symptoms usually subside at or around 2 hours plasma glucose < 200 mg/dl and the mainstem of therapy revolves around detection and management of diabetic complication and risk modification for DM associated diseases [13].

Patient education is an important part of daibetic care and it involves training regarding (i) self monitoring of blood/urine glucose, (ii) monitoring of urine ketones (type 1 DM), (iii) insulin administration, (iv) guidelines of diabetic management during illness, (v) recognition and management of hypoglycaemia, (vi) foot and skin care, (vii) risk factor modifying activity [14].

1.6.1 Nutrition :

Medical Nutrition Therapy is a term used by ADA to describe the optimal co-ordination of calorie intake with other aspects of diabetes therapy. Though imparted with some flexibility in recent time (like provision of calorie sweeteners like sucrose), diabetic diet remains a complicated, restrictive, and stoic one. MNT must be adjusted in accordance with individual needs [15].

Nutritional recommendations for all diabetics :

(1) Proteins to provide ~ 10-20% of KCal/d (~10% for those with nephropathy).

(2) Saturated fats to provide ~ 10% of KCal/d (~ 7% for those with ↑ LDL cholesterol).

(3) Polyunsaturated fats to provide ~ 10% of KCal/d.

(4) Remaining calories to be distributed between carbohydrate and monounsaturated fat based on medical needs and individual tolerence.

(5) Use of calorie sweeteners like sucrose is permissible provided the insulin demand they create is matched by available insulin.

(6) Fibres (20-35 gm/d) and sodium (≤3000 mg/d) levels as recommended for normal persons.

(7) Cholesterol intake ↟ 300 mg/d.

(8) Same precaution regarding alcohol as applicable in general population. As alcohol increases the risk of hypoglycaemia it should be taken with food.

Goal of MNT in type 1 DM is to match the calorie intake, temporarily and quantitatively with the appropriate amount of insulin. MNT in type 1 DM and self monitoring of glucose must be integrated to deduce the optimal insulin regimen. MNT must be flexible to account of exercise and insulin therapy must allow for deviation in calorie intake. MNT in type 1 DM must also minimize the weight gain often encountered in intensive diabetic management [16].

The goal of MNT in type 2 diabetes address the reduction in body wt. and reduction in increased frequency of cardiovascular disease like hypertension or dyslipidemia. MNT in type 2 diabetes includes modest calorie reduction, increased physical activity and reduction of hypertension and hyperlipemia [17]. Reduction in weight with hypocaloric diets sometimes result

in marked improvement of glycaemic control. About 50% of patients respond to MNT alone without pharmacological intervention (type 2 DM). All type 1 DM patients require exogenous insulin in addition to MNT, exercise and risk factor modification. The target glycaemic control and HbA1c level are individualised but a general guideline as adopted from ADA 2000 is given [18].

	Normal	Goal	Additional action suggested
Average Pre-prandial glucose	< 100 mgm/dl	80-120 mgm/dl	< 80 mgm/dl > 140 mgm/dl
Average bedtime glucose	< 110 mgm/dl	100-140 mgm/dl	< 100 mgm/dl > 160 mgm/dl
HbA1c %	< 6	< 7	>8

Normoglycaemia or near normal glycaemia is the goal of therapy. However, it is elusive in most cases. Sometimes it is required to achieve near normal glycaemia and there intensive diabetes management by techniques like multi component insulin, multiple daily injections (MDI) or continuous subcutaneous insulin infusions (CSII). Benefits of intensive management with improved glycaemic control include a reduction/delay in occurence of micro and macrovascular complications of DM, a sense of well-being, greater flexibility in diet and exercise. Intensive management in pregnancy reduces the frequency of foetal malformations and morbidity. However, intensive management require frequent interaction with health care professionals, excellent patient education, self monitoring of blood glucose (8 times per day minimally) and entails heavy financial cost. It is not suitable for most patients [19].

1.6.2 Indications for intensive management :

(1) Otherwise healthy adults with type 1 or type 2 DM (selected adolescents or older children).

(2) All pregnant women with diabetes, all women planning pregnancy.

(3) Management of labile diabetes.

(4) Patients who have had kidney transplantation for diabetic nephropathy [20].

1.6.3 Pharmacological Management of Type 2 DM :

Besides MNT (as described above) and an exercise program to induce weightloss, if glycaemic control is not achieved after 4 weeks, pharmacologic approach is needed. Oral glucose-lowering agents are used [21,22].

Depending on mechanism of action oral glucose lowering agents are divided into 3 categories.

(1) Insulin secretagogues like sulfonylureas of 1st and 2nd generation, effective in type 2 DM of < 5 years of duration, obese patients with some residual endogenous insulin secretion.

(2) (a) Biguanides (Metformin) reduce blood glucose by reducing hepatic glucose production, increasing peripheral glucose utilisation and inducing modest weightloss.

(b) α glucosidase inhibitors (Acarbose, Miglitol) reduce post-prandial hyperglycaemia by inhibiting glucose absorption from gut by inhibiting the enzyme which cleaves oligosaccharides to monosaccharides.

(3) Thiazolidinediones (Rosiglitazone, Pioglitazone) : They reduce insulin resistance. They reduce hyperglycaemia by improving peripheral glucose utilisation and insulin sensitivity.

All failures in treatment by oral agents or severely ill patients of type 2 DM are treated with exogenous insulin [23, 24, 25, 26, 27, 28, 29, 30].

Factors associated with increased morbidity in diabetes are :

(i) Duration (ii) early onset (iii) high HbA1c (iv) hypertension (v) proteinuria (vi) obesity (vii) hyperlipemia.

1.7 Complication :

Complications can be divided into two groups : Acute complications are (i) diabetic ketoacedosis (ii) non-ketotic hyperosmolar state (iii) Lactic acidosis (iv) Hypoglycaemia (Ketoogenic in most cases).

Chronic complications are : (i) Diabetic retinopathy (ii) Diabetic foot (iii) Diabetic neuropathy (iv) Diabetic nephropathy [31, 32, 33, 34, 35, 36, 37, 38].

1.8 Selenium and its role in human metabolism :

Globally selenium (Se) is an important element because it has wide application. Selenium was discovered by Berzelius in 1817. The Se atomic number is 34 and it is in the Group VI (A) elements of the Periodic Table. The Se atomic weight is 78.96. Selenium is a required micronutrient for animals and humans; excessive Se, however, can be toxic. Its requirement for plants is not clearly understood. The range between Se toxicity and deficiency for animals is rather narrow. Selenium toxicity problems have been documented as early as the twelfth century.

For example, Marco Polo travelling in China in 1295 was probably describing toxicity symptoms of Se in livestock. In 1560, Father Simon Pedro described what was probably Se toxicity symptoms in humans in Colombia. In 1857, T.W. Madison, U.S. Army Surgeon, described the suspected Se toxicity symptoms in horses near Fort Randall, South Dakota. In China, two types of Se endemic human diseases, cardiomyopathy (Se deficiency) and selenosis (Se toxicity) were reported [39].

Selenium occurs naturally in soils and groundwaters. The main geological source of Se in soils is derived from cretaceous age snales. However, soils derived from igneous rocks are expected to contain low Se. Selenium is expected to be found in higher concentrations in semiarid and arid environments of western United States, China, Mexico and Colombia. In some humid environments of eastern United States, China, United Kingdom, Japan, India and New Zealand, Se concentration is expected to be lower. The common range of total Se concentrations in most soils is between 0.01 and 2 mgm/kg. However, in some soils of the world, total Se concentrations can be as high as 1200 mgm/kg [40]. Such soils are commonly known as seleniferous soils.

Very limited information regarding the dissolved Se concentrations in natural waters is available. Dissolved Se concentrations in fresh water may range between 0.1 and 400 μg/L [41]. Dissolved Se concentrations between 16 and 231 ng/L for river waters in Japan have been reported [42]. Dissolved Se concentrations ranging between 60-70 ng/L in Finnish groundwaters, rivers and lakes have also been reported [43].

Human activities may introduce Se into soil-water systems. Burning fossil fuels (coal), disposal of coal residues, mining activities and application of fertilizers put Se into soil-water systems. Anthropogenic activities such as coal burning power plants and smelting and refining industries are the main sources of Se [44]. Worldwide total Se input to, fresh waters from coal burning power plants is 6-30 x 10^4 metric tones of Se per year. Smelting and refining industries contribute 3-20 x 10^4 metric tons of Se per year to fresh waters.

Se in natural waters (e.g., soil water, groundwater) exist in the form of free ionic species (e.g., selenate SeO_4^{2-}) and Selenite (SeO_3^{2-}), inorganic complexes (e.g., $CaSeO_4^6$, $MgSeO_3^6$), and dissolved organic carbon complexes (DOC-Se) [45].

Se levels in blood appear to reflect the large body Se pools of nutritional interest during static long-term intakes of Se, because erythrocyte and plasma Se levels were good indicators of muscle and liver Se content in rats fed constant amounts of dietary selenium for 8 weeks or

more [46]. Skeletal muscle and liver are important because these tissues contained the largest body pools of Se. The skeletal muscle appears to contain the largest body pool of Se in humans. If a person who weighs 70 kg has a skeletal muscle mass of 28 kg [47] with Se level of 0.24 mgm/kg [48], then this tissue contains about 6.7 mgm, or 46% of the total body Se content. In humans, the liver apparently has a smaller Se pool relative to muscle than in rats. A liver of 1.8 kg with a Se content of 0.54 mgm/kg would contain only about 1 mg of Se. Sodium selenite was less effective than selenomethionine in elevating blood Se levels in New Zealand subjects of low Se status [49]. Hair Se levels were better correlated with muscle or liver Se content than either plasma or red cell Se levels in rats. Toe nails have recently been suggested as a useful indicator of human Se status [50].

The use of urinary Se levels to assess Se status in humans has recently been reviewed [51]. Se exposure in industrial workers has been monitered by measuring Se in urine [52] and a good correlation between blood Se levels and urinary excretion was observed in New Zealand residents [53]. However, random urine samples are strongly affected by current dietary Se intake and also suffer from dilution effects, whereas 24 hrs samples are difficult to obtain. The excretion of trimethyl Selenonium ion may be influenced by dietary Se intake [54] and quantification of urinary Se metabolites may lead to a convenient way of assessing Se status in humans.

Arsenic, cadmium, lead and mercury have metabolic interaction with Selenium [55].

Keshan Disease - Keshan disease, a childhood cardiomyophy endemic in some regions of the People's Republic of China, has been attributed to selenium deficiency.

Kaschin-Beck disease has also been attributed to selenium deficiency.

Gallagher et al have found hair analysis to be a valuable tool for assessing selenium status. They found dietary supplementation with selenium enriched yeast was reflected by significant increase in hair selenium levels. While the supplementation resulted in increased hair levels, it was not reflected in blood selenium levels and there was no correlation between blood and hair selenium levels [56].

Selenium intoxication may result in Kidney and liver damage, as well as damage to the CNS and brain. Reports on selenium intoxication in China also show elevated selenium levels in the hair, blood and urine. The source of selenium in this case was the food crops from soils rich in this element [57, 58].

REVIEW OF LITERATURE

2.0 Review of Literature :

2.1 General Aspects :

Diabetes is an age old disease. With its varied complications it spreads its domain to different discipline of medicine. There are many well known journals exclusively on diabetes. Literatures on diabetes are published in many other journals too. There are more than hundred thousand publications on diabetes. Some are on the clinical approaches to diagnose cases, some on therapeutic variations for successful control of blood sugar, many on the invention of drugs, some on the complications, some to find out the aetiological basis of diabetes etc. Our goal is to find out the relation of Selenium on the biochemistry of streptozotocin induced diabetic mice. so we have concentrated only on the different biochemical parameters which are affected in the diabetes process and as we have tested in experimentally induced mice the literatures surveyed by us are all for animals with drug induced diabetes.

The distribution of LDH-isoenzymes from homogenate-supernatant of muscle soleus of normal and streptozotocin-diabetic male rats was investigated by Wohlrab and Schmidt in 1975 by agargel-electrophoresis. Five LDH-isoenzymes could be detected in the muscle of normal rats. Diabetes was induced by intravenous injection of 65 mgm streptozotocin per kg body weight. After 1-2 month duration of diabetes the LDH 1 was decreased and the LDH 4 was increased. The H-subunit value was decreased. In long term diabetes (11 months) the values of single fractions and the H-subunit value did not differ from those of controls [59].

Armstrong et al in 1976 studied succinate dehydrogenase activities of skeletal muscles in normal and streptozotocin induced diabetic rats. Enzyme activities in all muscles declined to a lower final level and exhibited a more rapid decay in animals receiving the larger dosage, both diabetic and Karela juice fed rats. In addition they also observed that Karela does not always reverse the effects on drug-metabolising enzymes in STZ-induced diabetes [60].

Adipose lactate dehydrogenase (LDH) (EC 1.1.1.27) isozyme distribution was altered in streptozotocin diabetic and fasting rats resulting from a relative reduction of subunit A. Treatment with insulin for 2 days partially restored the relative content of isozyme 5 to control values in the diabetic rats, and the effect of insulin was not inhibited by simultaneous injection of actinomycin D or puromycin. When the epididymal adipose tissues isolated from control animals were incubated in vitro with dibutyryl adenosine 3', 5' -cyclic monophosphate or epinephrine, a relative decrease in subunit A was observed; whereas either compound caused an increase in subunit A in diabetic tissues. Chang and Rothrock in 1977 suggested that the redistribution of

LDH isozyme under these conditions is to prevent excessive accumulation of lactate in the tissue [61].

Studies were undertaken by Nakayama and Nakagawa in 1977 to examine cholesterogenesis in the intestine of streptozotocin-diabetic rats by measuring incorporation of [2(-14)C] acetate into cholesterol and 3-hydroxy-3-methylgultaryl CoA reductase (HMG-CoA reductase, EC 1.1.1.34) activity. In these diabetic rats, the intestinal mucosal weight and food consumption were markedly high. The incorporation of [2(-14)C] acetate into cholesterol was significantly increased in all diabetic intestinal segments. However, the rates of production of fatty acids and carbon dioxide were not affected. Hepatic HMG-CoA reductase activities were markedly reduced during both the diurnal high and low periods in these diabetic rats, and there was no diurnal variation. In contrast, the specific activities of this enzyme in jejunal crypt cells during both the diurnal high and low periods were significantly higher in these diabetic rats without loss of diurnal variation. Total reductase activity per segment of intestine in jejunal and ileal mucose (villi + crypt cells) was increased in these diabetic rats. Control rats had higher total and specific activity of ileal mucosal (villi + crypt cells) reductase than of jejunal mucosal reducatse during the diurnal high period. The jejunal-ileal gradient in reductase activity and the incorporation of [2(-14)C] acetate into cholesterol did not change significantly with streptozotocin-diabetic rats. The results indicate that in streptozotocin-diabetic rats, hepatic cholesterogenesis decreases but intestinal synthesis increases [62].

The activities of UDP glucuronosyltransferase, microsomal epoxide hydrolase and cytosolic glutathione S-transferase were measured by Rouer et al in 1981 in the liver of spontaneously (db/db and ob/ob) or streptozotocin-induced diabetic mice. An important (2-3-fold) increase of most phase II activities was observed in streptozotocin-treated animals, whereas slighter changes were detected in spontaneously diabetic animals. The latter also exhibited physico-chemical modifications of the liver microsomal membranes, as shown by the temperature-induced variations of epoxide hydrolase activity [63].

Lenzen and Panten in 1983 showed that succinate dehydrogenase activities in homogenates of rat and ob/ob mouse pancreatic islets were only 13% of the activities in homogenates of liver and were also several times lower than in homogenates of pancreatic acinar tissue. This indicates that the content of mitochondria in pancreatic islet cells is very low. The very low activity of succinate dehydrogenase is in agreement with the low mitochondrial volume in the cytoplasmic ground substance of pancreatic islet cells as observed in morphometric studies. This may represent the poor equipment of pancreatic islet cells with electron transport

chains and thus provide a regulatory role for the generation of reducing equivalents and chemical energy for the regulation of insulin secretion. The activities of succinate dehydrogenase in tissue homogenates of pancreatic islets, pancreatic acinar tissue and the liver were significantly inhibited by malonate and diazoxide but not by glucose, mannoheptulose, streptozotocin or verapramil [64].

Verschoor et al have previously suggested that mechanisms other than reduced lipoprotein lipase (LPL) activity might contribute to the defect in plasma removal of very low density lipoprotein (VLDL)-triglyceride (TG) observed in insulin-deficient rats. To further evaluate this phenomenon, removal rates of TG in nonfractionated plasma, as well as in isolated lipoprotein fractions obtained from insulin-deficient and control rats, were compared in a new, sensitive in vivo bioassay system (estradiol-treated male rats with a consistently low endogenous VLDL-TG pool). Removal of TG in nonfractionated plasma from insulin-deficient rats was slower than that of control rats: 3.0 +/- 0.3 vs 1.6 +/- 0.2 min (P less than 0.001). No difference was found in removal rate of isolated VLDL-TG (2.5 +/- 0.3 vs 2.6 +/- 0.4 min), or in removal rates of TG carried in other lipoprotein fractions. Authors in 1984 determined the effect of injection into normal rats of aliquots of dialyzed lipoprotein-free (D greater than 1.215) plasma from insulin-deficient and control rats on the removal rate of normal VLDL-TG, and found that lipoprotein-free plasma from insulin-deficient rats significantly (P less than 0.01) prolonged removal of normal VLDL-TG (4.3 +/- 0.4 to 6.8 +/- 0.7 min). This same fraction did not interfere with the in vitro hydrolysis of normal VLDL-TG by post-heparin LPL. Thus, a factor in the D greater than 1.215 plasma fraction of insulin-deficient rats is present which interferes with the rate of removal of TG from plasma, unrelated to inhibition of LPL activity [65].

Agius and Gidari in 1985 demonstrated that Streptozotocin (STZ) increased the activity of mouse hepatic glutathione (GSH) S-transferases assayed with 1-chloro-2,4-dinitrobenzene. Nicotinamide administered prior to STZ prevented the hyperglycemia indicative of STZ-induced diabetes, but had no effect on the increase in GSH S-transferase activity caused by the drug. Another diabetogenic agent, alloxan, did not alter GSH S-transferase activity. Thus, streptozotocin may be increasing GSH S-transferase activity directly, and not as a result of the diabetic state the drug induces. Two transferases were characterized from mouse liver cytosol. One was a homodimer with a subunit molecular weight of about 28,000 and a pI of about 8.2. The other was also a homodimer with a subunit molecular weight of about 27,500 and a pI of about 9.2. The pI 8.2 GSH S-transferase was induced by STZ, while the pI 9.2 transferase was decreased by the drug. At least one other transferase appeared to be induced by STZ.

Two other nitroso compounds, chlorozotocin and diethylnitrosamine, also increased GSH S-transferase activity, suggesting that this effect may be nitroso related [66].

The activities and zonal distribution of key enzymes of carbohydrate metabolism were studied in livers of diabetic rats by Miethke et al in 1985. 48 h after alloxan treatment the following alterations were observed, intermediate values being reached after 24 h: Blood glucose, acetoacetate and beta-hydroxybutyrate were increased to more than 500%; liver glycogen was reduced to about 10%. Portal vein insulin was reduced to below 10%, portal glucagon was increased to almost 200%. The glucogenic enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase were enhanced to 320% and 150%, respectively. The glycolytic enzymes glucokinase and pyruvate kinase L (differentiated from the M2 isoenzyme with a specific anti-L-antibody) were lowered to 50% and 75%, respectively. The citrate cycle enzyme succinate dehydrogenase remained unchanged. The normal periportal to perivenous gradient of phosphoenolpyruvate carboxykinase of about 3:1, as measured in microdissected tissue samples, was enhanced to about 4:1 with activities elevated to 230% and 190%, respectively, in the two zones. The normal periportal to perivenous gradient of pyruvate kinase L of about 1:1.7, as determined with the microdissection technique, was reduced to about 1:1.4 with levels lowered to 55% and 45%, respectively, in the two zones. The even zonal distribution of pyruvate kinase M2 remained unaltered [67].

Favreau and Schenkman in 1987 measured the cytochrome P-450 dependent hydroxylation of testosterone in hepatic microsomes of control, diabetic and insulin treated diabetic rats. The observed decrease in testosterone 16α-hydroxylase activity in diabetes, an activity previously shown to be largely due to RLM 5, was accompained by a dramatic decrease in immuno detectable RLM 5. Diabetic rats which received insulin had elevated testosterone 16α-hydroxylase activity relative to the diabetic animals, which was accompained by a corresponding increase in the levels of RLM 5. These results provide evidence that specific constitutive cytochrome P-450 enzymes are altered in the diabetic state and that these changes are not permanent since they can be overcome, at least partially, by insulin replacement therapy [68].

These studies have been carried out by Golay et al in 1988 in rabbits with alloxan-induced diabetes in order to see if insulin deficiency affects low density lipoprotein (LDL) catabolism. The results showed that plasma LDL-cholesterol was lower in diabetic rabbits, associated with a fall in the cholesterol to protein ratio of LDL particles. In addition, 125I-LDL disappeared more slowly from plasma of diabetic rabbits, leading to a significant reduction in

fractional catabolic rate and a decrease in residence time of 125I-LDL. These data demonstrated that LDL composition and catabolism are greatly altered as a consequence of insulin deficiency [69].

Monospecific polyclonal antibodies to five constitutive hepatic microsomal cytochromes P-450 were prepared by Favreau and Schenkman in 1988. These antibodies were used to monitor alterations in the content of the enzymes in livers of diabetic male rats. Within 3 wk of onset of streptozocin induced diabetes, immunodetectable levels of RLM3 and RLM5 were decreased by 85 and greater than 95%, respectively. Insulin treatment for 1 wk reversed the decline in these isozymes and restored RLM3 to 60% and RLM5 to 53% of levels found in the untreated rat. After a 2nd wk of therapy, these levels were returned to 86 and 92%, respectively. In contrast, the levels of RLM5b and RLM6 were elevated in diabetes 1.7- and 8-fold, respectively. Insulin treatment for 1 wk only slightly decreased the levels of RLM5b but completely reduced RLM6 levels to those seen in age-matched untreated rats. After the 2nd wk of insulin treatment, the level of RLM5b was almost completely restored to normal, with no additional change in the RLM6 level. The level of a fifth enzyme, RLM5a, was not markedly altered by diabetes or by insulin treatment. The results suggest there are at least three types of responses by constituents of the cytochrome P-450 population to diabetes: no change in the microsomal content, a rapid increase when insulin level declines and restoration when insulin is supplied and a rapid decline when insulin level declines and a restoration by insulin treatment [70].

In the present investigation by Godin et al in 1988, it is shown that rats made diabetic with alloxan, an agent differing from streptozotocin both chemically and its mechanism of diabetogenesis, show virtually identical tissue antioxidant enzyme changes, which as is the case with streptozotocin, are preventable by insulin treatment. The finding that the patterns of antioxidant enzyme alterations in chemically induced diabetes are independent of the diabetogenic agent used and the presence of similar abnormalities in tissues of spontaneously diabetic (BB), Wistar rats (particularly when diabetic control is less than optimal) suggest that the changes observed are a characteristic feature of the uncontrolled diabetic state and that these may be responsible for (or predispose to) the development of secondary complications in clinical diabetes. The study also showed increased in glutathione reductase activity, decreased susceptibility to oxidative glutathione depletion and an increased production of malondialdehyde (an indirect index of lipid peroxidation) in diabetes. The extent of this increase in susceptibility of red cell lipids to oxidation paralleled the severity of diabetic complications. Authors view is

that increased oxidative activity may play an important role in the pathogenesis of complications associated with the chronic diabetic state [71].

Thomas et al in 1989 determined the activities of peroxisomal β-oxidation, cytosolic and microsomal epoxide hydrolase as well as soluble glutathione-s-transferases in the livers of alloxan and streptozotocin-diabetic mice. After initiation of diabetes serum glucose levels were elevated more than the increase in the activities of peroxisomal β-oxidation and cytosolic epoxide hydrolase. The activities of microsomal epoxide hydrolase and glutathione-s-transferase were reduced to about 71% and 80% of controls. Application of depot insulin twice a day for 10 days restored the initial glucose levels and enzyme activities except for peroxisomal β-oxidation. Starvation similarly resulted in increase in peroxisomal β-oxidation and cytosolic epoxide hydrolase activity. Microsomal epoxide hydrolase was significantly decreased whereas glutathione-s-transferase was only marginally reduced. Except for glutathione-s-transferases initial enzyme activities were restored upon refeeding within 10days. With this the authors predicted that this may indicate that high levels of free fatty acids or their metabolites which are known to accumulate in liver in both metabolic states may act as endogenous peroxisome proliferators [72].

Bollen et al in 1990 showed that isolated hepatocytes from streptozotocin-diabetic rats failed to respond to a glucose load with an activation of glycogen synthase. This lesion was associated with severely decreased activities of glycogen-synthase phosphatase and of glucokinase. All these defects were abolished after consumption for 13-18 days of drinking water containing Na_3VO_4 (0.7mgm/ml), and they were partially restored after 3.5 days, when the blood glucose concentration was already normalized. In all conditions the maximal extent of activation of glycogen synthase in cells closely parallelled the activity of glycogen-synthase phosphatase [73].

The effect of insulin-dependent diabetes on the hepatic microsomal activity of cytochrome P450 III and P 450 IV family proteins was investigated in rats pretreated with streptozotocin by Barnet et al in 1990. It was concluded that insulin-dependent diabetes induces proteins of the P 450 III and P 450 IV families and that the hyperketonaemia that accompanies diabetes is largely responsible for the changes in the latter family [74].

The effect of oral administration of sodium ortho vanadate for 5 weeks on hepatic glycogen metabolism was studied in control and streptozotocin induced diabetic rats by Pugazhenthi and Khandelwal in 1990. Diabetes caused hyperglycaemia (5-fold increase), hypo insulinemia (85% decrease), and hyperglucagonemia (4 fold increase). There were also marked decrease in liver

glycogen and activities of glycogen-metabolizing enzymes in liver. Although vanadate administration in control animals showed no significant effect on the various parameters measured except for a 70% decrease in plasma insulin, this treatment in diabetic rats restored these parameters to near control values. In conclusion the authors depicted an insulin like in vivo action of vanadate on various parameters related to hepatic glycogen metabolism [75].

Donahue and Morgan in 1989 showed the ability of sodium metavanadate to reverse the effects of streptozotocin induced diabetes on hepatic cytochrome P-450 isozymes in male rats. Streptozotocin caused P-450 h levels to fall 95%, and P-450 j and P-450 b levels to rise 8 and 40-fold, respectively, after 1 week. Furthermore, P-450 h m RNA levels correlated well with levels of P-450 h apoprotein for all treatment groups, indicating that P-450 h suppression in diabetic rats is under pretranslational control and is independent of the increased expressions of P-450 j and P-450 b, and of the hyperlipidemia and ketosis that occurs in diabetes. Vanadate is capable of separating the effects of diabetes on expression of individual P-450 isozymes [76].

Dumingo et al in 1990 in a letter to the editor pointed out that increased levels of vanadium in several tissues after orthovanadate (50 ppm v) or sodium metavanadate (50 ppm $NaVO_3$) administration. Signs of renal toxicity were also observed at 50 ppm $NaVO_3$. Moreover, ammonium metavanadate decreased erythropoiesis and maturation of erythrocytes when given to rats in drinking water for a period of 2, 4 or 8 weeks [77].

This study was performed by Jain et al in 1990 to determine whether or not hyperglycaemia in diabetes results in elevated levels of lipid peroxidation products in red blood cells (RBC). Diabetes was induced in rats by treatment with streptozotocin. The level of lipid peroxidation products was examined in fresh RBC by measuring their thiobarbituric acid (TBA) reactivity after 2 and 4 months of induction of diabetes. Hyperglycaemia was assessed by measuring the level of glycosylated hemoglobin and blood glucose. Results show that lipid peroxidation levels were significantly higher (50% to 84%) in RBC of diabetic rats than in controls. The increase in the level of lipid peroxidation was blocked in diabetic rats in which hyperglycaemia was controlled by insulin treatment [78].

A week after application of alloxan (200 mgm/kg. s.c.) Lackovic and Salkovic in 1990 found the concentration of serotonin, dopamine and norepinephrine to be increased in the brain of a diabetic rat. Accumulation of these monoamines, produced by inhibition of monoamine oxydase with pargyline (100 mgm/kg i.p.) suggesting a decrease in deamination rate. Surprisingly, however, after an intracerebro-ventricular administration of non-diabetogenic doses

of streptozotocin (5-20 mgm/kg) or alloxan (20 mgm/kg), changes in brain monoamines were similar to those observed in diabetic animals. This observation apparently suggests that the CNS effect of streptozotocin or alloxan is not necessarily related to a diabetogenic, beta-cytotoxic action of these substances [79].

Dash et al in 1991 found hyperglycemia due to experimental diabetes induced in rats, causes a decrease in the activity of Acetylcholinesterase in brain regions and heart; changes in the heart being more significant than the brain. Insulin administration reversed this effect in both the heart and the brain. Significant increase in the levels of catecholamines were also found in the brain regions in diabetes, which was reversed by insulin. The decreased activity of acetylcholinesterase observed in diabetes may be due to an early impaired glucose oxidation and glucose transport as a result of lack of insulin, which causes specific alterations in neurotransmitter levels, thereby effecting blood brain barrier transport, thus causing brain dysfunction [80].

This is a report by Kondoh et al in 1992 investigating the methylglyoxal (MG) bypass in animals, by which D-lactate is produced from triosephosphate via MG. Rats were made diabetic using streptozotocin or starved for 72 h. D-Lactate and various metabolites related to it, such as L-lactate, pyruvate, methylglyoxal, glucose and inorganic phosphate, were measured in the blood plasma, liver and skeletal muscle of the rats. Diabetic and starved rats had significantly higher levels of D-lactate in plasma, liver and skeletal muscle compared with the control group. In contrast, pyruvate levels in plasma, liver and skeletal muscle was markedly lower than normal in diabetic and starved rats. L-Lactate level lowered markedly in plasma, liver, and skeletal muscle of starved rats and elevated in liver of diabetic rats. Differences between plasma L-lactate level for diabetes and control were not significant. MG level was significantly elevated in plasma and depressed in livers and muscles of starved rats as well as livers of diabetic rats. Hepatic glycerol content was markedly increased in those states. Enzyme activities related to D- and L-lactate, such as pyruvate kinase, phosphofructokinase, aldolase and glyoxalase I, were measured in the livers of these rats. Pyruvate kinase activity decreased in these states, but other enzyme activities showed no significant changes. D-Lactate was much more excreted than L-lactate in the urine of diabetic and fasted rats compared with normal rats [81].

Wahba et al in 1992 estimated the activities of choline acetyltransferase and acetyl-cholinesterase in the seminal vesicle and in urinary bladder in streptozotocin induced diabetic male Sprague-Dawley rats. Diabetic rats exhibited significant increase in both the enzymes

compared to control animals in the detrusor muscles of urinary bladder. Significant increase in Choline acetyl transferase activity was observed only in the seminal vesicles [82].

Shibib et al in 1993 showed that Coccinia indica and Momordica charantia extracts lowered blood glucose by depressing its synthesis, on the one hand through depression of the key gluconeogenic enzymes glucose-6-phosphatase and fructose 1, 6, bisphosphatase and on the other by enhancing glucose oxidation by the shunt pathway through activation of its principal enzyme glucose-6-phosphate dehydrogenase in streptozotocin induced hyperglycaemic rats [83].

Levels of lipid peroxidation in liver, kidney, brain and blood, liver glutathione (GSH) and several enzymes in liver tissue associated with antioxidant defence mechanism, namely Catalase (EC: 1.11.1.6), GSH reductase (EC:1.6.4.2) and GSH-S-transferase (EC: 2.5.1.18), were investigated in streptozotocin-induced diabetic rats by Mukherjee et al in 1994. The single intraperitoneal injection of streptozotocin (65 mgm/kg) caused a four-, eight- and seven-fold increase in lipid peroxidation in brain, liver and kidney, respectively. A decline in GSH levels both in blood (two-fold) and liver (16%) compared with normal counterparts was also observed. A marginal increase in catalase activity, a 20% decrease in GSH reductase and an increase of GSH-S-transferase activity was also found in this experimental diabetic condition. These results suggest experimental diabetes, induced by streptozotocin, can produce biochemical changes not only in pancreas but also in liver, kidney and brain tissue [84].

Plasma levels of fibrinogen, alpha 1-acid glycoprotein (AG) and albumin, pancreatic insulitis quantitative scores, and erythrocyte velocity in the mesoappendix microvessels were measured by Guillot et al in 1994 in BB diabetic (BBD) and streptozotocin-diabetic rats (WSTZ) in order to answer the following questions : (a) Does hyperfibrinogenemia or increase in AG plasma level occur in BBD and WSTZ rats, and if so, are these alterations secondary to the hyperglycemia or to an inflammatory process such as insulitis? (b) Is there a decrease in microcirculatory flow in the BBD and WSTZ rats, and if so, is it secondary to the hyperfibrinogenemia and/or the hyperglycemia? Insulitis was present in the BBD rats after 5 weeks of disease (with a score of 2.9 +/- 0.1 vs. 1.4 +/- 0.6 in the normoglycemic controls), but absent in WSTZ rats after 5 months of disease (1.2 +/- 0.06 vs. 1.1 +/- 0.06). Increase in fibrinogen and AG plasma levels was observed in the BBD rats only and appears linked to the insulitis. The major acute phase protein AG level is increased in BBD rats already on the first day of appearance of glycosuria. In the WSTZ rats, without insulitis, chronic hyperglycemia alone did not induce an increase in fibrinogen and AG plasma levels. A decreased microcirculatory erythrocyte velocity has been found in both BBD and WSTZ rats. Thus an increase in fibrinogen

or AG plasma levels is not necessary for inducing a decrease in erythrocyte velocity. Hyperglycemia is probably the main factor responsible for the decrease in microcirculatory flow in the BBD and WSTZ rats [85].

This study by Rasschaert et al in 1994 aimed to compare the metabolic and secretory responses of pancreatic islets from animals with non-insulin dependent diabetic to D-glucose with the effects of the methyl esters of succinic acid and glutamic acid. In contrast unaltered activities of glutamate dehydrogenase and succinate dehydrogenase in the islets of diabetic animals were found [86].

Liu et al studied in 1994 the effect of acute, streptozotocin induced diabetes on hepatic microsomal glucose-6-phosphatase activity and m-RNA expression in young, juvenile and adult ratio. In control rats the m-RNA expression and enzyme activity was similar among the three age groups. The enzyme activity was increased in the streptozotocin induced diabetic rats in all groups. Glucose-6-phosphatase m-RNA expression was increased in the diabetic rats as well. So they concluded that acute streptozotocin- diabetes increase expression of glucose-6-phosphatase m-RNA and thus contributes to the increased glucose-6-phosphatase activity seen with diabetes mellitus [87].

The monomethyl ester of succinic acid (SME) was proposed as a novel tool for stimulation of proinsulin biosynthesis and insulin release in animal models of non-insulin-dependent diabetes mellitus. In this study by Giroix et al in 1994, either saline or SME (14 m mol/day) was infused for 3 days to control rats, animals injected with streptozotocin during the neonatal period and Goto-Kakizaki rats with inherited diabetes. The infusion of SME failed to correct the anomalies found in the islets of diabetic rats. These findings raise the question of whether a more prolonged administration of SME is required to raise the insulin store and improve the secretory potential of the endocrine pancreas in animals with type 2 diabetes [88].

Prickarto et al in 1995 studied the effects of chronic treatment with Acetyl-L-Carnitine on spatial discrimination learning and choline acetyl transferase activity of middle aged streptozotocin treated rats. Chronic treatment with Acetyl-L-Carnitine attenuated both the STREP induced impairment in spatial bias and the decrease in hippocampal choline acetyl transferase activity [89].

Glucose production and utilization and activities of key enzymes involved in liver and muscle glucose metabolism were studied by Burcelin et al 1995 in post absorptive streptozotocin diabetic rats after 12 hour of severe hyperglycaemia and insulinopenia. Basal glucose production

was increased; liver glycogen concentration was decreased and liver glucose-6-phosphatase activities were increased [90].

Kakkar et al in 1995 hypothesized that oxygen free radicals (OFRs) may be involved in pathogenesis of diabetic complications. They therefore investigated the levels of lipid peroxidation by measuring thiobarbituric acid reactive substances (TBARS) and activity of antioxidant enzymes [superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT)] in tissues and blood of streptozotocin(STZ)-induced diabetic rats. The animals were divided into two groups: control and diabetic. After 10 weeks (wks) of diabetes the animals were sacrificed and liver, heart, pancreas, kidney and blood were collected for measurement of various biochemical parameters. Diabetes was associated with a significant increase in TBARS in pancreas, heart and blood. The activity of CAT increased in liver, heart and blood but decreased in kidney. GSH-Px activity increased in pancreas and kidney while SOD activity increased in liver, heart and pancreas. The findings suggest that oxidative stress occurs in diabetic state and that oxidative damage to tissues may be a contributory factor in complications associated with diabetes [91].

Tormo et al in 1995 observed that intestine has a high glycolytic activity but its metabolic role could be altered in diabetes mellitus. They supplied glucose to the intestine only by the vascular route and investigated in vivo the glucose retained and the lactate produced by the intestine of normal and diabetic rats and in vitro the effect of different arterial glucose concentrations on glucose utilization and lactate, alanine and pyruvate production in normal and diabetic rats. The lactate produced was significantly higher in diabetic than in normal rats [92].

Reddy et al studied in 1995 the status of glycogen and pyruvate and the activity of glucose-6-phosphatase and alanine amino transferase in liver under the influence of sepia shell extract in both normal and streptozotocin induced diabetic mice. The glycogen concentration was elevated steeply in both and the pyruvate concentration increased substantially in diabetic mice, while the activity of glucose-6-phosphatase and alanine amino transferase was inhibited in normal and diabetic mice [93].

Bitter melon (momordica charantia) commonly known as karela, has been reported to have hypoglycaemic, antiviral, antidiabetic and anti tumour activities. Raza et al in 1996 investigated the effects of oral feeding of karela fruit juice on the hepatic cytochrome P 450 and glutathione-s-transferase drug metabolising enzymes in the streptozotocin induced diabetic rat. The cytosolic glutathione concentration was decreased in diabetic rats, and karela juice

feeding normalised the effect. However, an increase of 20-30% in the glutathione-s-transferase activity was observed in both diabetic and karela juice fed rats. In addition they also observed that karela does not always reverse the effects on drug-metabolising enzymes in STZ-induced diabetes [94].

Yao et al in 1997 showed that controversial reports on the efficacy and possible toxicity of vanadium obtained from various studies may be attributed to differences in the method of diabetes induction and (or) to differences in animal strains. The objective of this study was to evaluate the contribution of these two factors to the effects of vanadium in the treatment of experimental diabetes. Two methods of streptozotocin induction of diabetes in rats have been used for studying the antidiabetic effects of vanadium. One involves a single intravenous injection of 60 mg/kg streptozotocin, and the other uses two subcutaneous injections of 40 mg/kg streptozotocin, to either Wistar or Sprague-Dawley rats. In a 7-week chronic study, Sprague-Dawley rats appeared to develop a more severe diabetes (indicated by higher plasma cholesterol and higher fasting plasma glucose levels) following the single intravenous injection of streptozotocin than rats made diabetic by two subcutaneous injections of streptozotocin. Irrespective of the method of diabetes induction, the responses of all the diabetic animals to chronic vanadyl sulphate treatment were similar. In an acute study, Wistar diabetic rats were more responsive than Sprague-Dawley diabetic rats to vanadyl sulphate and to lower doses (0.6 and 0.8 mmol/kg) of a new organic vanadium compound, bis(maltolato)oxovanadium (i.v.) [95].

Tatsuki et al in 1997 studied the relationship between changes in lipid peroxides and those in catalase activity in pancreases, livers and hearts of streptozotocin-induced diabetic rats. Animals were killed 2 or 7 weeks after saline or streptozotocin (32 mgm/kg, i.v.) injection. The levels of blood glucose and plasma insulin in the 2-week streptozotocin-treated rats were 176.8+/-20.5 mg/dl and 29.9+/-3.2 microU/ml, respectively. In the pancreas, the lipid peroxide level significantly decreased and the catalase activity significantly increased 2 weeks after streptozotocin injection. These changes recovered after 7 weeks. In the heart, the lipid peroxide level significantly increased without any change of catalase activity 2 weeks after the initiation of diabetes. After 7 weeks, the catalase activity significantly increased and the lipid peroxide level returned to the control level. In the liver, there was no change in the lipid peroxides and catalase in the 2-week streptozotocin-treated rats, whereas the catalase activity significantly increased 7 weeks after the injection. It was suggested that the defense system in the pancreas to oxidative stress may be evoked in an early stage of streptozotocin-induced diabetes [96].

It is known that streptozotocin, penetrating into the organism generates nitrogen monoxide (NO). Therefore, it is justified to presume that in beta-cell destruction thereby induced, peroxinitrite resulting from NO and superoxide (O_2^-) reaction has an important role. Matkovics et al in 1997 tested and compared antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase activities) glutathione reductase activity regenerate reduced glutathione. The oxidized, reduced glutathione values and lipid peroxidation changes were measured. From the studies, it has appeared that streptozotocin treatment generally induces an oxidative predominance in tissues [97].

Oxygen free radicals have been suggested to be a contributory factor in complications of diabetes mellitus. There are many reports indicating the changes in parameters of oxidative stress in diabetes mellitus. In this study Kakkar et al in 1998 aimed to identify whether oxidative stress occurs in the liver and pancreas in the initial stages of development of diabetes. They therefore investigated the lipid peroxide level (thiobarbituric acid-reactive substances, TBARS) and activities of antioxidant enzymes [superoxide dismutase (SOD), catalase and glutathione peroxidase] in liver and pancreas of control and streptozotocin-induced diabetic rats at various stages of development of diabetes. Male Sprague-Dawley rats were divided into two groups: group I, control (n = 42) and group II, diabetic (n = 42). Each group was further subdivided into seven groups consisting of six rats each. Rats in these subgroups were studied at weekly intervals (0 to 6 weeks). Plasma glucose levels, TBARS levels and activities of antioxidant enzymes were measured in liver and pancreas at various time intervals. There was a significant ($P < 0.05$) and progressive increase in TBARS levels of liver and pancreas in the diabetic group. Total SOD and Cu-Zn-SOD activity increased ($P < 0.05$) with progression of diabetes while Mn-SOD activity showed no significant change in either tissue. Catalase and glutathione peroxidase activities increased significantly ($P < 0.05$) in liver and pancreas. Immunohistochemical study of pancreatic islet revealed a decrease in the expression of insulin with progression of diabetes. However, glucagon and somatostatin showed an increase in immunoreactivity and a difference in their distribution pattern. The findings of the present study suggest that oxidative stress starts at early onset of diabetes mellitus and increases progressively. In conclusion, the structural damage to these tissues or complications of diabetes mellitus may be due to oxidative stress [98].

The glycogen concentration in liver is altered in various pathophysiologic states. In fasted rats, it is higher in diabetic, and lower in adrenalectomized rats compared to control animals. In fed rats, it is lower in diabetic, and little changed in adrenalectomized animals compared to

controls. Gannon et al in 1998 determined whether the activity of glycogenin, a self-glycosylating protein that initiates the synthesis of new glycogen molecules, could explain these differences in liver glycogen concentration. Glycogenin activity was measured by the incorporation of 14C-glucose from UDP-U-14C-glucose into an acid-precipitable product before and after amylase treatment of liver extracts. The glycogenin activity was similar in normal, diabetic and adrenalectomized fasted animals, regardless of the hepatic glycogen concentration. In fasted rats, glycogenin was present predominantly as the free-form of the enzyme, i.e., not attached to an amylase-digestible glycan, presumably glycogen. In contrast, in fed rats, the majority, if not all of the glycogenin was incorporated into a glycogen-like (proteoglycan) molecule. Proteoglycan synthase activity, previously identified in normal fed rats, also was present in diabetic and adrenalectomized fed rats, and the activity was similar. Thus, the altered ability to store hepatic glycogen in diabetic fed and fasted and adrenalectomized fasted rats cannot be explained by decreases in glycogenin or proteoglycan synthase activities, at least as measured using the present assays [99].

Poucheret et al demonstrated in 1985 that vanadium administered in the drinking water to streptozotocin (STZ) diabetic rats restored elevated blood glucose to normal. Subsequent studies have shown that vanadyl sulfate can lower elevated blood glucose, cholesterol and triglycerides in a variety of diabetic models including the STZ diabetic rat, the Zucker fatty rat and the Zucker diabetic fatty rat. Long-term studies of up to one year did not show toxicity in control or STZ rats administered vanadyl sulfate in doses that lowered elevated blood glucose. In the BB diabetic rat, a model of insulin-dependent diabetes, vanadyl sulfate lowered the insulin requirement by up to 75%. Vanadyl sulfate is effective orally when administered by either single dose or chronic doses. It is also effective by the intraperitoneal route. Authors have also been able to demonstrate marked long-term effects of vanadyl sulfate in diabetic animals following treatment and withdrawal of vanadyl sulfate. Because vanadyl sulfate is not well absorbed they have synthesized and tested a number of organic vanadium compounds. One of these, bismaltolato-oxovanadium IV (BMOV), has shown promise as a therapeutic agent. BMOV is 2-3x more potent than vanadyl sulfate and has shown less toxicity. Recent studies from their laboratory have shown that the effects of vanadium are not due to a decrease in food intake and that while vanadium is deposited in bone it does not appear to affect bone strength or architecture. The mechanism of action of vanadium is currently under investigation. Several studies indicate that vanadium is a phosphatase inhibitor and that vanadium can activate serine/threonine kinases distal to the insulin receptor presumably by preventing dephosphorylation

due to inhibition of phosphatases Short-term clinical trials using inorganic vanadium compounds in diabetic patients have been promising [100].

To better define the modifications of liver gluconeogenesis and citric acid cycle, or Krebs' cycle, activity induced by insulin deficiency and the effects of metformin on these abnormalities, Large and Beylot in 1999 infused livers isolated from postabsorptive or starved normal and streptozotocin-induced diabetic rats with pyruvate and lactate (labeled with [3-13C]lactate) with or without the simultaneous infusion of metformin. Lactate and pyruvate uptake and glucose production were calculated. The 13C-labeling pattern of liver glutamate was used to calculate, according to Magnusson's model, the relative fluxes through Krebs' cycle and gluconeogenesis. These relative fluxes were converted into absolute values using substrate balances. In normal rats, starvation increased gluconeogenesis, the flux through pyruvate carboxylase-phosphoenolpyruvate carboxykinase (PC-PEPCK), and the ratio of PC to pyruvate dehydrogenase (PDH) flux ($P < 0.05$); metformin induced only a moderate decrease in the PC:PDH ratio. Livers from postabsorptive diabetic rats had increased lactate and pyruvate uptakes ($P < 0.05$); their metabolic fluxes resembled those of starved control livers, with increased gluconeogenesis and flux through PC-PEPCK. Starvation induced no further modifications in the diabetic group. Metformin decreased glucose output from the liver of starved diabetic rats ($P < 0.05$). The flux through PC-PEPCK and also pyruvate kinase were decreased ($P < 0.05$) by metformin in both groups of diabetic rats. In conclusion, insulin deficiency increased in this model of diabetes gluconeogenesis through enhanced uptake of substrate and increased flux through PC-PEPCK; metformin decreased glucose production by reducing the flux through PC-PEPCK [101].

Antioxidant enzymes in liver and small intestine were investigated by Giron et al in 1999 using control and streptozotocin diabetic rats fed diets with 5% olive, sunflower or fish oil for five weeks. In liver, glutathione peroxidase and superoxide dismutase decreased and in intestine glutathione-s-transferase (GST) increased by diabetes. In isolated jejunum and ileum, this increase in GST activity was due to an increase in GST-alpha and mu isoenzymes in jejunum and GST-alpha, mu and pi in ileum. Since GST plays an important role in protecting tissues from oxidative damage, the results highlight the role of the intestine against free radicals in physiological or pathological situations [102].

The aim of this study by Kinalski et al in 2000 was to evaluate lipid peroxidation and scavenging enzyme activity in streptozotocin-induced diabetes, and then to establish whether moderate doses of nonenzymatic antioxidant vitamin E play a role in the antioxidant defence

system in diabetic pregnant rats and their offspring. The study group consisted of 30 normal female Wistar rats, which were given a single dose of streptozotocin (40 mgm/kg) and were mated 7 days later. Subsequently, the diabetic animals were divided into two matched groups: the first supplemented with vitamin E (30 mgm/100 g chow), and the other fed with a standard diet lacking vitamin E. Controls consisted of 15 pregnant rats. On the first day after delivery, the rats were decapitated and homogenates of maternal liver and uterus as well as neonatal lungs and liver were prepared. Then the following parameters were measured: malondialdehyde (MDA) concentrations in the homogenates and blood serum, glutathione (GSH) levels, the activity of CuZn-superoxide dismutase (SOD) and glutathione peroxidase (GPx), and glycaemia. The neonates of diabetic rats were smaller than the healthy ones and serum glucose concentration was markedly higher in the diabetic animals. MDA levels were significantly increased, whereas GSH, SOD and GPx were markedly diminished in the diabetic adult rats and their offspring in comparison to the control group. In the animals supplemented with alpha-tocopherol, MDA concentrations were significantly lower, GSH content and SOD activities were markedly elevated most tissues studied, whereas GPx remained unchanged. By monitoring the activity of selected scavenging enzymes, information on ongoing biological oxidative stress and thereby on the fetus/neonate status may be obtained. The results suggest that diabetic pregnant rats and their neonates are exposed to an increased oxidative stress and that vitamin E supplementation may reduce its detrimental effects [103].

Compounds of the trace element vanadium have been shown to mimic insulin in in vitro and in vivo systems. These compounds have been found to exert anti-diabetic effects in rodent models of type 1 and type 2 diabetes mellitus as well as in a limited number of studies in human diabetic subjects. Thus, vanadium compounds have emerged as agents for potential use in diabetes therapy. However, treatment of diabetic animals with inorganic vanadium salts has also been associated with some toxic side-effects such as gastrointestinal discomfort and decreased body weight gain. In addition, vanadium salts have been reported to exert toxic effects on the liver and kidney. More recently, it was shown that organic vanadium compounds were much safer than inorganic vanadium salts and did not cause any gastrointestinal discomfort, hepatic or renal toxicity. This review by Srivastava in 2000 briefly summarizes the anti-diabetic and toxic effects of vanadium compounds [104].

Clore et al in 2000 determined rates of endogenous glucose production and the activities of glucose-6-phosphatase and glucokinase in obese patients scheduled for gastric by pass surgery. Hepatic glucose-6-phosphatase activity determined from freshly isolated microsomes

was significantly increased in the type 2 diabetic patients compared with the obese control subjects [105].

In streptozotocin (STZ)-induced diabetic rats, the authors previously showed an increased heparin-releasable (luminal) lipoprotein lipase (LPL) activity from perfused hearts. To study the effect of this enlarged LPL pool on triglyceride (TG)-rich lipoproteins, Sambandam et al in 2000 examined the metabolism of very-low-density lipoprotein (VLDL) perfused through control and diabetic hearts. Diabetic rats had elevated TG levels compared with control. However, fasting for 16 h abolished this difference. When the plasma lipoprotein fraction of density <1.006 g/ml from fasted control and diabetic rats was incubated in vitro with purified bovine or rat LPL, VLDL from diabetic animals was hydrolyzed as proficiently as VLDL from control animals. Post-heparin plasma lipolytic activity was comparable in control and diabetic animals. However, perfusion of control and diabetic rats with heparinase indicated that diabetic hearts had larger amounts of LPL bound to heparan sulfate proteoglycan-binding sites. [(3)H]VLDL obtained from control rats, when recirculated through the isolated heart, disappeared at a significantly faster rate from diabetic than from control rat hearts. This increased VLDL-TG hydrolysis was essentially abolished by prior perfusion of the diabetic heart with heparin, implicating LPL in this process. These findings suggest that the enlarged LPL pool in the diabetic heart is present at a functionally relevant location (at the capillary lumen) and is capable of hydrolyzing VLDL. This could increase the delivery of free fatty acid to the heart, and the resultant metabolic changes could induce the subsequent cardiomyopathy that is observed in the chronic diabetic rat [106].

It has recently been shown that food intake is not essential for the resynthesis of the stores of muscle glycogen in fasted animals recovering from high-intensity exercise. Because the effect of diabetes on this process has never been examined before, Ferreira et al in 2001 undertook to explore this issue. To this end, groups of rats were treated with streptozotocin (60 mgm/kg body mass ip) to induce mild diabetes. After 11 days, each animal was fasted for 24 h before swimming with a lead weight equivalent to 9% body mass attached to the tail. After exercise, the rate and the extent of glycogen repletion in muscles were not affected by diabetes, irrespective of muscle fiber composition. Consistent with these findings, the effect of exercise on the phosphorylation state of glycogen synthase in muscles was only minimally affected by diabetes. In contrast to its effects on nondiabetic animals, exercise in fasted diabetic rats was accompanied by a marked fall in hepatic glycogen levels, which, surprisingly, increased to preexercise levels during recovery despite the absence of food intake [107].

Streptozotocin (STZ), an analogue of GlcNAc, inhibits purified rat spleen O-GlcNAc-selective N-acetyl-beta-D-glucosaminidase (O-GlcNAcase), the enzyme that removes O-GlcNAc from protein. STZ increases pancreatic islet O-linked protein glycosylation. In light of these data, Konrad et al in 2001 investigated the possibility further that STZ causes beta-cell death by inhibiting O-GlcNAcase. In isolated islets, the time course and dose curve of STZ-induced O-glycosylation correlated with beta-cell toxicity. STZ inhibition of rat islet O-GlcNAcase activity also paralleled that of its beta-cell toxicity, with significant inhibition occurring at a concentration of 1 mM. In contrast, STZ inhibition of rat brain O-GlcNAcase and beta-TC3 insulinoma cell O-GlcNAcase was significantly right-shifted compared with islets, with STZ only significantly inhibiting activity at a concentration of 5 mM, the same concentration required for beta-TC3 cell toxicity. In comparison, N-methyl-N-nitrosourea, the nitric oxide-donating portion of STZ, did not cause increased islet O-glycosylation, beta-cell toxicity or inhibition of beta-cell O-GlcNAcase. Enhanced STZ sensitivity of islet O-GlcNAcase compared with O-GlcNAcase from other tissues or an insulinoma cell line suggests why actual islet beta-cells are particularly sensitive to STZ. Confirming this idea, STZ-induced islet beta-cell toxicity was completely blocked by GlcNAc, which also prevented STZ-induced O-GlcNAcase inhibition, but was not even partially blocked by glucose, glucosamine or GalNAc. Together, these data demonstrate that STZ's inhibition of beta-cell O-GlcNAcase is the mechanism that accounts for its diabetogenic toxicity [108].

2.2 Role of Selenium on Diabetes :

Accepted methods of treatment of Diabetes Mellitus in patients vary in their effects in producing normoglycaemia. Normoglycaemia or near normal blood sugar level is difficult to attain with any of the antidiabetic regimes and is even more difficult to maintain over a period of time. Moreover, antidiabetic regimes are associated with considerable possible side effects and they require strict clinical monitoring, imparting a burden of considerable cost, restriction in life style and training on patient's part. Maintenance of normoglycaemia over a period of time is, however, nodal in management of diabetes and prevention of diabetic complication. It is mandatory to maintain normoglycaemia in certain conditions like pregnant diabetic and diabetes with renal transplantation. Regimes to maintain normoglycaemic level strictly over a period of time are costly, cumbrous and restrictive.

It is clear from above discussion that search of a relatively cheap, non-toxic, less restrictive and inexpensive management regime of diabetes other than the accepted one, is a matter of great practical interest. Study of selenium intake in experimentally produced diabetic mice as a model, gives us important insight in problems of diabetic management and metabolism. A gamut of previous work discussed below entails the effect of selenium in diabetic mice.

Slonim et al in 1983 studied the effect of antioxidant vitamin E, administered prior to administration of diabetogenic agents, in mice. This was done in addition to study of diabetic susceptibility in mice, fed on vit. E and selenium deficient diet. It was observed that the mice, fed on vit E, Selenium deficient diet, showed increased susceptibility to diabetes development by ordinarily non-diabetogenic doses of streptozotocin. It pointed out that selenium in diet might have a role in prevention of diabetes in animal model. In our study we got results corroborated with the said hypothesis more so because our study examined about reversal of diabetic state in diabetic mice by selenium in diet [109].

Dohi et al in 1988 studied the activity of tissue glutathione peroxidase, a selenium dependent enzyme, in rats 4 & 8 months after injection of streptozotocin. Some other enzymes like catalase and superoxide dismutase were also studied. Selenium dependent glutathione peroxidase activity was increased in kidney, unaltered in lung & liver and significantly decreased in aorta. Catalase activity was uninfluenced by diabetic state in rat aorta. Superoxide dismutase activity was less than detectable. Catalase and superoxide dismutase activity was unaltered in kidney in diabetic state. The observed anomalies in this study are corrected by reversal of diabetic state by insulin treatment in animal model. It is depicted from the study that repletion of selenium dependent

glutathione peroxidase in rat aorta is dependent on reversal of diabetic state and hence are interrelated. In part of the study they studied the effect of selenium feeding in diabetic mice on liver catalase activity and found no significant alteration [110].

Flechner et al in 1990 studied the effect of radical scavengers in the prevention of β cell destruction by streptozotocin in animal model of BB rat. Eight compounds were tested either in isolation or in combination. No effect was seen on diabetic state for 3 aminobenzamide, N acetyl DL homocysteine thiolactone, ebselen and butylated hydroxyanisole whereas partial suppression of hyperglycaemia was seen with cysteamine. In BB rats diabetes development was delayed and hyperglycaemia partially suppressed by administration of ebselen and vit E. and Max EPA (fish lipid concentrate). Authors conclude that diabetic state was not readily modifiable by exogenous radical scavengers. In the study, however, intervention with oral selenium interfered significantly with development of diabetic state in animal model [111].

Mc Neill et al in 1991 found that the treatment of streptozocin (STZ)-induced diabetic rats with sodium selenate (10-15 mumol.kg-1.day-1) for 7 wk resulted in a decrease in plasma glucose, food intake, and water intake to control or near control levels. Plasma insulin was reduced in control rats given sodium selenate to the level found in the diabetic and treated diabetic group. Treatment did not affect control rats with regard to the other measurements cited. Sodium selenate enhanced weight gain in responding diabetic rats to that seen in controls; sodium selenate's actions thus resembled those of insulin. Thus selenate, like vanadium, appears to have insulinlike effects when administered in vivo [112].

A study in 1992 showed more valuable insight in interaction between selenium & development of diabetic state in animal model (Mukherjee et al 1992). It showed considerable beneficial effect in reduction of hyperglycaemia in streptozotocin induced diabetic mice. Blood glucose level and uptake of selenium in pancreas were monitored and matched with normal controls. Selenium uptake in pancreas was increased in diabetic mice in relation to control as well as hyperglycaemia was suppressed [113].

In an impressive work Iizuka et al in 1992, studied the effect of selenium on blood sugar and insulin levels in different varieties of diabetic rats, one induced by streptozotocin and the other by pancreatectomy. Direct action of selenium on islet tissues of diabetic rat was also studied. Drastic and fast reduction of very high blood sugar level in acute diabetic state was shown to occur by single parenteral injection of selenium as well as increase in serum insulin. However, rats with chronic diabetic state showed a normoglycaemic effect on parenteral injection

of selenium for 4 days without any significant increase in insulin levels. Pancreatectomised diabetic rats show a tendency of reduction of blood sugar level without any increase in insulin level. Selenium was shown to increase insulin release from islets in a dose dependent manner. This study showed important potential of selenium as a blood glucose lowering agent in animal model in different diabetic states as well as throws light on the mechanism of such action through pancreas by release of insulin or on effect independent of release of insulin [114].

Ghosh et al in 1994 studied the effect of oral selenium on setreptozotocin induced diabetic mice. Hyperglycaemia, decrease in hepatic glycogen, increase in glucose 6 phosphatase activity and significant decrease in plasma insulin levels and protein kinase activity are studied as established parameters in diabetic mice. Reversal to near normal values is observed in diabetic mice on oral selenium but no effect on control sample. It shows the potential of selenium as an intervention agent in diabetic with little chance to overcorrect the parameters and development of side effects like hypoglycaemia [115].

Insulin is known to be capable of regulating cellular and metabolic processes through action on gene expression and subsequent effect on enzyme activity. This knowledge gives rise to use insulin mimetic agents in experimental model in studying the effect of activity on different enzymes, to gain insight into mechanism of action of insulin on metabolism as a whole as well as on the specific enzyme activity. Berg et al made an important work in 1995 in which they used Vanadate and Selenate, known to control blood sugar level in experimental diabetic model in vivo (an insulin mimetic action). Though Vanadate is known to influence expression of several enzymes in vitro and in vivo, studies about Selenate have not been reported. The authors showed that administration of Vanadate or Selenate in streptozotocin induced diabetic rats restore activity of glucose-6-phosphate dehydrogenase and fatty acid synthetase in tissues in addition to normalisation of blood sugar levels. A normal control and a streptozotocin induced diabetic rat, treated with insulin, are also studied. Comparison of activity of G6PD and FAS activity are markedly reduced in streptozotocin induced diabetic rats than in normal control with restoration of activity by insulin or Vanadate/Selenate administration upto 80-90% of normal level with no significant difference in result from insulin and Vanadate/Selenate group in between increase in G6PD or FAS are due to increase in cellular mRNA level. Increase in cellular mRNA, responsible for increased synthesis of G6PD & FAS, suggests the effect of insulin or mimetics (Vanadate/Selenate in this case) occurs pretranslationally through gene regulation [116].

Saito et al in 1995 found that tissue selenium was decreased in streptozotocin treated old rats and this decrement was probably related to the peroxidation in tissue damage [117].

Further evidence of insulin mimetic action of selenium independent of insulin was obtained from a breakthrough work of Becker et al in 1996. On feeding with sodium selenate (Na_2SeO_4) for 10 weeks, streptozotocin induced insulin deficient diabetic rats show amelioration of hyperglycaemia (appx. 25 mmol) and glucosuria (appx. 85 mmol/day) by 50% and 80% respectively when compared to a similar untreated diabetic control group. Comparison of oral and intravenous GTT (glucose tolerance test) shows reduction of 40-50% in blood glucose levels after a glucose load when compared to untreated control. These activities were not accompanied by a rise in plasma insulin levels. In fact insulin reserves are reduced more than 90% by streptozotocin in treated and untreated diabetic rats. The hepatic activity of two key glucolytic enzymes, glucose and L pyruvate kinase as well as corresponding mRNA activity were increased approximately two-three fold to reach 40-75% of normal. In diabetic rats activity of these two enzymes were blunted. In addition gluconeogenic enzyme phosphoenolpyruvate carboxykinase, elevated in streptozotocin induced diabetic rats, were reduced by selenate treatment with parallel changes in correspondent mRNA. Selenium, an anorexigenic, induces reduction of body weight up to a moderate extent, a calorie restricted weight matched similar control of diabetic rats were run and show no improvement in glucose homeostasis and enzyme effects compared to selenate group. In addition no obvious untoward effect on kidney or liver was observed for selenium treatment in diabetic rats. The study showed selenium improved glucose homeostasis and diabetic state in animal model through an insulin like action but independent of insulin by correction of pretranslational regulatory mechanism in hepatic metabolism of glucose [118].

Douillet in 1996 studied the effect of selenium and selenium supplemented with Vit. E on kidney in diabetic rats. Some protection against development of pathology in reduction of renal hyperfiltration and dissemination in number and severity of diabetic glomerular lesion is evidenced [119].

A study of selenium supplement (selenomethionine) along with Vit. E on platelet activity in diabetic rats in vitro was shown by Douillet in 1996 to effect a reduction in platelet thrombin, ADP induced aggregation, in adhesiveness to fibronectin and in sorbitol content. Platelet selenium is significantly increased alongwith prevention of diabetic oxidative damage to platelet membrane [120].

Fifty-two healthy Swiss Male Albino rats aged two mo were used in the study by Gumuslu et al in 1997 . They were divided into four groups: control (C), diabetic (D), cadmium (Cd), and diabetic + Cd (D + Cd) groups. Diabetic condition was induced in D and D + Cd groups

by administration of alloxane (5 mg/100 g). After this treatment, Cd and D + Cd groups were injected with CdCl2 i.p. (2 mg/kg/wk). At the end of the 2-mo experimental period, thiobarbituric acid reactive substances (TBARS), plasma and erythrocyte selenium (Se), plasma ceruloplasmin (Cp), and vitamin E (vit E) were determined in four groups of rats. The erythrocyte Se was lower in the experimental groups than in the controls. Plasma Se was significantly decreased in the D and D + Cd groups compared with the control group. Plasma Cp was unaltered. Plasma vit E was significantly decreased in Cd group in comparison with the C, D, and D+Cd groups [121].

Battell in 1998 substantiated again the event of normoglycaemia in streptozotocin induced diabetic rat by selenium independent of insulin release. At 8 weeks of diabetes selenium treated diabetic group are found to have normal heart functions in comparison to untreated diabetic group [122].

Benefical effects of seleniomethionine and Vit. E supplements were impresively demonstrated in tissues like liver, kidney, heart and aorta of diabetic rat after 24 weeks of induction by streptozotocin in a study by Douillet in 1998. Diabetes induced increase in thiobarbituric acid reactive substances, conjugated dienes and decrease in triglyceride and phospholipid levels in liver were beneficially corrected to a large extent by supplement. Decrease in 18 : 2n-6 and increase in 22 : 6n-3 observed in diabetic rat liver as a reflection of altered glycaemic control were beneficially reversed. The increase in cardiac triglyceride induced by diabetes was reversed alongwith increase in 18 : 0 ether linked alcohol, 20 : 4n-6 and 22 : 5n-3 level in cardiac lipids. In aorta 20 : 5n-3 was increased. These polyunsaturated fatty acids are precursors of PGI_2 and PGI_3 seemingly involved in cardiovascular protection. In kidney decreased 20 : 4n-6, the precursor of thromboxane A_2 by selenium supplements confers nephroprotection as thromboxane A_2 is implicated conversely to glomerular injury. Thus selenium or selenium and Vit E play a role in controlling oxidative status and altered lipid metabolism in liver, producing favourable fatty acid distribution in major tissues, affected by diabetic complication [123].

Interrelation of blood glucose, lipid peroxidation, glutathione, glutathione peroxidase and glutathione S transferase activity and blood selenium levels in streptozotocin induced diabetic mice, treated with selenium is studied by Mukherjee et al in 1998 to gain important insight in beneficial activity of selenium and the mechanism of action. Diabetes induced hyperglycaemia (2.8 fold increase), increase in malondialdehyde levels (89% in liver and 83% in blood), marked decrease in glutathione (approximately 73% in blood and 79% in liver), increase in glutathione

S transferase activity (55%) after 5 weeks of streptozotocin treatment, were reverted to normal by sodium selenite supplement. It is suggestive of a major role of selenium in reducing oxidative stress associated with diabetes [124].

Metabolic abnormalities observed in retina and in cerebral cortex were compared in diabetic rats and experimentally galactosemic rats by Kowluru et al in 1999. Diabetes or experimental galactosemia of 2 months duration significantly increased oxidative stress in retina, as shown by elevation of retinal thiobarbituric acid reactive substances (TBARS) and subnormal activities of antioxidant defense enzymes, but had no such effect in the cerebral cortex. Activities of sodium potassium adenosine triphosphatase [(Na, K)-ATPase] and calcium ATPase became subnormal in retina as well as in cerebral cortex. In contrast, protein kinase C (PKC) activity was elevated in retina but not in cerebral cortex in the same hyperglycemic rats. Dietary supplementation with an antioxidant mixture (containing ascorbic acid, Trolox, alpha-tocopherol acetate, N-acetyl cysteine, beta-carotene, and selenium) prevented the diabetes-induced and galactosemia-induced elevation of retinal oxidative stress, the elevation of retinal PKC activity and the decrease of retinal ATPases. In cerebral cortex, administration of the antioxidant diet also prevented the diabetes-induced decreases in (Na,K)-ATPase and calcium ATPases, but had no effect on TBARS and activities of PKC and antioxidant-defense enzymes. The results indicate that retina and cerebral cortex differ distinctly in their response to elevation of tissue hexose, and that cerebral cortex is more resistant than retina to diabetes-induced oxidative stress. The greater resistance to oxidative stress in cerebral cortex, as compared to retina, is consistent with the resistance of cerebral cortex to microvascular disease in diabetes, and with a hypothesis that oxidative stress contributes to microvascular disease in diabetes. Dietary supplementation with these antioxidants offers a means to inhibit multiple hyperglycemia-induced retinal metabolic abnormalities [125].

Naziglu in 1999 showed protective effect of Vit C, Vit E and selenium against oxidative damage to lens. However, the effect of Vit C here far outweighed the effect of other two supplements. In peritoneally administered Vit C, Vit E and selenium were shown to have protective effects by estimation of lipid peroxidation, glutathione peroxidase, reduced glutathione activities in lens of diabetic rats [126].

Effects of hyperglycemia (both diabetes and experimental galactosemia) on cardiac metabolism have been determined by Kowluru et al in 2000. In addition, the effect of supplemental antioxidants on these hyperglycemia-induced abnormalities of cardiac metabolism has been investigated. Diabetes or experimental galactosemia of 2 months duration in rats

significantly increased oxidative stress in myocardium, as demonstrated by elevation of thiobarbituric acid reactive substances (TBARS) and lipid fluorescent products in left ventricle. Activity of protein kinase C (PKC) was elevated in the myocardium, and the activities of (Na,K)-ATPase and calcium ATPases were subnormal. Administration of supplemental antioxidants containing a mixture of ascorbic acid, Trolox; alpha-tocopherol acetate, N-acetyl cysteine, beta-carotene, and selenium prevented both the diabetes-induced and galactosemia-induced elevation of oxidative stress and PKC activity, and inhibited the decreases of myocardial (Na,K)-ATPase and calcium ATPases. The results show that these metabolic abnormalities are not unique to diabetes per se, but are secondary to elevated blood hexose levels, and supplemental antioxidants inhibit these metabolic abnormalities. Our findings suggest that antioxidants inhibit abnormal metabolic processes that may contribute to the development of cardiac disease in diabetes, and offer a potential clinical means to inhibit cardiac abnormalities in diabetes [127].

Goemen et al in 2000 studied the effect of vitamin E and sodium selenate treatment on the neurogenic and endothelium-dependent relaxation of isolated corpus cavernosum obtained from streptozotocin-induced diabetic mice. Relaxant responses of corpus cavernosum precontracted by phenylephrine to electrical field stimulation and to acetylcholine were significantly decreased in diabetic mice. There was no significant difference between diabetic and non-diabetic groups for the relaxant response of corpus cavernosum to sodium nitroprusside and papaverine. Treatment with sodium selenate, but not vitamin E, partially prevented the impairment of the neurogenic relaxation, whereas both had a significant, partial restorative action on endothelial dysfunction in corpus cavernosum obtained from diabetic groups. Neither agent exhibited a significant action on the relaxant responses of corpus cavernosum obtained from non-diabetic mice. A decrease in the sensitivity of the neurogenic impairment to antioxidant action may develop more rapidly than that of endothelial dysfunction in streptozotocin-induced diabetic mice [128].

Severe steroidogenic and spermatogenic alterations are reported by Unlucerci et al in 2000 in association with diabetic manifestations in humans and experimental animals. This study was planned to determine whether oxidative stress is involved in diabetes-induced alterations in the testes. Diabetes was induced in male rats by injection of 50 mg/kg of streptozotocin (STZ). Ten weeks after injection of STZ, levels of selenium and activities of selenium dependent-glutathione peroxidase (GPx) and phospholipid hydroperoxide glutathione peroxidase (PHGPx) were measured in rat testis. Lipid and protein oxidations were evaluated as measurements of testis malondialdehyde (MDA) and protein carbonyl levels, respectively. Testis sulfydryl (SH)

levels were also determined. The control levels of GPx and PHGPx activities were found to be 46.5 +/- 6.2 and 108.8 +/- 19.8 nmol GSH/mgm protein/min, respectively. Diabetes caused an increase in testis GPx (65.0 +/- 21.1) and PHGPx (155.9 +/- 43.1) activities but did not affect the levels of selenium or SH. However, the testis MDA and protein carbonyl levels as markers of lipid and protein oxidation, respectively, did not increase in the diabetic group. Aminoguanidine (AG) treatment of diabetic rats returned the testis PHGPx activity (136.5 +/- 24.9) to the control level but did not change the value of GPx activity (69.2 +/- 17.4) compared with diabetic group. MDA and protein carbonyl levels in testis were not affected by AG treatment of diabetic rats, but interestingly AG caused SH levels to increase. The results indicate that reactive oxygen radicals were not involved in possible testicular complications of diabetes because diabetes-induced activations of GPx and PHGPx provided protection against oxidative stress, which was reported to be related to some diabetic complications [129].

Naziroglu et al in 2001 determined the protective effects of intraperitoneally administered vitamin E and selenium (as Na2SeO3, Se) on the lipid peroxidation as thiobarbituric acid reactive substances (TBARS) and vitamin E levels, glutathione peroxidase (GSH-Px), reduced glutathione (GSH) activities in the plasma, red blood cell (RBC), liver, and muscle,of rats with streptozotocin-induced diabetes. Fifty adult male Wistar rats were used and all rats were randomly divided into five groups. The first group was used as a control and the second group as a diabetic control. A placebo was given to first and second groups by injection. The third group was intraperitoneally administered with vitamin E (20 mg over 24 h), the fourth group with Se (0.3 mg over 24 h), and the fifth group with vitamin E and Se combination (COM) (20 mg vitamin E + 0.3 mg Se over 24 h). This administration was done for 25 days and the TBARS, vitamin E, GSH-Px, GSH levels in the plasma, RBC, liver, and muscle samples were determined. The vitamin E level in the plasma and liver was significantly ($p < 0.05$) higher in the control than in the diabetic control group. Also, the TBARS levels in the RBC, liver and muscle were significantly ($p < 0.05$) lower in the control than in the diabetic control group. However, GSH-Px and GSH activities in RBC, liver and muscle were not statistically different between the control and the diabetic control groups. The vitamin E levels in plasma and liver ($p < 0.01$ and $p < 0.001$) and GSH-Px activities ($p < 0.01$, $p < 0.001$) in RBC were significantly higher in vitamin E, Se, and COM groups than in both control and diabetic control groups. However, the TBARS levels of RBC, muscle, and liver in vitamin E and Se administered groups were significantly ($p < 0.05$-$p < 0.001$, respectively) decreased. These results indicate that intraperitoneally administered vitamin E and Se have significant protective effects on the blood, liver, and muscle against oxidative damage of diabetes [130].

Diabetes results in various biochemical abnormalities in the retina, but which of these abnormalities are critical in the development of retinopathy is not known. Kowluru et al in 2001 examined the effect of antioxidant supplementation on diabetes-induced alterations of retinal glutamate, and explored the inter-relationship between alterations of retinal glutamate, oxidative stress, and nitric oxide (NO) in diabetes. Glutamate was measured in the retina at 2 months of diabetes in rats receiving diets supplemented with or without a mixture of antioxidants containing ascorbic acid, Trolox, DL alpha-tocopherol acetate, N-acetyl cysteine, beta-carotene and selenium. The relationship between glutamate, oxidative stress and NO was evaluated using both bovine retinal endothelial cells and normal rat retina. In diabetes, retinal glutamate was elevated by 40, thiobarbituric acid-reactive substances (TBARS) by 100, and NO by 70%, respectively. Administration of antioxidants inhibited the diabetes-induced increases in glutamate, TBARS and NO. Incubation of bovine retinal endothelial cells or normal rat retina with glutamate significantly increased TBARS and NO, and addition of either antioxidant (N-acetyl cysteine) or a NO synthase inhibitor prevented the glutamate-induced elevation in oxidative stress and NO. Incubation of retina with a glutamate agonist, likewise elevated oxidative stress and NO, and memantine inhibited such elevations. Thus, the alterations of retinal glutamate, oxidative stress and NO appear to be inter-related in diabetes and antioxidant therapy may be a suitable approach to determine the roles of these abnormalities in the development of diabetic retinopathy [131].

BACKGROUND : Oxidative stress has been implicated in the pathogenesis of diabetic nephropathy. Although glucose itself can initiate oxidative stress, deficiency of essential trace elements such as selenium (Se) may exacerbate this oxidative stress in diabetic rats. The mechanism by which Se deficiency causes oxidative stress and renal injury is not completely understood. This study by Reddi et al in 2001 tested the hypothesis that Se deficiency induces renal oxidative stress and renal injury via transforming growth factor-beta1 (TGF-beta1). METHODS: Fifty-four male Wistar rats were used. Diabetes was induced in 27 rats by streptozotocin, and the other 27 rats received buffer only. Ten weeks after induction of diabetes, both normal and diabetic rats were killed, their kidneys removed, and glomeruli were isolated. Glomeruli from normal and diabetic rats were incubated in the presence of TGF-beta1 alone or its neutralizing antibody. Antioxidant enzyme (Cu-Zn) superoxide dismutase (Cu-Zn SOD), catalase, and glutathione peroxidase (GSH-Px) activities; total glutathione; and lipid peroxidation were determined. For Se studies, 15 normal and 15 diabetic rats were divided into groups of five each and fed either a regular, Se-deficient, or Se-supplemented diet one week after induction

of diabetes. Ten weeks after feeding these diets, rats were killed and glomeruli were isolated. Oxidative stress was examined by determining the mRNA expressions for antioxidant enzymes and also for TGF-beta1. Plasma glucose and albuminuria were determined. Histology of the kidney and interlobular artery was evaluated by light microscopy. RESULTS: In vitro studies showed that TGF-beta1 significantly reduced glomerular catalase and GSH-Px activities as well as total glutathione levels with an increase in lipid peroxidation in both normal and diabetic rats. Antibody to TGF-beta abrogated these changes. There was no effect of TGF-beta1 on Cu-Zn SOD. Like TGF-beta1, a Se-deficient diet caused a significant decrease in glomerular mRNA expression for Cu-Zn SOD, catalase, and GSH-Px, but a significant increase in TGF-beta1 mRNA expression. Also, a Se-deficient diet caused an increase in albuminuria, glomerular sclerosis, and plasma glucose levels in both normal and diabetic rats. The deficient diet caused a decrease in the lumen size of the interlobular artery. Se supplementation to diabetic rats up-regulated mRNA expression for antioxidant enzymes, and significantly reduced but did not normalize that of TGF-beta1. Glomerular sclerosis was normalized and the interlobular artery lumen size was greatly enlarged in diabetic rats by Se supplementation. Also, the tubulointerstitium was preserved by Se supplementation in diabetic rats. CONCLUSIONS: The data show that TGF-beta1 is a pro-oxidant and Se deficiency increases oxidative stress via this growth factor. In addition, Se deficiency may simulate hyperglycemic conditions. Se supplementation to diabetic rats prevents not only oxidative stress but renal structural injury, as well [132].

The reference ranges of the trace elements Al, As, Be, B, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Li, Rb, Se, Sr, and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS) in sera of a group of free-ranging plains viscachas of the pampa grasslands of Argentina. The values were compared with those of a small group of captive plains viscachas of the Zurich Zoo with diabetes and bilateral cataracts. In addition, a method for digestion of whole-blood samples is described for the trace element determination. Significant differences in the concentration of trace elements in the two groups of animals are discussed. No correlation was found by Forrer et al in 2001 between the levels of selenium and of other trace elements compared to the formation of cataracts [133].

Kowluru et al in 2001 administered antioxidants to diabetic rats and experimentally galactosemic rats to evaluate the ability of these agents to inhibit the development of diabetic retinopathy. Alloxan diabetic rats and nondiabetic rats that were fed 30% galactose randomly received standard diets or the diets supplemented with ascorbic acid and alpha-tocopherol (vitamins C+E diet) or a more comprehensive mixture of antioxidants (multi-antioxidant diet),

including Trolox, alpha-tocopherol, N-acetyl cysteine, ascorbic acid, beta-carotene, and selenium. Diabetes or galactose feeding of at least 12 months resulted in pericyte loss, acellular capillaries, and basement membrane thickening. Compared with diabetic controls, the development of acellular capillaries was inhibited by 50% ($P < 0.05$) in diabetic rats that received supplemental vitamins C+E, and the number of pericyte ghosts tended to be reduced. The vitamins C+E supplement had no beneficial effect in galactosemic rats, but these rats consumed only approximately half as much of the antioxidants as the diabetic rats. The multi-antioxidant diet significantly inhibited (approximately 55-65%) formation of both pericyte ghosts and acellular capillaries in diabetic rats and galactosemic rats ($P < 0.05$ vs. controls), without affecting the severity of hyperglycemia. Parameters of retinal oxidative stress, protein kinase C activity, and nitric oxides remained elevated for at least 1 year of hyperglycemia, and these abnormalities were normalized by multi-antioxidant therapy. Thus, long-term administration of antioxidants can inhibit the development of the early stages of diabetic retinopathy, and the mechanism by which this action occurs warrants further investigation. Supplementation with antioxidants can offer an achievable and inexpensive adjunct therapy to help inhibit the development of retinopathy in diabetes [134].

In order to study the metabolism of essential trace elements in diabetics, Feng et al in 2001 studied alloxan-diabetic rats for the distribution patterns of chromium (Cr), cobalt (Co), iron (Fe), selenium (Se), and zinc (Zn) in the liver, kidney, pancreas, and testes, as well as in the organ subcellular fractions. Normal rats were used as controls. Cr 50-enriched stable isotopic tracer solution was given by intravenous injection to avoid the difficulties of estimation of Cr status. Data showed that the concentrations of Zn in liver and kidney, of Co, Fe, and Zn in pancreas, and of Fe and Zn in testes of the diabetic rats were significantly higher than in the control rats. Nevertheless, the concentrations of Cr in pancreas, Fe in kidney, and Cr and Se in testes of the diabetic rats were significantly lower than in the controls. Furthermore, they observed significant alterations of element concentrations in subcellular fractions of various organs in the diabetic rats. These results suggest that changing hormone levels may interfere with the accumulation of some trace elements both in the organs and in the subcellular fractions of rats [135].

So this review shows that study of the effect of antioxidant like Vit E, selenium etc. on experimentally induced diabetes was the first line of research work started on free radical scavengers on diabetes.

This was a chance finding that selenium showed to have a normoglycaemic effect in experimentally induced hyperglycaemia.

Vanadium like selenium has an insulin like effect to lower the blood sugar level in diabetes. Studies then looked for different biochemical markers and enzymes related to different biochemical pathways in diabetes and the effects of selenium in regulating these pathways.

Gradually the researchers found interest in the complication of diabetes and started working on the beneficial effect of antioxidants on the long term complications like cataract, nephropathy, retinal metabolism, cardiomyopathy etc.

Considering these facts we studied the effects of selenium on carbohydrate, fat, protein metabolism and the action on hepatic microsomal enzymes and the peroxidative processes.

AIMS & OBJECTIVES OF PRESENT STUDY

3.0 Aims & Objectives of present study :

Diabetes is tackled by diet control, oral hypoglycaemics and insulin injection. Sulphonylureas act primarily by stimulating the beta cells of the islets of Langerhans of pancreas to release stored insulin. They are, therefore; ineffective in totally insulin deficient patients and for successful therapy probably require about 30% of normal beta cell function.

Biguanides reduce absorption of carbohydrates from the gut and increase the utilisation of glucose in peripheral tissues, provided insulin is present and they reduce hepatic gluconeogenesis.

Both groups of drugs are only effective in the presence of insulin. Long term administration of these drugs probably cause cardiovascular morbidity.

Insulin's drawbacks :

1. Promotes allergic reaction.
2. Hypoglycaemia leading to coma and death.
3. Lipoatrophy at the injection site.
4. Injectable preparation.

So it is seen that inspite of diabetes being a burning threat to population all over the world there are drawbacks of therapy and no existing biochemical marker which is directly proportional to the severity of the disease is available.

A number of laboratory studies in animals and epidemiological studies relating to humans have appeared that attempt to link decreased selenium status with increased incidence of cancer [136] and its use as an anticarcinogenic agent has been studied in detail [137, 138, 139, 140 & 141]. Selenium is in GroupVI A of the Periodic Table and has properties intermediate between those of a metal and non-metal. Selenium has a highly specific metabolism and its functional role has been well documented. Selenium (Se), being an integral part of the enzyme glutathione peroxidase is the first line of defence in protecting various cells from injurious consequences, Glutathione peroxidase plays an important role in preventing lipid degradation and membrane disordering [142, 143 & 144]. Recent studies have suggested that in a cell free system glucose can enolize and reduce molecular oxygen radicals [145, 146 & 147] and hyperglycaemia may cause peroxidative injury to membranes. This prompted us to focus our attention on the possible hidden role of selenium as a therapeutic agent since selenium prevents the initiation of peroxidation of membrane lipids and free radical attack.

A few interesting points emerge from the recent studies on diabetes. Many workers have already stated the role of selenium for normoglycaemia in experimental diabetic mice. There are few more reports which showed the effect of selenium on different antioxidants in mice. There are only few reports which stated the effect of selenium on the parameters of different metabolic pathways as well as on the liver microenzyme system.

In the light of the above literature review the present work was aimed towards the understanding of the response of different metabolic pathways under selenium treatment in diabetic mice. Efforts will also be given to draw a correlation between the response of these parameters with the prominent antioxidants like catalase, glutathione.

Mice were made diabetic by intraperitoneal injection of streptozotocin. Selenium was fed to the diabetic mice. It was observed that normoglycaemia occurs after a certain period of time. This time period varies directly with the dose of selenium. Three doses in multiple of ten were taken. These doses were much lower than the toxic dose of selenium. A dose response study of the different parameters were observed to find out the overall normalization effect of selenium in experimental diabetes.

The following parameters were studied :

1. Glucose
2. Glucose 6 Phosphatase
3. Glucose 6 Phosphate dehydrogenase
4. Succinic dehydrogenase
5. Lactic acid
6. Pyruvic acid
7. Glycogen
8. Lactate dehydrogenase
9. Urea
10. L.D.L.
11. H.D.L.
12. V.L.D.L.
13. Cholesterol
14. Triglyceride
15. UDP Glucoronyl transferase
16. Cytochrome p 450
17. Catalase
18. Mono Amine Oxidase
19. Acetyl choline transferase
20. Fibrinogen
21. Glutathione
22. Glutathione s transferase
23. Glutathione reductase
24. Lipid peroxidation
25. HMG CoA reductase
26. Protein
27. Selenium
28. Vanadium
29. Chromosome preparation
30. Histology of pancreas

3.1 Plan of Work :

The following plan of works were undertaken during the course of investigations.

Phase-I : Selection of Experimental animals :

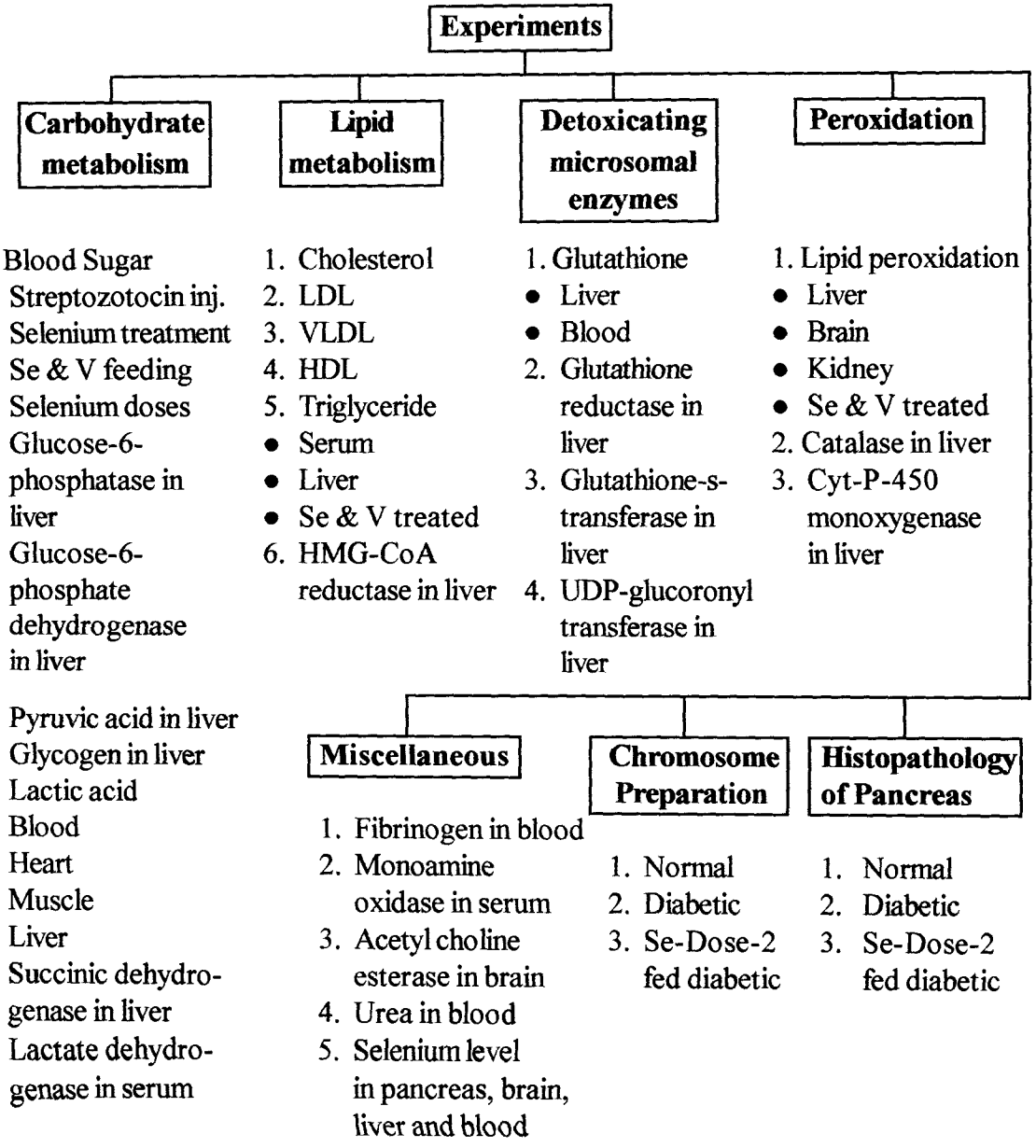
Seven to eight weeks old inbred male swiss mice, average weight of each being 20 ± (SD2) gm were taken.

Phase-II : Mode of treatment :

Mice were injected intraperitoneally 65 mg/kg body weight of streptozotocin (Sigma, USA) dissolved in 0.5 M citrate buffer (pH 4.5).

Phase-III : Design of experiments :

The details of experimental design is given in the scheme below :



MATERIALS & METHODS

4.0 Materials & Methods :

4.1.1 Experimental animals :

Seven to eight weeks old inbred male swiss mice, average weight of each being 20 ± (SD2) gm, were housed in a temperature controlled (25.5 ± 0.5°C) room with 50-60% humidity and were exposed to 14 hr. cycle of light and darkness. All the animals had access to mouse feed (pellets) supplied by Lipton India Ltd., Calcutta and Water ad libidium.

4.1.2 Mode of treatment :

Mice were injected intraperitoneally 65 mg/kg body weight of streptozotocin (Sigma, USA) dissolved in 0.5 M citrate buffer (pH 4.5) [148]. Hyperglycaemia was observed within 24-48 hrs.

4.1.3 Design of Experiments :

Animals were divided into two groups of fifteen. One group was kept as control and another was made diabetic. Blood samples were drawn from orbital veins and heart. Experiments were performed after 3 weeks of injection of Streptozotocin. Animals were sacrificed after proper anaesthesia with ether. Mice with blood sugar over 250 mgm% were taken.

4.2 Carbohydrate Metabolism :

4.2.1 Estimation of blood sugar :

Blood sugar was estimated by glucose oxydase method [149].

Blood glucose was measured by One Touch instrument of Life Scan inc. Johnson & Johnson Company, Milpitas, California, 95035, USA.

Procedure :

1. Insert test strip and close door.
2. Press on /off button.
3. Check code.
4. Obtain blood sample.
5. Open door, apply blood and close door.
6. Reading.

4.2.2 Estimation of liver glucose-6-phosphatase :

Test sample - 5% weight/volume of liver homogenate in sucrose solution [150].

Reagents :

(i) Sucrose solution (0.25 M) - 8.56 gm in 100 ml of water.

(ii) Glucose-6-phosphate solution (0.1 M) - 0.0336 gm. in 1 ml. water.

(iii) Maleic acid buffer - 116 mgm. Maleic acid is dissolved in water, pH is adjusted to 6.5 with 1(N) NaOH and made upto 10 ml.

(iv) 10% TCA.

(v) Ammonium Molybdate solution - 0.75 gm of ammonium molybdate is dissolved in 20 ml. of water, 10 ml of 10 (N) H_2SO_4 is added, and volume made upto 40 ml with water.

(vi) Metol solution - 1 gm in 100 ml of 3% sodium bisulphite.

(vii) Standard phosphate solution - 0.022 gm of H_2PO_4 is dissolved in 100 ml of water. Few drops of chloroform is added.

(viii) Ascorbic acid.

Procedure :

Centrifuge tubes are taken and reagents are added according to following protocol (All in ml.)

Tubes	Sucrose EDTA Sol.	Gl-6-PO_4	Buffer		Sample	Standard PO_4 Sol.	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
	0.2	0.1	0.1	Mix and bring to 37°C in water bath	0.1	0.1	Mix and incubate at 37°C for 10 mins.

Ascorbic Acid TCA Sol.	Sample		Supernatant fluid	Ammonium Molybdate sol.	D.W.	O.D. 680nm
(9)	(10)	(11)	(12)	(13)	(14)	(15)
2	0.1	1. Keep in ice for 5 mins. 2. Centrifuge for 3 mins. at 3000 rpm. 3. Pipette clear supernatant fluid to another centrifuge tube.	1	0.5	1	

$$\text{Volume Activity} = \frac{\text{E sample - E control}}{\text{E standard}} \times \frac{1500}{t}$$

[U/L] at 37°C
t = 10 mins

4.2.3 Estimation of liver Glucose-6-phosphate dehydrogenase :

Reagents :

(i) Physiological saline with EDTA :

0.9 gm of NaCl was dissolved in distilled water and made to 100 ml. 0.025 gm EDTA - Na_2H_2, $2H_2O$ was dissolved in physiological saline and made to 100 ml.

(ii) Triethanol amine buffer (50 mM; pH 7.5)

0.93 gm of triethanolamine hydrochloride and 0.2 gm EDTA- Na_2H_2, $2H_2O$ were dissolved in 50 ml distilled water, pH was adjusted at 7.5 with 0.1 (N) NaOH and diluted to 100 ml with distilled water.

(iii) Glucose-6-phosphate solution (40 mM)

13 mg or 0.013 gm Glucose-6-phosphate, sodium salt (Gl-6-PO_4, Na_2) was dissolved in 1 ml distilled water.

(iv) $NADP^+$ solution - (30 mM β-NADP)

0.0025 gm NADP, Na_2H was dissolved in 0.1 ml 1% $NaHCO_3$ solution.

Procedure :

(i) Preparation of Enzyme

0.35 gm of liver tissue and EDTA physiological saline solution were taken in an ice cold homogeniser and homogenised for 2 min in an ice bath (The EDTA physiological buffer was added according to 0.04 ml/mg of wet liver tissue (i.e., 2.5% w/v). Then the content was centrifuged for 20 min. (30 min.) at 0-15°C (0°C) and 15,000 rpm (7,600 rpm) smallest unit of cold centrifuge (lower gradient) = 400 rpm and clear supernatant fluid was decanted. The time between the biopsy and the start of centifugation should not be more than 5 min [151].

(ii) Assay Procedure (in ml)

Tubes (1)	Triethanol Amine Buffer (2)	Sample (3)	NADP Sol. (4)	(5)	Glucose-6-phosphate Sol. (6)
	2.4	0.5	0.05	Mix and incubate for 5 min. at 25°C	0.05

After setting 100% T at 365 nm with the blank test solution was placed in cuvette. After waiting for an extinction increase of about 0.020 readings were taken at every 2 min interval for 10 mins.

4.2.4 Estimation of Liver Pyruvic acid :

Lu, 1939; Friedman and Flangen, 1943; Nath Mukherjee, 1957.

This method is based on the formation of hydrazone of Pyruvic acid with 2'4 dinitro-phenyl hydrazone. This gives a reddish colour with strong alkali which is estimated by colorimeter. Other associated substances like acetone, aceto acetic acid etc. giving the same colour in lesser degree are eliminated by extraction in an organic solvent like toluene and re-extraction in sodium carbonate solution [152].

Preparation of Reagents :

1. Trichloro acetic acid- (TCA), 10% (w/v) : prepare by dissolving 10 gm in water and making to 100 ml. Keep in refrigerator.

2. 2'4 - dinitrophenyl (DNPH), 100 mg/100 ml : Dissolve 100 mg of finely powdered substance in 2 (N) HCl and make to 100 ml with the same solvent, filter if necessary and keep in refrigerator.

3. Toluene, Pure grade, benzene or xylene also may be used.

4. Sodium carbonate solution, 10% : Dissolve 10 gm of Na_2CO_3 in water and make to 100 ml. Store in a polythene bottle.

5. Sodium hydroxide, 1.5 (N) : Prepare from more concentrated solution of known strength.

6. Standard solution of Pyruvic acid, 100 mgm/100 ml of 0.1 (N) H_2SO_4. Dissolve 100 mgm of freshly distilled pyruvic acid (or 125 mgm of Sodium pyruvate or 107 mgm of lithium pyruvate) in 100 ml of 0.1 (N) H_2SO_4.

Method :

1. Take 8 ml of TCA in a 15 ml graduated centrifugal tube.
2. Deliver 2 ml of liver homogenate into TCA.
3. Mix immediately and thoroughly and centrifuge. Keep in refrigerator till use.
4. Bring the supernatant and TCA to room temperature. Transfer 3 ml of supernatant to tube marked T.
5. Add 3 ml of TCA to another tube marked C.
6. Add 1 ml of DNPH to each. Mix and keep for 10 min at 37^0C.
7. Add 3 ml of toluene to each tube and mix the liquids by bubbling and current of air for 5 min. using a cappilary pipette.
8. Remove the lower aquaous layer as completely as possible.
9. Add 6 ml of Sodium Carbonate and mix thoroughly by passing air.
10. When 2 layers separate out clearly, separate the lower carbonate layer with a pasteaur pipette into 2 similarly marked pipette.
11. Add 5 ml of 1.5 (N) NaOH and mix.
12. Wait for 10 min at 37^0C.
13. Take reading of T against C at 520 nm.

Interpretation :

Normal range of whole blood pyruvic acid by the above method is 0.3 - 0.9 mgm/100 ml. There is no sex difference. Newborns have higher values. Values also increase after meals and exercise. Plasma has slightly higher concentration than whole blood.

In thiamine deficiency the value may be increased to 2.3 mgm/100 ml. Thiamine is an essential factor for the oxidation of pyruvic acid and an increase in the level of pyruvic acid indicates thiamine deficiency. It is also elevated in diabetes mellitus, congestive heart failure, diarrhoea, in some liver disease and some infections.

4.2.5 Estimation of liver Glycogen :

Biochemistry Laboratory Techniques, Sterling Chaykin John Wiley and Sons, Inc. 1966, Page 88.

The characterisation of Glycogen [153, 154, 155] **:**

The anthrous method (a general colorimetric method for the determination of carbohydrates). Carbohydrates are dehydrated by conc. H_2SO_4 to form furfural and a variety of other degradation products. Furfural will condense with anthrone (9, 10-dehydro-9, oxanthracene) to form a blue coloured complex. The blue colored complex is subject to quantitation through colorimetry. Since various carbohydrates have different dispositions with regard to the formation of furfural, the colour yield varies from one carbohydrate to another. This method is particularly useful in the estimation of di and polysacharide, since hydrolysis of these compounds prior to assay is unnecessary. For example the colour yield per mole of glucose is the same for glucose, maltose and glycogen.

Experimental procedure :

To 1.0 ml of a solution containing carbohydrate (0-100 μg) add 4.0 ml of anthrone reagent (0.2% w/v anthrone dissolved in conc. H_2SO_4). Quickly and thoroughly mix the two solutions, taking care not to spatter the concentrated acid, cover each tube with a marble to prevent loses of water by evaporation. Heat the mixture in a boiling water bath for 10 mins allow the reaction tubes to cool and read their adsorbance at 620 mμ against a reagent blank.

1. Verify that Beer's law hold over the concentration range suggested above (various number of the class should choose different standard sugars, such as glucose, maltose, glycogen, a methyl α-glucoside)

2. Determine the purity of the glycogen isolated from mouse liver.

$$\text{Glycogen concentration} = \frac{\text{glucose std. conc.}}{1.11} \times \frac{E_{620} \text{ of glycogen reac. mixture}}{E_{620} \text{ of glucose reac. mixture}}$$

4.2.6 Estimation of Lactic Acid :

4.2.6.1 Blood lactic acid estimation :

Serum lactic acid was estimated by the method of Barker and Summerson (1941)[156].

In this method protein free filtrate is treated with copper sulphate and solid calcium hydroxide to remove glucose. A portion of the filtrate from this process is treated with sulphuric acid to convert the lactic acid into acetaldehyde, the amount of which is measured by means of the purple colour given by p-hydroxy diphenyl in the presence of copper.

Calculation :

Since 5 ml of standard (Standard solution of zinc or lithium lactate. Dissolve 0.213 gm of pure dry lithium lactate in about 100ml of water, add 1ml of conc. H_2SO_4 and make up to a litre with water. Dilute 1 in 20 to obtain a standard containing 10 μg/ml) contains 50 μg (0.05 mgm), $^1/_{10}$th of which is used for developing the colour and since the colour developed in the determination corresponds to 0.02 ml of blood.

$$\text{Mgm. lactic acid per 100 ml blood} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 0.005 \times \frac{1007}{0.02} \text{ i.u. x } 25$$

4.2.6.2 Tissue lactic acid estimation :

Reagents :

(i) 20% $CuSO_4$, $5H_2O$

(ii) 4% $CuSO_4$, $5H_2O$

(iii) Solid $Ca(OH)_2$ powder

(iv) Conc. H_2SO_4

(v) 1.5% p-hydroxy diphenyl in 0.5% NaOH

1.5gm of p-hydroxy diphenly was dissolved in 100 ml of 0.5% NaOH.

The sample : Liver/muscle homogenate 10% w/v in distilled water.

Procedure :

1. Removal of protein :

(i) To 2 ml of tissue homogenate, 1ml of 10% TCA was added and shaken well.

(ii) The tube was centrifuged at 1000 g for 15 mins. and the supernatant was removed.

2. Treatment with copper and calcium :

(i) To 1 ml of supernatant 1ml of 20% $CuSO_4$, 5 H_2O and 8 ml distilled H_2O were added to make total volume 10 ml.

(ii) Approximately 1 g powdered $Ca(OH)_2$ was added and then the tube was shaken vigorously by a cyclomixer.

(iii) The mixture was allowed to stand at room temperature for 30 mins with occasional shaking.

(iv) Then it was centrifuged at 1000 g for 15 mins. and supernatant was removed.

3. Formation of Acetaldehyde :

(i) To 1ml of the supernatant fluid (taken in a test tube) 0.05ml of 4% $CuSO_4$, $5H_2O$ was added, tube was kept in an ice and water bath for 2 min.

(ii) Then 6.0 ml of conc. H_2SO_4 was added slowly with occasional shaking.

(iii) Then the test tube was placed in a boiling water bath for 5 mins.

(iv) Then it was cooled below 20^0C

4. Development of colour :

(i) To the test tube 0.1 ml of p-hydroxydiphenyl was added and shaken well.

(ii) The tube was placed in a water bath (25-30^0C) for 30 minutes and meanwhile it was shaken at least once to redisperse the precipitated reagent.

(iii) Excess reagent was dissolved by heating the tube in a boiling water bath for 90 sec. and cooled in cold water.

5. Measurement of colour :

The resultant solution was read at 560 nm against conc. H_2SO_4 as blank

4.2.7 Estimation of liver Succinic dehydrogenase :

Reduction of indophenol - The enzymatic oxidation of succinate by indophenol has been followed spectrophotometrically at 600 mμ. A cuvette of 1 ml capacity was filled with 0.98 ml containing 10 μmoles of phosphate (pH - 7.4), 0.5 mgm of H. Serum albumin, 2 μmoles of KCN, 10 μmoles of succinate and 20 γ [γ = 0.001 mgm] of 2, 6-dichloroindophenol. Then (0.02 mgm) 5-15 γ of enzyme in 0.02ml were added and reading were taken at 30 sec. intervals against blank containing all components except succinate [157].

Specific activity to be defined as micromoles of indophenol reduced/mgm of protein/min. at 38^0C. The O.D. change at 600 mμ divided by 19.1 is the value for micro moles of indophenol reduced.

Reagents :

Sodium Succinate (0.1M) = 0.27 gm/10 ml of distilled water

Dichlorophenol indophenol = 0.6 mgm/1 ml of distilled water

KCN - 0.0195 gm/5ml

BSA - 10 mgm in 2ml

Protocol :

	Blank (ml)	Test (ml)
Buffer	0.1	0.1
Ser Albumin	0.5	0.5
KCN	0.1	0.1
Succinate	-	0.1
Indophenol	-	0.1
Distilled water	2.2	2.0
Enzyme	0.1	0.1

4.2.8 Estimation of Serum Lactate Dehydrogenase :

The spectrophotometric method measures the increase or decrease in extinction at 340 mμ due to the change in the amount of $NADH_2$. The reduced forms of nicotinamide-adenine dinucleotide and nicotinamide-adenine dinucleotide phosphate have an absorption peak at the wave length whereas the oxidized forms have very little absorption. Wroblewski and La Due (1955) using pyruvate and $NADH_2$ measured the decrease in extinction [158].

$$\underset{\text{Pyruvate}}{CH_3COCOO^-} + NADH_2 \rightleftharpoons \underset{\text{Lactate}}{CH_3CHOH.COO^-} + NAD$$

Reagents :

(i) Phosphate buffer, pH 7.4, 0.1 M : Dissolve 18.97 gms. of anhydrous dipotassium hydrogen phosphate & 2.69 gms. of anhydrous potassium dihydrogen phosphate in water to 1 lit.

(ii) Reduced nicotinamide-adenine dinucleotide, 2.5 mg per ml phosphate buffer (freshly prepared).

(iii) Sodium pyruvate 2.5 mgm/ml. (keep in refrigerator and have to discard if any contamination occur) dissolved in H_2O.

Technique :

2.7 ml of phosphate buffer into a spectrophotometer cell (1 cm.) add 0.1 ml of serum, 0.1 ml of $NADH_2$. Allow to stand for 20 minutes to reduce any keto acids already present in the serum. Add 0.1 ml of pyruvate.

Read the extinction for 5 min at intervals of 15 to 30 seconds at room temperature (24-27°C)

[0.1 ml Serum + 0.1 ml of $NADH_2$ → 20 mins. + 0.1 ml of pyruvate]

Calculation :

The unit of activity is defined as that which produces a decrease of 0.001 in extinction/ minute at 340 mμ and is reported per ml. of serum.

$$\text{Hence : Units/ml} = \frac{\text{Change in extinction/min}}{0.001} \times \frac{1}{\text{Volume of serum used}}$$

$$= \text{Change in extinction/min} \times 10{,}000$$

To convert to International unit.

4.3 Lipid metabolism :

4.3.1 Estimation of Blood Cholesterol :

Blood cholesterol was estimated by Sackett method [159]. This is a modification of Bloor's method in which blood is added to an ethanol-ether mixture, which precipitates proteins and extracts the cholesterol. The supernatant fluid obtained in centrifuging is evaporated, the cholesterol taken up in chloroform and determined colorimetrically by the Libermann-Buschard reaction.

4.3.2 Estimation of serum LDL, VLDL, and HDL :

Serum lipoproteins were estimated by selective precipitation method of Miller et. al.(1982). Different fractions of lipoprotein were precipitated by sodium dodecyl sulphate and centrifuged. Clear supernatant was analysed for total cholesterol by using Ferric chloride [160].

4.3.3 Estimation of serum and liver triglyceride :

Serum and liver (10% w/v) was dissolved in isopropanol and shaken with active alumina to remove phospholipids, triglycerides remaining in solution. This is then hydrolysed by alkali and liberated glycerol is oxidised to formaldehyde by periodate. The formaldehyde is condensed with acetyl acetone in presence of ammonia to form yellow colored. 3, 5-diacetyl, 1,6 dihydrobetidine which is measured in a colorimeter [161].

Preparation of reagents :

1. Isopropanol, analytical grade, aldehyde free.

2. Aluminium oxide, active : This should be thoroughly washed with isopropanol to remove fine granules and then dried.

3. Saponification mixture : Dissolve 0.5 g of KOH in 3.75 ml of water and 1.25 ml of isopropanol and mix. Prepare fresh.

4. Periodate solution : Dissolve 0.95 gm of ammonium acetate in 8 ml of water. Add 0.75ml of glacial acetic acid and 8 mgm of sodium metaperiodate. Dilute 12.5 ml with water and mix. Prepare fresh.

5. Acetyl acetone solution : Mix 0.4 ml of acetyl acetone in 100 ml of isopropanol and shake to dissolve. Prepare fresh.

6. Standard solution of triolein, 100 mgm per 100 ml. Dissolve 0.1 g of triolein in 100 ml of isopropanol.

Method :

Arrange 5 centrifuge tubes fitted with glass stoppers and label them B (for blank), S (for standard) and others.

		B	S	Others
Aluminium oxide	gm	0.8	0.8	0.8
Isopropanol	ml	4.8	4.8	4.8
Serum/Liver	ml	-	-	0.2
Water	ml	0.2	-	-
Standard Triolein solution	ml	-	0.2	-

Put the stoppers shake vigorously for 15 to 20 mins. Mechanical shaking is preferable, without removing stoppers, at 2000 r.p.m. for 10 min. to get a clear supernatant in each. Next arrange another 5 tubes marked as before and add as under.

		B	S	Others
Respective supernatant	ml	2	2	2
Saponification mixture	ml	0.5	0.5	0.5
		Mix and wait for 10 mins and then add		
Periodate solution	ml	1.5	1.5	1.5
Acetyl acetone solution	ml	1.5	1.5	1.5

Mix and incubate in the dark at 65-70^{0}C for 15 min. Cool to room temperature and take readings for unknown and standard respectively against blank at 415 nm (violet filter).

Calculation :

$$\text{mgm of triglyceride per 100 ml of serum/liver} = \frac{v}{S} \times 100 \ (v = \text{unknown})$$

Results may also be obtained from a calibration curve prepared with different concentrations, say from 30 to 150 mgm of triolein per 100 ml in isopropanol.

4.3.4 Estimation of liver HMG CoA reductase :

HMG CoA reductase was estimated by homogenising the liver in potassium phosphate buffer pH 7.2 containing 20 mM dithiothritol. Reductase activity was determined on the whole homogenate to optimize the recovery of the enzymatic activity as recommended by Field. (1982) [162].

Total activity was calculated by the protein recovered. Protein was estimated by the method of Lowry. All statistical analysis were done by Student's T test.

4.4 Detoxicating microsomal enzymes :

4.4.1 Estimation of glutathione in liver and blood :

Glutathione level was determined by the method of Grunert and Philips (1951) [163] in liver tissue homogenate and blood.

Principle :

The observation by Grunert and Philips that the colored complex in the analysis for glutathione was stable for only 15 secs. prompted an investigation for the purpose of determining the factors which would prevent the rapid fading of color. The instability of the complex has been noted by Biorich. when the use of nitroprusside for the analysis of glutathione was first

reported. The qualitative inhibition of fading by the use of cyanide suggested the posibility that the reagent would be of value in the quantitative determination of glutathione. With this in mind the nitroprusside method of analysis for glutathione was reinvestigated in order to obtain the maximum degree of stability for the coloured complex and to determine the optimum concentration of reagent and condition.

Reagents :

Metaphosphoric acid solution - 3% Metaphosphoric acid in saturated solution of sodium chloride - 2%

Sodium chloride granules

Sodium nitroprusside solution - 0.067 M 20 mgm/ml (stored in brown bottle from sunlight)

Sodium carbonate - Sodium cyanide solution - 1.5 M sodium carbonate and 0.067 M sodium cyanide. This solution was stable.

Procedure :

Blood (0.5 ml) obtained by heart or venous puncture was haemolysed in 1ml of water containing a pinch of saponin in a centrifuge tube. Upon completion of haemolysis 2.5 ml of 3% metaphosphoric acid was added followed by sufficient sodium chloride to saturate the solution (1.5 gm). After shaking well the sample was centrifuged, filtered and 2 ml aliquot removed from the filtrate for analysis when liver and other tissues were analysed.

100 - 200 mgm of freshly excised CO_2 frozen tissue was homogenised with Polter-Elrehjen homogeniser in 3 ml of 3% metaphosphoric acid and 1 ml of water. The solution was then saturated with sodium chloride and treated as the blood sample. All manipulations were carried out at 3^0C.

To an Evelyn tube containing 6 ml of saturated sodium chloride was added the 2 ml aliquot. After equilibration at 20^0C for 5-10 min 1 ml of sodium nitroprusside solution was added followed immediately by 1 ml of the sodium cyanide solution. The intensity of the resulting colour was measured in the Evelyn colorimeter with a 520 mμ filter within 1 min. 2 ml of 2% metaphosphoric acid saturated with sodium chloride was used for reagent blank.

4.4.2 Estimation of Glutathione reductase in liver :

Glutathione reductase activity was measured by a modification of the method of Carlburg and Manervik [164].

The reaction mixture (1 ml) consisted of 0.1 M potassium phosphate (pH 7.6), 0.1 nM NADPH, 0.5 nM EDTA, 1 nM oxidised glutathione and a suitable amount of cytosol preparation (6 mgm protein per ml). The reaction mixture was incubated at 30°C for 5 min. before initiating the reaction by the addition of cytosol. The enzyme activity was determined by measuring the disappearance of NADPH at 340 nm.

4.4.3 Estimation of Glutathion-S-Transferase in liver [165] :

Glutathione-S-transferases (E.C. 2.5.1.18) are thought to play a physiological role in initiating the detoxication of potential alkylating agents including pharmacologically active compounds. These enzymes catalyse the reaction of such compounds with the -SH group of glutathione, there by neutralizing their electrophilic sites and rendering the products more water soluble. Glutathione conjugates are thought to be metabolised further by cleavage of the glutamate and glycine residues followed by acetylation of the resultant free amino groups of the cystenyl residue to provide the final product a mercapturic acid. The mercapturic acids i.e., S-alkylated derivatives of N-acetyl cystein are then excreted.

Spectrophotometric assay methods :

Enzyme activity with aromatic substrates was usually determined by monitoring changes in absorbance in a Cary dual beam spectrophotometer. A complete assay mixture without enzyme was used as control. Assays were conducted in a thermostated cell compartment at 25°C in 0.1 M potassium phosphate at a pH at which the non enzymatic reaction was minimal.

The concentration of GSH was 5 nM, except in systems with trans-4-phenyl-3-butene-one and ethacrynic acid (0.025 GSH) and with 1-Chloro-2-4 dinitrobenzene (1 nM GSH). The concentration of the specific substrates was limited by either high absorbance or low solubility. All assays were linear function of protein concentration and of time for atleast 3 min when the amount of enzyme used resulted in an absorbance change of less than 0.05/min substrates of limited water solubility were prepared in ethanol. The final ethanol concentration in the assay solution was always less than 4%.

Activity units : A unit of activity is defined as the amount of enzyme catalysing the formation of 1 μmole of product per min.under the condition of the specific assay. Specific activity is defined as the units of enzyme activity per mgm of protein as measured by the method of Lowrey et al.

Conditions for spectrophotometric enzyme assays in 0.1 M potassium phosphate and 5 mM GSH at 25°C.

Substrate	Conc. in in mM	pH	λ max (nm)	$mM^{-1} cm^{-1}(\lambda\varepsilon)$
1, 2 Dichloro 4-nitro benzene	1.0	7.5	345	8.5
1-Chloro, 2, 4-dinitro benzene	1.0	6.5	340	9.6
4-nitro pyriamic-N-amide	0.2	7.0	295	7.0
p-nitro benzyl chloride	1.0	6.5	310	1.9

Detailed method using 3 : 4 dichloronitrobenzene as substrate :

The substrate chosen for the estimation of enzyme activity was 3 : 4 dichloronitro benzene, becuase of the change in the ultraviolet absorption spectrum which occurs when this compound is converted in S-(2-Chloro-4-nitrophenyl) glutathione.

A cell containing all the constituents of the reaction mixture except 3 : 4 dichloro nitro benzene was used as the blank so that the rate of increase of E at 344 mμ is a measure of the rate of formation of S-(2-Chloro-4-nitophenyl) glutathione.

This increase was measured on a Unicom S.P.500 spectrophotometer with a Unicom SP 570 constant temperature cell housing. The reaction mixtures contained GSH (5 mM), various concentrations of 3 : 4 dichloronitro benzene (added 0.1 ml of ethanol and enzyme in a total volume of 3 ml of 0.1M pyrophosphate buffer, pH 2.0. The enzyme was diluted so that the rate of increase in E was less than 0.2/min. The reactions were carried out at 37°C the constituents being brought to this temperature before the determination and the reaction was started by the addition of the enzyme solution to the cell.

After stirring, readings at 344 mμ were taken at 30 secs. interval for 5.5 min. begining 30 secs. after mixing. Under these conditions the initial reaction rates were constant for at least 1.0 min and 1 unit of enzyme is defined as that amount which will form 1 μmole of S (2-Chloro-4-nitrophenyl) glutathione/min. Specific activity is expressed as units/mgm of protein.

Protein was estimated by Lowry et al method.

Method followed in this experiment :

Reagent :

(i) 0.1 M potassium phosphate buffer (pH 7.5)

(ii) GSH (5 mM)

(iii) 2-5 dichloronitro benzene 1 mM (dissolved in phenol) in a total volume of 3 ml.

GSH 5 mM :

$$\frac{5}{1000} \times 307.33 \text{ g in } 1000 \text{ ml}$$

1000 ml - 5.1 gm

3 ml - 0.004609 gm

Now dissolve (0.004609 x 10) g GSH i.e., 0.04609 g in 10 ml water.

Then 1 ml will contain 0.004609 g and this will satisfy the conc. reqd. in 3 ml.

2, 5-Dichloronitro benzene 1 mM or 0.001 M

MW - 192 g

1000 ml - 0.192

$$3 \text{ ml} - \frac{0.192 \times 3}{1000} = 0.000576 \text{ gm}$$

0.12 ml - 0.000576 gm

$\therefore$ 2 ml - 0.0096 gm.

Weigh 0.0096 g and dissolve in 2 ml ethanol 0.1 M potassium phosphate buffer calculated in the same manner.

(Liver was homogenised in 0.1 M potassium phosphate buffer).

10% homogenate

Protocol :

Tubes	Buffer	GSH	2, 5-Dichloronitro benzene	Enzyme
Blank	4 ml	2 ml	0.24 ml	-
Test	2 ml	1 ml	0.12 ml	0.1 ml

Reading at 344 nm.

Calculation :

$$\frac{\text{Mean extinction coefficient}}{\text{mgm of protein}} = \text{Unit activity of GSH-s-trans/mgm of protein/min.}$$

4.4.4 Estimation of hepatic UDP- glucoronyl transferase activity :

Mice liver microsomes were prepared by homogenising the liver tissue in 4 vols. of ice cold 0.1 M KCl. The homogenate was filtered through nylon gauze and rotated at 8000 g for 30 mins. The supernatant was then spun at 1,05,000 g for 1 hour in an ultracentrifuge. The microsomal pellet was resuspended in a vol. of 0.1 M KCl equivalent to the original net weight of liver with p-nitrophenol as the substrate, glucoronyl transferase activity was measured spectrophotometrically at 400 mμ [166]. Statistical evaluation was carried out using Student's T test.

4.5 Peroxidation :

4.5.1 Estimation of lipid peroxidation in liver, brain and kidney :

Lipid peroxidation in liver, brain and kidney was measured by the thioberbeturic acid assay [167]. In this method malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids reacts with thiobarbituric acid to give a red species absorbing at 535 nm. Tissues were homogenised in KCl (potassium chloride, pH 7.4) solution in a Potter-Elvehjem Teflon glass homogeniser for 1 minute to make a 10% w/v homogenate. The malondialdyhyde concentration of the sample can be calculated by using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ Cm^{-1}$ according to Wills, 1969.

Reagent :

(1) TCA-TBA-HCl Reagent-15 g TCA and 0.375 g TBA (Thio barbituric acid) were dissolved in 100 ml 0.25 (N) HCl. This solution was mildly heated to assist in the dissolution of the thiobarbituric acid.

(2) 0.15 M KCl

Test sample -Tissue homogenate in 0.15 M KCl.

Liver-10%, Kidney-10%, Brain-10%.

Tube	Tissue homogenate	Distilled water	TCA-TBA-HCl
B	-	1.0 ml	2.0 ml
Sample	1.0 ml	-	2.0 ml

(i) All tubes were vortexed for few seconds.

(ii) Then all were heated for 15 mins in a boiling water bath.

(iii) All were cooled in room temperature/refrigerator.

(iv) All were centrifuged at 1000 g for 10 min.

(v) Supernatant liquid in each tube was removed by pasteur pipette in cuvette and read at 535 nm against blank.

Results :

Molar extinction coefficeient = 1.56×10^5

1 (M) 1000 ml → 72 g MDA (Malondialdehyde)

1 ml → 72/1000 MDA (Malondialdehyde)

3 ml → $\frac{3 \times 72}{1000} = 0.216$ g MDA

1.56×10^5 O.D. ≡ 0.216 g MDA

1 O.D. ≡ $\frac{0.216}{1.56 \times 10^5}$ g MDA

X O.D. ≡ $X \times 0.13846 \times 10^{-5}$ g MDA

10% Tissue homogenate.

∴ 1 ml tissue homogenate or 0.1g tissue gives = $X \times 0.13846 \times 10^{-5}$ g MDA

1 g tissue gives $= X \times 0.13846 \times 10^{-4}$ g MDA

$$\therefore \text{ n moles of MDA} = \frac{X \times 0.13846 \times 10^{-4}}{72} \times 10^{-9} = X \times 192.305$$

$\therefore$ n moles of MDA per gm. of tissue = O.D. x 192.305

4.5.2 Estimation of Catalase in liver :

Catalase activity was determined by the method of Cohen et al 1970 [168].

Ethanol increases the observable catalase level by decomposing complex II which is an inactive complex of catalase with H_2O_2.

Preparation of enzyme sample :

1. An isotonic solution containing NaCl and 0.01 M sodium phosphate buffered at pH 7.4. Prepare a stock concentration solution containing
   (a) 0.405 gm NaCl
   (b) 0.061425 gm. Na_2HPO_4
   (c) 0.010935 gm. NaH_2PO_4
   (d) Make upto 4.5 ml with distilled water
   (e) Dilute 9 : 100 to obtain required buffer.
2. Individual livers were removed and homogenised in the cold (10 strokes) in 10 volumes of isotonic buffer in a glass homogeniser tube equipped with a Teflon pestle.
3. The homogenate was centrifuged for 5-10 mins. at 700 g to remove nuclei and cell debris.
4. To an aliquot of the supernatant fluid ethanol was added to a final concentration of 0.17 M (0.01 ml Ethanol/ml).
5. The samples were then incubated for 30 mins. in ice water bath. This procedure decomposes Complex II.
6. After 30 mins., 10% Triton x-100 was added to a final concentration of 1.0%.
7. Cold isotonic buffer was then added to produce a 100 fold dilution of the original homogenate. The solubilisation of catalase by Triton is rapid and dilution can be made within minutes.

Reagents for the next step :

1. 0.01 M phosphate buffer, pH 7.0. Prepare stock concentrated solution by mixing 9 parts 1 M KH_2PO_4 with 10 parts 1 M K_2HPO_4. Dilute 1 : 100 to obtain required buffer.

2. 6 mM H_2O_2 (0.06 ml 30% H_2O_2/100 ml, 0.01 M phosphate buffer pH 7.0).

3. 6 (N) H_2SO_4

4. 0.01 (N) $KMnO_4$ (316 mgm/ltr.)

Procedure :

Reactions are carried out in an ice water bath (0-2°C). The enzyme catalysed decomposition of H_2O_2 is measured.

1. Place duplicate 0.5 ml aliquots of cold catalase samples (A) into cold 20-40 ml test tubes.

2. Include duplicate blanks consisting of 0.5 ml distilled water.

3. Initiate the enzymic reactions sequentially at fixed intervals by adding 5.0 ml of cold 6 mM H_2O_2 and mix thoroughly (vortex).

4. After exactly 3 mins. stop the reactions sequentilly at the same fixed intervals by rapidly adding 1.0 ml 6 (N) H_2SO_4 and mixing (vortex).

Spectrophotometry - The H_2O_2 is measured by reacting it with a standard excess of $KMnO_4$ and then measuring the residual $KMnO_4$ spectrophotometrically.

(i) Prepare a spectrophotometric standard by adding 7 ml of 0.01 (N) $KMnO_4$ to a mixture of 5.5 ml buffer and 1.0 ml 6 (N) H_2SO_4.

Read the absorbance at 480 nm. (For convenience in performing later calculations we adjust the digital read out of the Gylford model 300 spectrophotometer to read 1.0 absorbance unit for the standard. Distilled water then reacts in the range of 0.3 absorbance unit). A distilled water reading is checked at intervals to ensure spectrophotometer stability.

(ii) The remaining enzyme reaction samples (A) and the blanks (B) are taken one at a time. To each add rapidly 7 ml of $KMnO_4$ reagent. Mix thoroughly (vortex). Read at 480 mμ within 30-60 secs. On longer standing turbidity developes. To avoid precipitation of MnO_2 in the cell flush with distilled water between samples.

Tube	Enzyme	Dist. H_2O ml	H_2O_2 buffer (ml)	Buffer (ml)		H_2SO_4 (ml)	$KMnO_4$ (ml)
Blank	-	0.5	5.0	-	Vortex	1.0	7.0
Stamd.	-	-	-	5.5	and after	1.0	7.0
Test	0.5	-	5.0	-	3 mins. add	1.0	7.0

Calculation and expression of results :

Under the conditions described the decomposition of H_2O_2 by catalase follows first order kinetics as given by the equation.

$K = \text{Log}(S_0/S_3) \times 2.3/t$

K = First order reaction rate constant

t = time interval over which reaction is measured (viz. 3 mins).

S_0 = Substrate concentration at zero time.

S_3 = Substrate concentration at 3 mins.

S_0 = Subtract the absorbance of the reaction system blanks from the spectrophotometric standard (St.)

S_3 = Subtract the absorbance of the reaction samples (A) from (St). Results can now be expressed in terms of the first order reaction rate constant (K).

4.5.3 Estimation of Cyt-P-450 mono oxygenase in liver :

The enzyme assay was performed using the method of Omura and Sato [169]. Cytochrome P 450 mono oxygenase is the carbon monoxide binding pigment of liver microsomes.

Experimental procedure :

Microsomal preparation - Liver of male mice was thoroughly perfused in situ with 0.9% NaCl solution. The liver was homogenised with 4 volumes of isotonic (1.15%) KCl solution.

(1) The homogenate was centrifuged at 12,000 g for 25 min in a refrigerated centrifuge and the precipitate was discarded.

(2) The microsome was sedimented by centrifugation at 78,000 g for 90 min in a Hitachi Model 40 P preparative ultra centrifuge.

(3) The firmly packed pellet of microsomes was resuspended in isotonic KCl solution and again centrifuged as above.

The washed microsomes were finally suspended in isotonic KCl, usually at a concentration of 10 mgm of protein per ml. The resultant microsomal suspensions were stored at 4^0C and used within 2-3 days.

The microsomal preparations thus obtained were found to be practically free of absorbed haemoglobin.

Measurements of difference spectra :

Difference spectra of microsomal preparations were measured in a Cary Model 14 spectrophotometer with cuvettes of 1 cm. optical path. Microsomal preparations usually containing 2 mgm. of protein per ml. of 0.1 M phosphate buffer (pH 7.0), were placed in both the sample and reference cells. After recording the base line, the content of sample cell was treated with various reagents and the spectral difference thereby induced was measured. When CO was used, it was carefully bubbled through the sample for about 20 seconds, this was sufficient to saturate the sample with the gas. Reduction of samples with dithionite was effected with a few milligrams of solid $Na_2S_2O_4$.

All spectrophotometric measurements were made at room temperature (20-25^{0}C).

Photo dissociation of CO compound of P-450 :

Microsomes suspended in 0.1 M phosphate buffer, pH 7.0, were placed in a 1 cm. square cell and reduced by a few milligrams of solid sodium dithionate. A suitable amount of water saturated with CO was added to the suspension to convert reduced P-450 to the CO compound.

The final concentration of CO was calculated from the solubility of CO in water at 20^{0}C.

The sample cell was then placed in the sample compartment of a Cary Model 14 spectrophotometer and illuminated from the side with a 100 watt tungsten lamp through a set of condenser lens and a red glass filter. The filter transmitted only red light of wave lengths longer than 520 mμ. An interference filter with a sharp transmittance max at about 450 mμ was inserted between the sample cell and the measuring photo tube to absorb the red light and to pass the light from the monochromater (450 mμ). The reference cell containing the same suspension was placed in the reference cell compartment without illumination. The photo dissociation was

measured by following the absorbance change at 450 mμ caused by illumination of the sample with the red light. Control experiments using reduced microsomes were carried out without CO addition to eliminate the effect of any red light leaking through the blue interference filter or blue light leaking through the red glass filter.

Reagents :

(1) $Na_2S_2O_4$ - Sodium dithionite.

(2) CO was prepared from formic acid and concentrated sulphuric acid by the conventional method and purified by bubbling through a KOH solution.

Calculation :

The concentrations of various Cyt-P-450 fractions were determined by the method of Onura and Sato from the reduced, CO complexed difference spectrum using an extinction coefficient of 91 mM^{-1}. The specific content of Cyt-P-450 in the various fractions was expressed as nmoles per mgm of protein.

4.6 Miscellaneous :

4.6.1 Estimation of Blood Fibrinogen :

Fibrinogen was determined by the method of Lempert [170].

Reagents :

(1) Calcium Chloride - 2.5% w/v (0.5 ml) [Calcium Chloride in dehydrated form must be used, otherwise significant error will occur].

(2) Sodium hydroxide - N/4 (10 ml).

(3) Sulphuric Acid - N (amount required for neutralization)

(4) Folin ciocaltin reagent (undiluted) - 1 ml.

(5) Sodium carbonate - 20% w/v - 6 ml.

(6) Standard tyrosine - 0.5 ml of tyrosine solution containing 20 mgm of tyrosine per 100 ml of 0.1 (N) HCl.

Apparatus and instruments :

(1) Centrifuge tube
(2) Pipette
(3) Small beaker
(4) Glass rod
(5) Incubator (37°C)
(6) Filter paper & funnel
(7) Digestion tube
(8) Boiling water bath
(9) Colorimeter

Method :

Blood is drawn from heart and taken in a centrifuge tube containing sodium citrate (as anticoagulant). It is better if 2 ml blood is taken. It is then centrifuged for 15 minutes. Plasma is separated and 0.5 ml of it is taken in a small beaker. It is diluted with 14 ml of distilled water and then mixed with 0.5 ml of 2.5 % Calcium chloride solution. A fine glass rod is placed in the liquid and its is allowed to stand in an incubator at 37°C for overnight in order to get a clot.

The rod is rotated to collect the clot into it. Then the rod is pressed against the side of a beaker to squeeze out any solution and to compress the clot. Care should be taken to pick up the rod as any small pieces of clot may have become detached. The clot is dried by pressing carefully against a piece of filter paper. The clot is transferred to the tube in which the digestion is carried out. The clot is washed with several proportions of distilled water, dissolved in 5 ml of $^{N}/_{4}$ sodium hydroxide (in a boiling water bath) nutralized with normal H_2SO_4 and then 0.5 ml of Folin ciocaltin reagent is added followed by 3 ml of 20% soidum carbonate. Volume is made upto 25 ml of distilled water and the solution is finally transferred into a small beaker or conical flask and it is placed in an incubator at 37°C for an hour.

As standard 0.5 ml of a tyrosine solution is used and treated in the same way as the test, Standard and unknown (test) solution are read at 680 nm wave length with water as blank.

Calculation :

Since 1 mgm of tyrosine is equivalent to 16.4 mgm of fibrinogen.

mgm of fibrinogen/100 ml of plasma

$$= \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times \frac{100}{0.5} \times 0.1 \times 16.4$$

$$= \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 328$$

4.6.2 Estimation of serum Mono amine oxidase :

$$RCH_2NH_3 + O_2 + H_2O \rightarrow RCHO + NH_4 + H_2O_2$$

Assay Method :

Benzaldehyde production from the oxidative deamination of Benzylamine allows a convenient spectrophotometric assay of enzyme activity [171].

Reagents :

Phosphate buffer (Na_2HPO_4 - KH_2PO_4) 0.2 M, pH 7.2 Benzylamine in 0.2 M phosphate buffer. 0.1 M final pH 7.2 may be prepared from either redistilled benzylamine or recrystallized benzylamine hydrochloride.

Procedure :

Serum or purified enzyme preparations and 0.1 ml of buffered 0.1 M benzylamine and a sufficient volume of 0.2 M phosphate buffer pH 7.2 to provide a final volume of 3.0 ml are added and a silica cell (1 cm light path) increases in optimal density at 250 nm are measured at 25°C in a recording.

Spectrophotometer, with thermostatically controlled cuvette chambers against a reaction mixture that contains no benzylamine.

Definition of Enzyme units and specific activity, one unit of activity is defined as the amount of enzyme catalysing a change in absorbance of 0.001/minute at 250 nm corresponds to the production of 0.25 nM of benzyldehyde/min. at 25°C. Specific activity expressed as units of enzyme/milligram of protein.

Protocol :

	Phosphate buffer + Benzylamine	**Phosphate buffer**	**Enzyme**
Blank	0.5 ml	14.5 ml	-
Test	0.1 ml	2.8 ml	0.1 ml.

4.6.3 Estimation of Acetyl Choline Esterase in brain :

Assay was made by the method of Biggs et al 1958 [172]. Cholinesterases hydrolyses esters of choline to give choline and an acid. Acetylcholine esterase would produce choline and acetic acid from acetyl choline bromide.

$$CH_3COO\ (CH_2)N^+\ (CH_3)_3 + H_2O \rightarrow CH_3COOH + CH_2OH.CH_2N^+\ (CH_3)_3.$$

The change of color of the indicator, bromothymol blue caused by the liberated acetic acid is read in the photoelectric colorimeter.

Preparation of reagent :

(1) Buffer : Dissolve 3.09 g of Na-barbitone, 0.34 g of Potassium dihydrogen phosphate and 43.84 g of NaCl in water and make to 250 ml.

(2) Buffer indicator solution : Dissolve 100 mgm of bromothymol blue in 2 ml of 2 (N) NaOH and wash into a litre volumetric flask with 150 ml of buffer. Dilute to about 950 ml with water. Adjust pH to 8 by adding 0.5 (N) HCl (about 16 ml necessary) and then dilute to a litre with water.

(3) Acetylcholine bromide solution : Prepare a 15% solution in water.

(4) Acetic acid : Prepare accurate by 0.15 (N) solution. Dilute 1 ml of this with 9 ml of water before use.

Method :

Measure in a tube 2 ml of buffer indicator solution and 2.2 ml of water; mix and then add 0.1 ml of tissue homogenate and 0.2 ml of acetylcholine bromide solution. Mix throughly and immediately read in the photoelectric colorimeter against red filter (620 nm). Incubate exactly for 30 min at 37°C and read again.

The difference of the 2 readings (u) corresponds to the amount of acid liberated.

The units of cholinesterase activity is defined to be the micromoles of acetic acid liberated from acetyl choline by 1 ml of tissue homogenate in 30 min at 37°C.

Preparation of standard curve :

To get a calibration curve, prepare a series of dilutions of acetic acid by measuring 0, 1, 2, 3, 4 .....14 ml of 0.015 (N) acid and making up the volume of each to 15 ml with water. 1 ml of each solution is mixed separately with 2 ml of buffer indicator. 1.4 ml of water and 0.1 ml

of tissue homogenates. These tubes will then represent 0, 10, 20, 30 .....140 units of activity. Read the tubes using the 1st tube with no acetic acid, as blank in the colorimeter against red filter (620 nm) and plot the readings against units of activity. Results are obtained from this curve.

Interpretation :

Normal range - 90 to 150 units per or 3.0 - 5.0 I.units/ml.

Low values are found in Uraemia, Tuberculosis and malnutrition and cachexia.

Reduced value in liver disease has limited significance. Low values are also found in poisoning by organo phosphorus compound.

4.6.4 Estimation of Blood Urea :

Estimation of Blood Urea by Diacetyl Monoxime Method [173] :

When urea is heated with substances such as diacetyl - (CH_3-C-C-CH_3), containing two adjacent carbonyl groups, coloured complexes are formed. Techniques employing this reaction have been devised for determining urea. Readings are taken at 480 nm wavelength against blank.

4.6.5 Estimation of Protein :

Protein was estimated by the method of Lowery et al [174].

4.6.6 Estimation of Selenium in Pancreas, Liver, Blood :

Selenium content was measured according to the method given by Mabuchi and Nakahara [175]. Sodium selenite was purchased from Loba Chemie Indoaustranal Co., Bombay-5, India. Selenium feeding was started 1 week before streptozotocin injection.

4.6.7 Vanadium :

Ammonium monovanadate was purchased from Sigma. Dose of vanadate solution used was 0.05 μg/0.1 ml. Vanadium feeding was started 1 week before streptozotocin injection.

4.7 Chromosome preparation and G banding technique from mice [176] :

(1) 40 mg of choline powder dissolved in 100 ml of distilled water followed by pasting in morter and stored at 4°C temperature (0.04% Colchicine).

(2) 1% sodium citrate solution - 1 gm sodium citrate in 100 ml single distilled water - hypotonic solution.

(3) Fixative - Glacial acetic acid one part and 90% ethanol or methanol (Acetoethanol = 1 : 3)

(4) Giemsa stain - stock solution - 1 gm of giemsa powder is pasted with 66 ml glycerol and kept at 60°C. Another 66 ml methanol is to be added later. Glycerol incubation at 60°C for 2 hours.

Filter the solution and store at 4°C for 15 days.

Pretreatment :

0.4% Colchicine is to be injected intraperitoneally in the adenoma at a rate of 1 ml/100 gm body weight after cleaning the site with ethyl alcohol. Leave for 45 mins. to 1 hour and 15 minutes.

Tissue extraction :

Colchicine treated animal is to be anaesthetized by cervical dislocation or by chloroform treatment. Femur of either side is to be dissected out and the bone marrow tissues is to be dissected out and the bone marrow tissues is to be collected in glass centrifuge by flushing the tissue with 1% sodium citrate at 37°C (by glass syringe with needle). The suspension is left for 10 mins. and centrifuged at 2000 rpm for 10 mins. Supernatant is to be discarded. Pellet is collected and mixed with acetomethanol. (3-4 ml at 1 : 3 ratio) and centrifuge again for 10 mins. at 2000 rpm. Discard supernatant. Mix aceto alcohol with the pellet. Leave for some time.

Preparation of slides :

Clean grease free glass slides are to be kept in chilled 50% ethanol for 4-5 hours. The suspension was placed in drops one after another uniformly along chilled slides held in horizontal position (1 foot height) to spread them out. The slides were flame dried.

Staining :

Staining is to be done in diluted Giemsa (one part Giemsa and 10-20 parts of distilled water) at pH 6.8 for about $1^1/_2$ hour. Slides were rinsed in distilled water and dried in air and leave for at least two days prior to observation.

4.8 Histopathology of Pancreas :

Procedure :

(i) Fix the small tissue (Pancreas) from mice in Bouin's fixative for 24 hours.

(ii) Dehydrate through 70% alcohol overnight.

(iii) Dehydrate through 90% alcohol (4 changes at 30 min interval).

(iv) Dehydrate through 100% alcohol (4 changes at 30 min interval).

(v) Immerse in Cedar wood oil for 7 days.

Microtomy :

(i) Remove oil with xylol for 10 minutes.

(ii) Infiltrate in paraffin (melted) - xylol mixture (1 : 1) in paraffin bath (incubator) at 58°C for 1 hour.

(iii) Infiltrate in paraffin in paraffin bath at 58°C for $1^1/_2$ hour.

(iv) Embed in paraffin, trim the block and make 5 μm thick paraffin section by microtome.

Staining :

(i) Dissolve paraffin in xylol for 10 minutes.

(ii) Hydrate through 100% alcohol (1 min), 90% alcohol (1 min), 70% alcohol (1 min) and distilled water (5 min).

(iii) Stain in haematoxylin (Delafield) (aqueous) for 5 minutes.

(iv) Wash in tap water for 5 minutes.

(v) Dehydrate through 70% alcohol (10 min), 90% alcohol (5 min).

(vi) Stain in eosin for 1 min.

(vii) Wash in 90% alcohol for 1 min.

(viii) Dehydrate in 100% alcohol for 10 minutes.

(ix) Immerse in xylol for 5 minutes.

(x) Mount in DPX, Examine.

Photomicrography : Film : ILFOR ASA : 100

RESULTS

5.0 Results :

5.1 Carbohydrate Metabolism :

A couple of independent experiments were performed to find out the effect of various doses of Selenium treatment on different aspects of carbohydrate metabolism in diabetic mice. The experimental results of each of the experiment thus described seperately below.

5.1.1 Experiments related to blood sugar :

Experiment 1 : Changes of blood sugar following intraperitoneal injection of Streptozotocin :

This experiment was done to show the effect of intraperitoneal injection of Streptozotocin in experimental mice. A group of mice were taken. The average blood sugar was found to be 61mgm%. In the 2nd week Streptozotocin was injected intraperitoneally. After 48 hours the blood sugar started rising and weekly sugar level was estimated. At the end of 10th week the sugar level was 245 mgm% on an average. This level was fixed for many months (Table-1, Fig.1).

Table 1 : Blood Sugar changes following Streptozotocin injection :

Week	Blood Sugar Level (mgm%)
1st	61
2nd	Injection Streptozotocin given intraperitoneally
3rd	84
4th	136
5th	179
6th	213
7th	234
8th	238
9th	241
10th	245

From 48 hours onwards the injection, the blood sugar started rising and rose to 4 to 5 times the noraml blood sugar level and remained at that high level for ever. This corroborates the theory that Streptozotocin produces permanent damage to β cells of Islets of Langerhans of Pancreas.

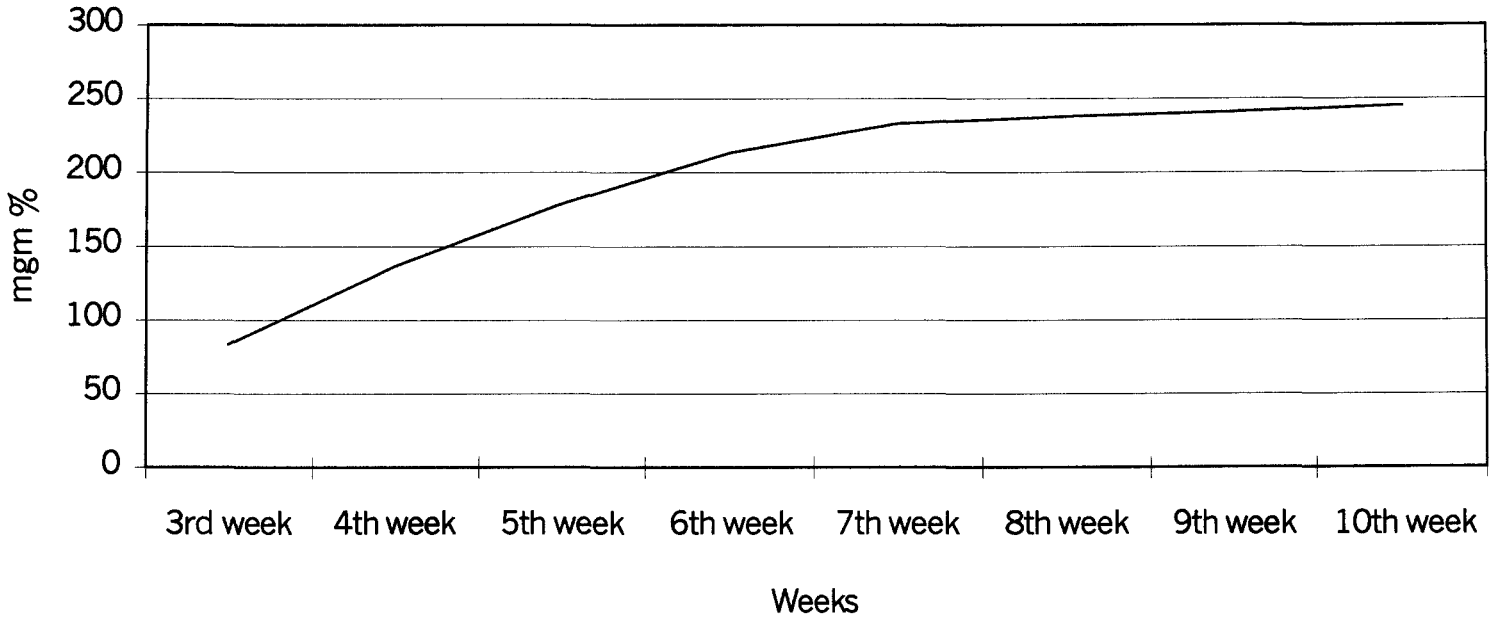


Fig. 1 : Blood Sugar changes following Streptozotocin injection.

Experiment 2 : Effect of Selenium treatment on blood sugar changes :

This experiment was designed to assess the blood sugar changes induced by Selenium treatment. A group of 5 mice were taken. Normal sugar level was found to be 60.92 ± 4.32 mgm%. Following intraperitoneal injection of Streptozotocin the blood sugar started rising and after 10 weeks the blood sugar level was 223.10 ± 6.85 mgm%. Three seperate groups of mice were made diabetic by Streptozotocin injection. To the first group Dose 1 of Selenium was fed and at 10 weeks the blood sugar level dropped to 119.90 ± 8.16 mgm%. The second group having selenium Dose 2 was normoglycaemic at 10 weeks and the blood sugar ranged at 65.20 ± 3.51 mgm%. In the third group which were fed with Selenium Dose 3 the blood sugar level went to 144.70 ± 7.14 mgm% (Table-2, Fig. 2).

Table 2 : Blood sugar changes induced by Selenium treatment :

Treatment Condition	Blood Sugar Content after 10 weeks		p Value with respect to normal
	(mgm%)	± SD	
Normal adult mice	60.92	± 4.32	
Diabetic Mice	223.10	± 6.85	
Se-Dose - 1 fed diabetic mice	119.90	± 8.16	0.0001
Se-Dose - 2 fed diabetic mice	65.20	± 3.51	0.1254
Se-Dose - 3 fed diabetic mice	144.70	± 7.14	0.0004

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

10 weeks after injection of Streptozotocin the blood sugar level rose to 4 times the normal value in experimental mice. Oral feeding with 3 different doses of Selenium recorrected the hyperglycaemia. Dose-2 found to be most effective in producing almost near normal blood sugar level.

So this Dose 2 i.e., 0.05 micro gm/0.1 ml has been chosen in the subsequent experiments.

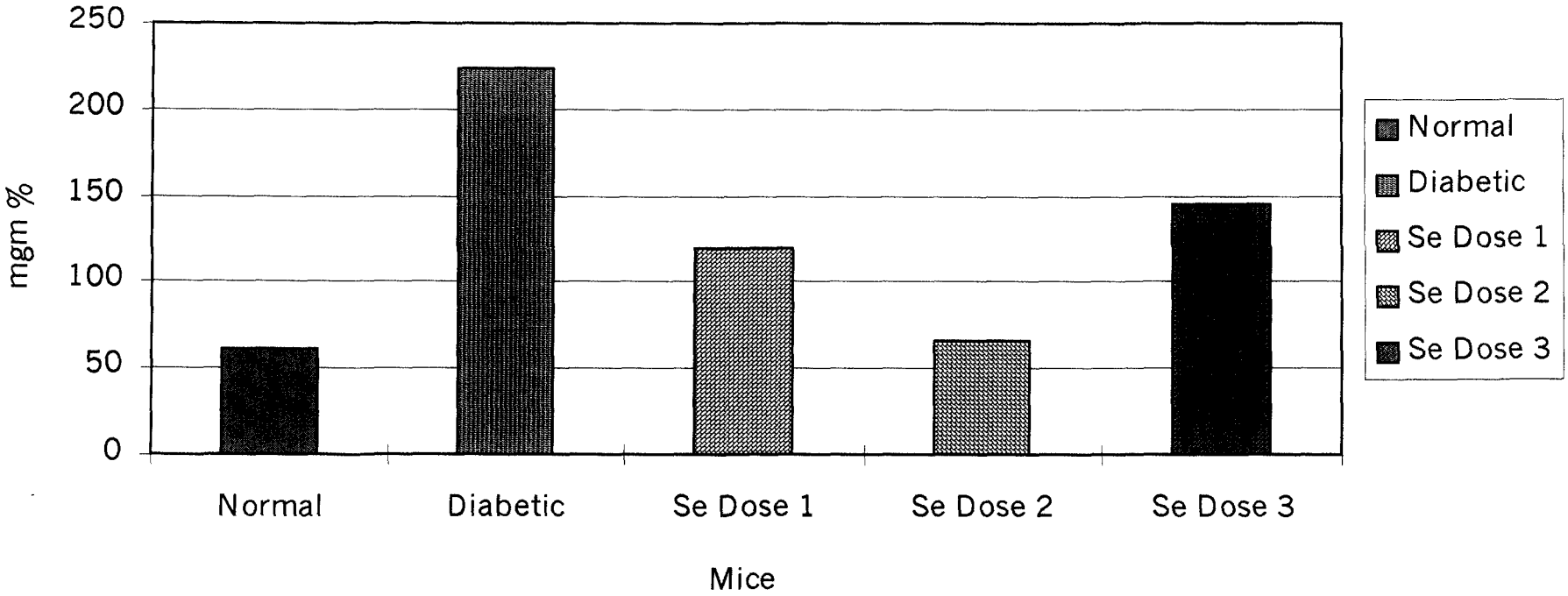


Fig. 2 : Blood sugar changes induced by Selenium treatment.

Experiment 3 : Changes of blood sugar following Selenium, Vanadium and Selenium plus Vanadium feeding :

This experiment was done to compare the normoglycamic effect of Selenium, Vanadium and the combination of two elements feeding in Streptozotocin induced diabetic mice. 4 groups of mice were taken. The first group was kept as control. The second group was fed with Selenium dose 2. The 3rd group was fed with Vanadium. The 4th group was fed with Selenium and Vanadium. All the animals were made diabetic by Streptozotocin injection in the 2nd week. In the first group the blood sugar went rising like the experiment 1. In the other 3 groups blood sugar started rising but gradually fell from 3rd week onwards and by 5th week it was near 84mgm%. In the 6th week Se & V feeding was stopped and the blood sugar started rising and by 8th week it was 184mgm%. Again refeeding of Se started and the raised blood sugar started falling and by 10th week it was 94mgm% (Table 3, Fig. 3).

Table 3 : Changes of blood sugar on Selenium and Vandium feeding :

Treatment Condition	Blood Sugar Content (mgm%)									
	1st wk	2nd wk	3rd wk	4th wk	5th wk	6th wk	7th wk	8th wk	9th wk	10th wk
Control	61	↑	84	136	179	213	234	238	241	245
Se fed	60	Injection	125	111	84	73	130	184	106	94
V fed	64	Streptozotocin	141	112	84	Feeding of Se, V		Refeeding of Se		
Se + V fed	62	↓	122	123	85	stopped	94			

Se Dose 2 = 0.05 micro gm/0.1 ml

Vanadium dose = 0.05 micro gm/0.1 ml

Selenium and Vandium both have normoglycaemic action. As our interest was in Se, so we continued with the Se feeding. The experiments showed that when Se is started it reduced the raised blood sugar in diabetic mice within weeks. Stoppage of feeding raised the sugar which again came down when the Se feeding was restarted. So Se is directly related to the normoglycaemia.

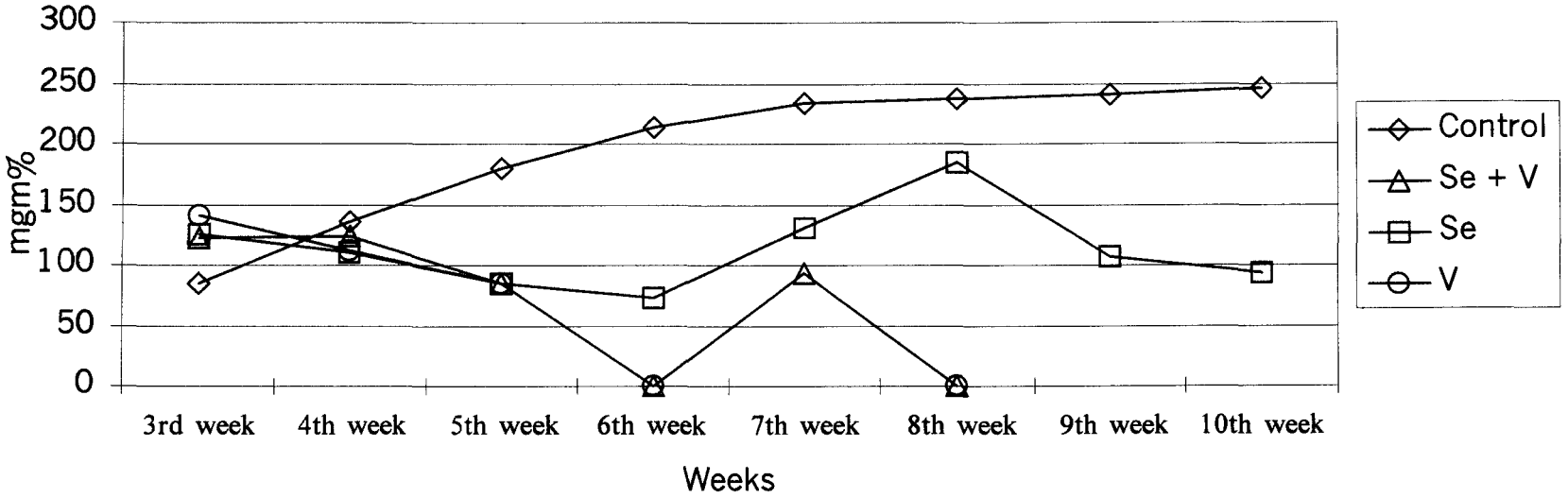


Fig. 3 : Changes of blood sugar on Selenium and Vandium feeding.

Experiment 4 : Comparative study of changes of blood sugar on feeding of 3 different doses of Selenium :

This experiment was designed to compare the normoglycaemic action of 3 different doses of Selenium. Three groups of mice were taken. To each group one different dose of Selenium was fed. On the 2nd week Streptozotocin was injected intraperitoneally. The blood sugar started rising. In the first group with the Selenium Dose-1 the blood sugar rose upto 234 mgm% in the 7th week and from 8th week onwards started normalising but reached only 133 mgm% in the 10th week. In the 2nd group having Selenium Dose-2 the blood sugar rose only 118 mgm% on the next week of injections Streptozotocin but from the subsequent weeks it started declining and came to normal in the 8th week and remained stationary. In the 3rd group with Selenium Dose 3 the blood sugar rose upto 240 mgm% in the 7th week and then started declining only to reach 144 mgm% in the 10th week (Table-4, Fig. 4).

Table 4 : Change of blood sugar on different doses of Se feeding in mice :

Treatment Condition	Blood Sugar Content (mgm%)									
	1st wk	2nd wk	3rd wk	4th wk	5th wk	6th wk	7th wk	8th wk	9th wk	10th wk
Se Dose-1 feeding	62	↑	121	145	187	198	234	189	167	133
Se Dose-2 feeding	64	Injection Streptozotocin	118	102	94	78	72	69	67	64
Se Dose-3 feeding	60	↓	127	148	185	214	240	194	178	144

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

All the doses of Selenium have normoglycaemic effect but the Dose-2 i.e., 0.05 micro gm/0.1 ml has the quickest blood sugar lowering effect. The normoglycaemia continues so long the Dose of Selenium is fed. So Selenium has an Insulin-mimetic action.

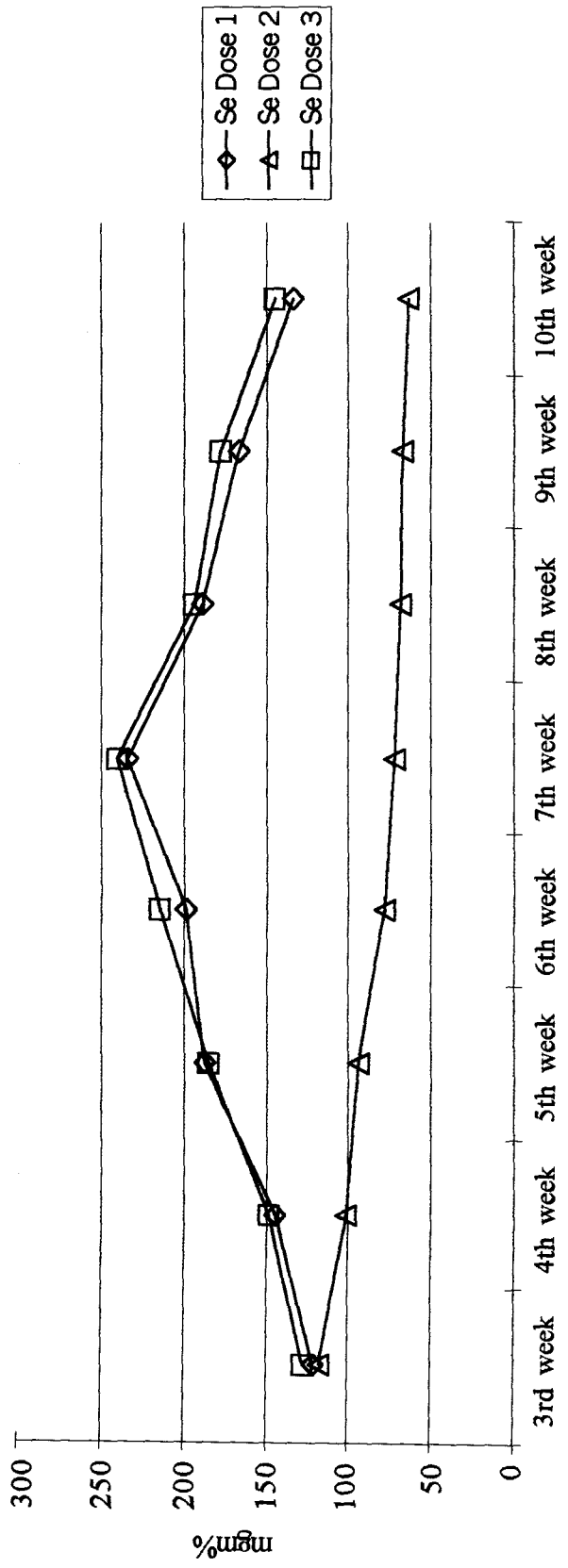


Fig. 4 : Change of blood sugar on different doses of Se feeding in mice.

5.1.2 Experiment related to glucose-6-phosphatase in liver :

Experiment 5 : Changes of Glucose-6-phosphatase in liver on Selenium treatment :

This experiment was designed to estimate the changes of level of liver Glucose-6-phosphatase with changes of doses of Selenium, fed in diabetic mice. The normal level was 9.33 ± 0.84 micron/litre. With injection of Streptozotocin the level was 13.38 ± 1.03 micron/litre. With feeding of different doses of Selenium the Glucose-6-phosphatase level was reduced. In Dose-1 fed the amount was 9.91 ± 0.89 micron/litre. In Dose-2 it was 7.09 ± 1.17 micron/litre and in Dose-3 fed it was 7.45 ± 0.66 micron/litre (Table-5, Fig. 5).

Table 5 : Changes of Glucose-6-phosphatase in liver on Selenium treatment :

Treatment condition	Glucose-6-phosphatase content in Liver		p Value with respect to normal
	micron/litre	± SD	
Normal adult mice	11.03	± 0.84	
Diabetic mice	13.38	± 1.03	
Se-Dose 1 fed diabetic mice	11.72	± 0.89	0.2451
Se-Dose 2 fed diabetic mice	7.09	± 1.17	0.0004
Se-Dose 3 fed diabetic mice	8.81	± 0.66	0.0019

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Glucose-6-phosphatase was elevated by 21.3% after production of experimental diabetes in mice and on Selenium treatment the level fell to or below normal of which Dose 2 was most efficient.

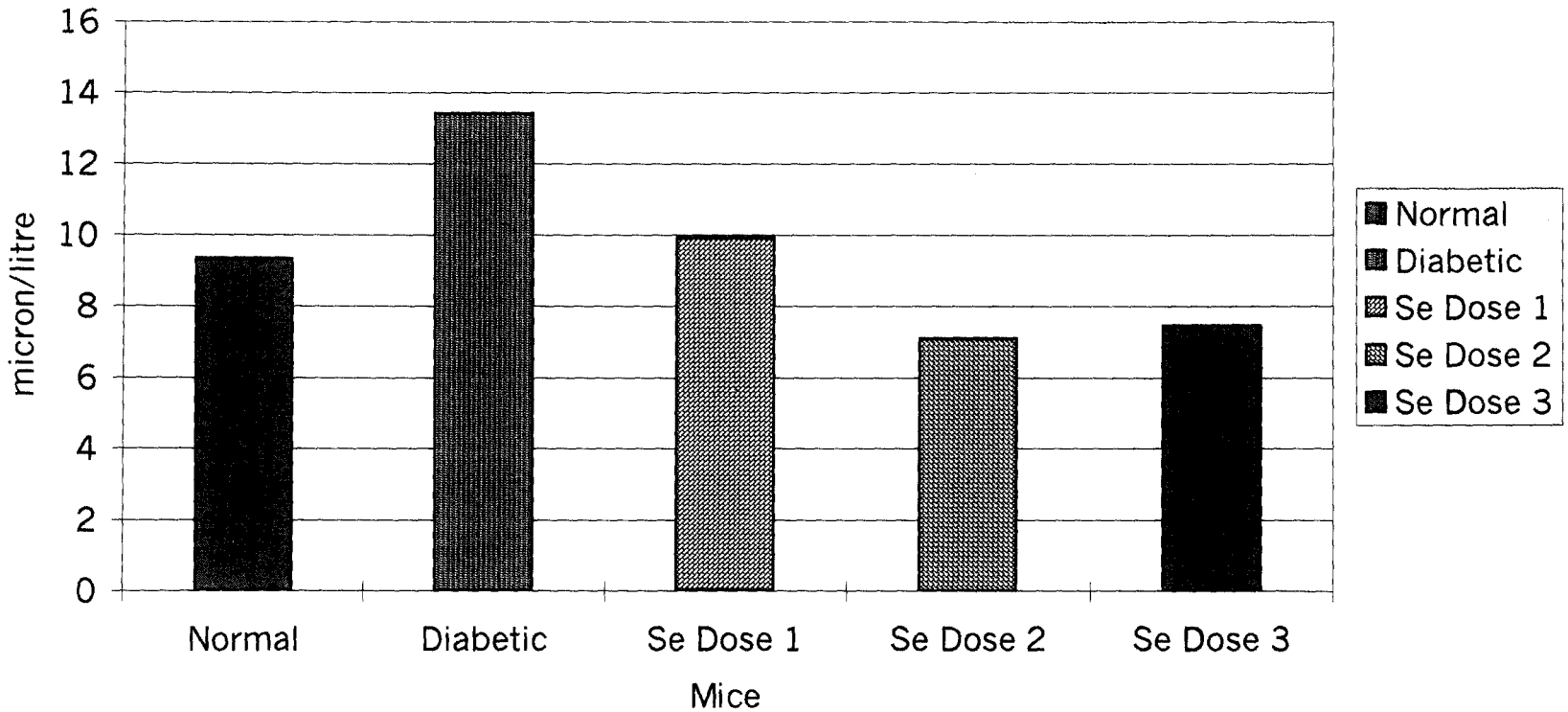


Fig. 5 : Changes of Glucose-6-phosphatase in liver on Selenium treatment.

5.1.3 Experiment related to Glucose-6-phosphate dehydrogenase in liver :

Experiment 6 : Changes of Glucose-6-phosphate dehydrogenase in liver on Selenium treatment :

This experiment was designed to understand the changes of level of Glucose-6-phosphate dehydrogenase in liver with respect to feeding of 3 different doses of Selenium in diabetic mice. The normal level in adult mice was 220.52 ± 20.68 micron/litre. In Streptozotocin induced diabetic mice the level was diminished to 174.62 ± 21.23 micron/litre. With oral feeding of different doses of Selenium the level of glucose-6-phosphate dehydrogenase rose to certain extent. With Dose-1 it was 186.99 ± 15.46 micron/litre. In Dose-2 the level increased to 210.48 ± 22.59 micron/litre. In Dose-3 the level was 185.56 ± 15.67 micron/litre (Table-6, Fig. 6).

Table 6 : Changes of Glucose-6-phosphate dehydrogenase in liver on Selenium treatment :

Treatment condition	Glucose-6-phosphate dehydrogenase content in Liver		p Value with respect to normal
	micron/litre	± SD	
Normal adult mice	220.52	± 20.68	
Diabetic mice	174.62	± 21.23	
Se-Dose 1 fed diabetic mice	186.99	± 15.46	0.0439
Se-Dose 2 fed diabetic mice	210.48	± 22.59	0.5365
Se-Dose 3 fed diabetic mice	185.56	± 15.67	0.0385

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Glucose-6-phosphate dehydrogenase was diminished by 20.8 % in diabetic mice and on Selenium treatment the level increased. In Dose-2 feeding group the level was almost near normal.

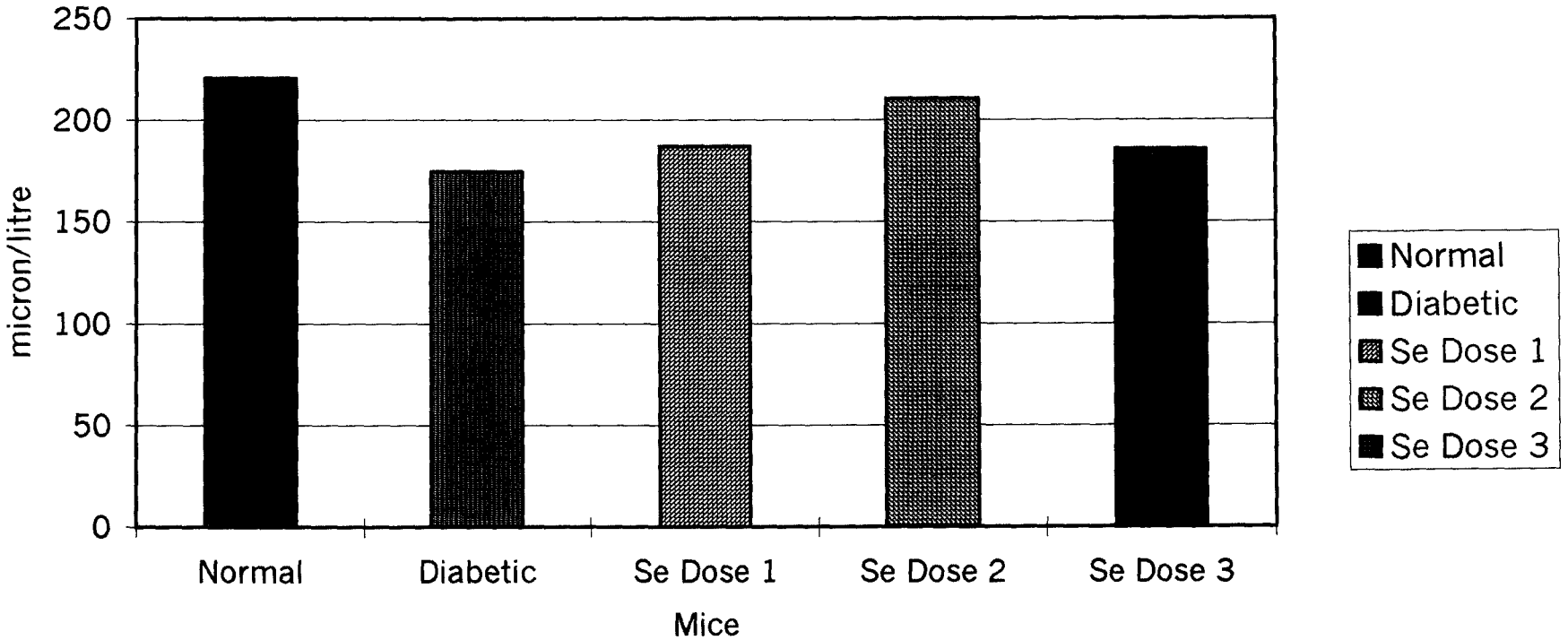


Fig. 6 : Changes of Glucose-6-phosphate dehydrogenase in liver on Selenium treatment.

5.1.4 Experiment related to Pyruvic acid in liver :

Experiment 7 : Changes of Pyruvic acid in liver on Selenium treatment :

This experiment was done to relate the changes of Pyruvic acid in liver with changes of amount of Selenium feeding in Streptozotocin induced diabetic mice. The normal Pyruvic acid in liver was 0.19 ± 0.06 mgm/ml. With induction of diabetes the Pyruvic acid level went down to 0.04 ± 0.03 mgm/ml. On feeding of 3 different doses of selenium the Pyruvic acid level rose as 0.06 ± 0.03 mgm/ml in Dose-1, 0.13 ± 0.09 mgm/ml in Dose-2 and 0.10 ± 0.08 mgm/ml in Dose-3 (Table-7, Fig. 7).

Table 7 : Changes of Pyruvic acid in liver on Selenium treatment :

Treatment Condition	Pyruvic acid level in Liver		p Value with respect to normal
	mgm/ml	± SD	
Normal adult mice	0.19	± 0.06	
Diabetic mice	0.04	± 0.03	
Se-Dose 1 fed diabetic mice	0.06	± 0.03	0.0060
Se-Dose 2 fed diabetic mice	0.13	± 0.09	0.2492
Se-Dose 3 fed diabetic mice	0.10	± 0.08	0.0836

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Pyruvic acid in liver was decreased to 78.9% in Streptozotocin induced diabetic mice. On Selenium treatment the level rose and in Dose-2 feeding the Pyruvic acid level was almost near normal.

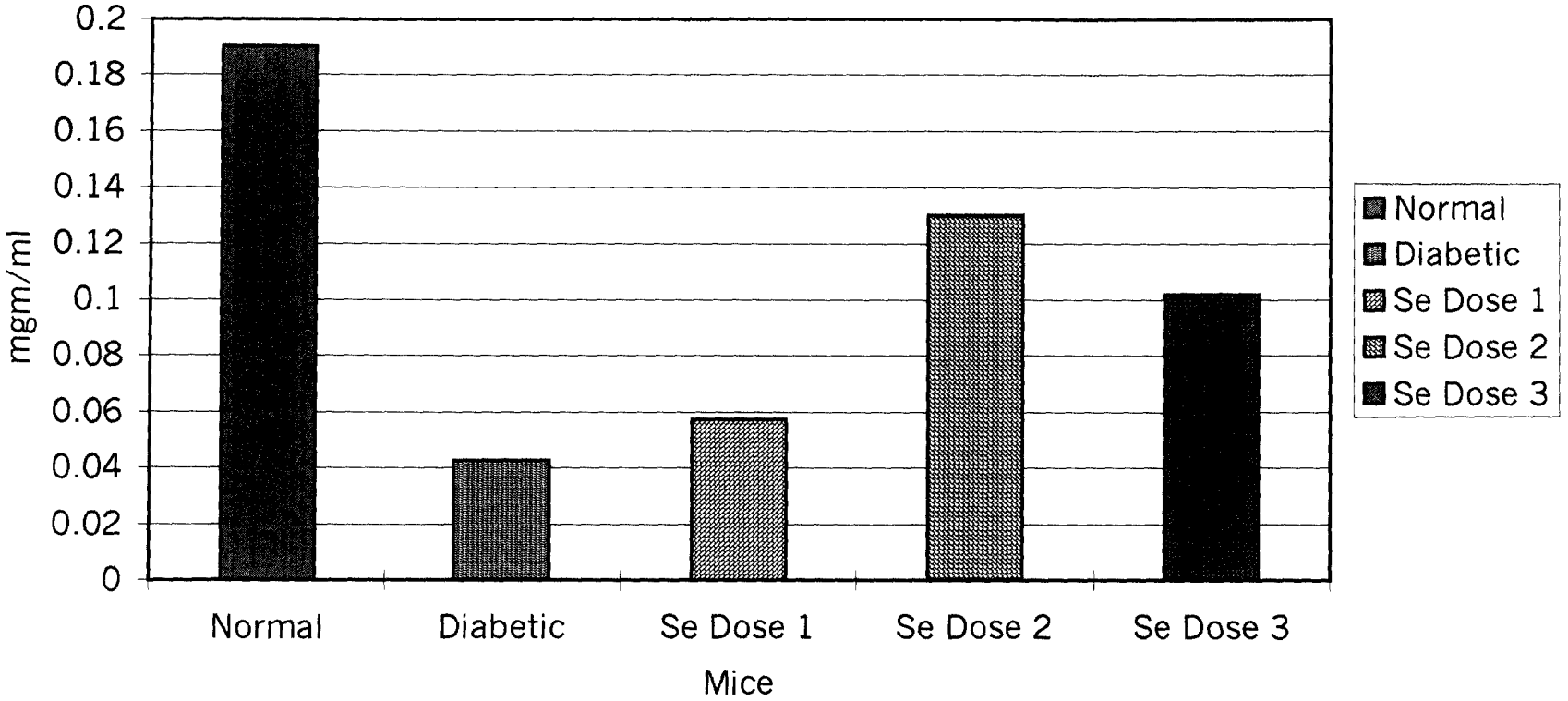


Fig. 7 : Changes of Pyruvic acid in liver on Selenium treatment.

5.1.5 Experiment related to Glycogen in liver :

Experiment 8 : Changes of Glycogen in liver on Selenium treatment :

This experiment was designed to show the changes in liver Glycogen with changes in different doses of Selenium feeding in diabetic mice. The normal Glycogen level in liver was 464.23 ± 72.45 microgm/ml. The level fell to 332.16 ± 61.30 microgm/ml in Streptozotocin induced diabetic mice. On Selenium feeding the glycogen amount rose. In Dose-1 fed mice the amount was 343.17 ± 62.11 microgm/ml, in Dose-2 fed mice the Glycogen level was 418.84 ± 75.70 microgm/ml and in Dose-3 fed mice the Glycogen content of liver was 357.94 ± 65.79 microgm/ml (Table-8, Fig. 8).

Table 8 : Changes of Glycogen in liver on Selenium treatment :

Treatment Condition	Glycogen level in Liver		p Value with respect to normal
	microgm/ml	± SD	
Normal adult mice	464.23	± 72.45	
Diabetic mice	332.16	± 61.30	
Se-Dose 1 fed diabetic mice	343.17	± 62.11	0.0451
Se-Dose 2 fed diabetic mice	418.84	± 75.70	0.4196
Se-Dose 3 fed diabetic mice	357.94	± 65.79	0.0733

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Glycogen in liver was decreased to 28.4 % in experimentally induced diabetic mice. On Selenium treatment the level of glycogen gradually recovered. Dose-2 was most efficient in raising the level.

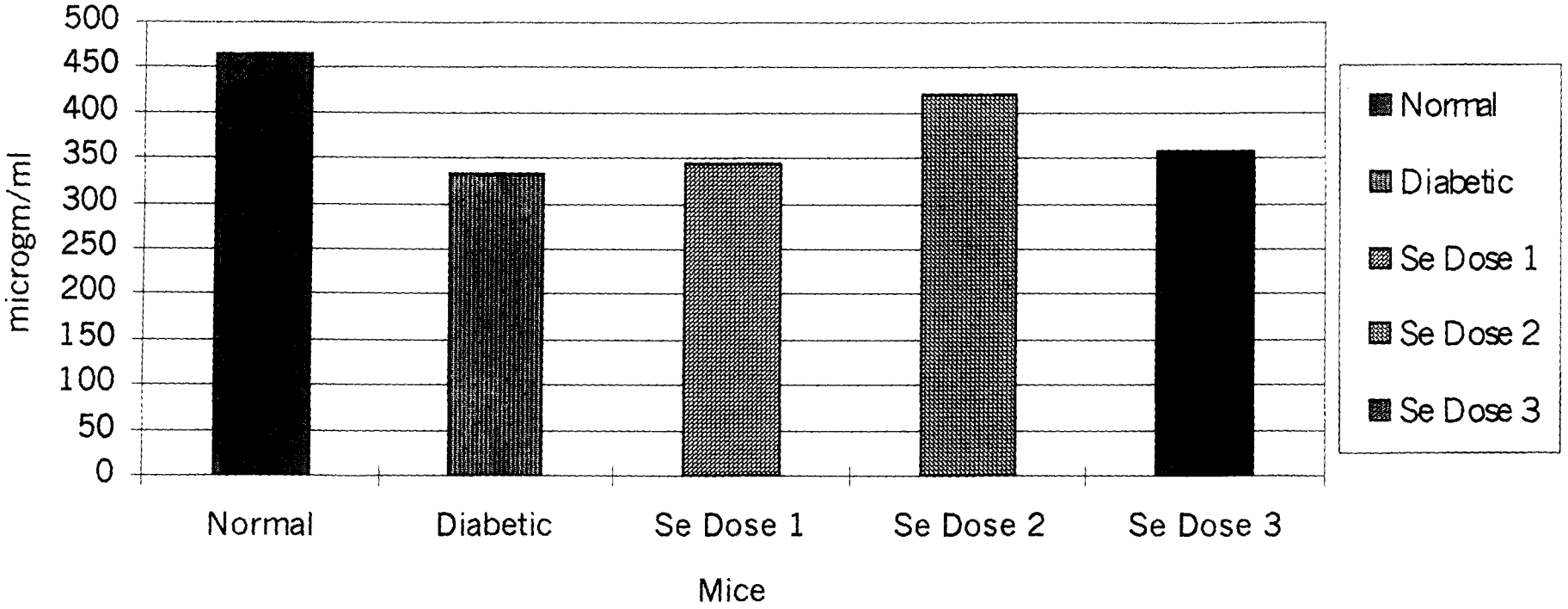


Fig. 8 : Changes of Glycogen in liver on Selenium treatment.

5.1.6 Experiments related to Lactic acid :

5.1.6.1 Blood :

Experiment 9 : Changes of Lactic acid in blood on Selenium treatment :

This experiment was the first group of experiment where attempt was made to know the changes of Lactic acid level in blood following changes of doses of Selenium in Streptozotocin induced diabetic mice. The normal level in adult mice was 87.52 ± 3.66 mgm%. With induction of diabetes the level rose to 136.14 ± 3.36 mgm%. In Selenium Dose-1 fed animals the level of Lactic acid in blood was 101.59 ± 2.68 mgm%. In Dose-2 fed mice the level was 72.24 ± 2.97 mgm% and in Dose-3 fed mice the Lactic acid content was 91.73 ± 4.12 mgm% (Table-9, Fig. 9).

Table 9 : Changes of Lactic acid in blood on Selenium treatment :

Treatment condition	Lactic acid level in Blood		p Value with respect to normal
	mgm%	± SD	
Normal adult mice	87.52	± 3.66	
Diabetic mice	136.14	± 3.36	
Se-Dose 1 fed diabetic mice	101.59	± 2.68	0.0002
Se-Dose 2 fed diabetic mice	72.24	± 2.97	0.0001
Se-Dose 3 fed diabetic mice	91.73	± 4.12	0.1267

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Lactic acid in blood rose to 55.5% in diabetic mice with respect to normal animal. The level fell with Selenium treatment. In Dose-2 fed mice the level was almost the normal control value.

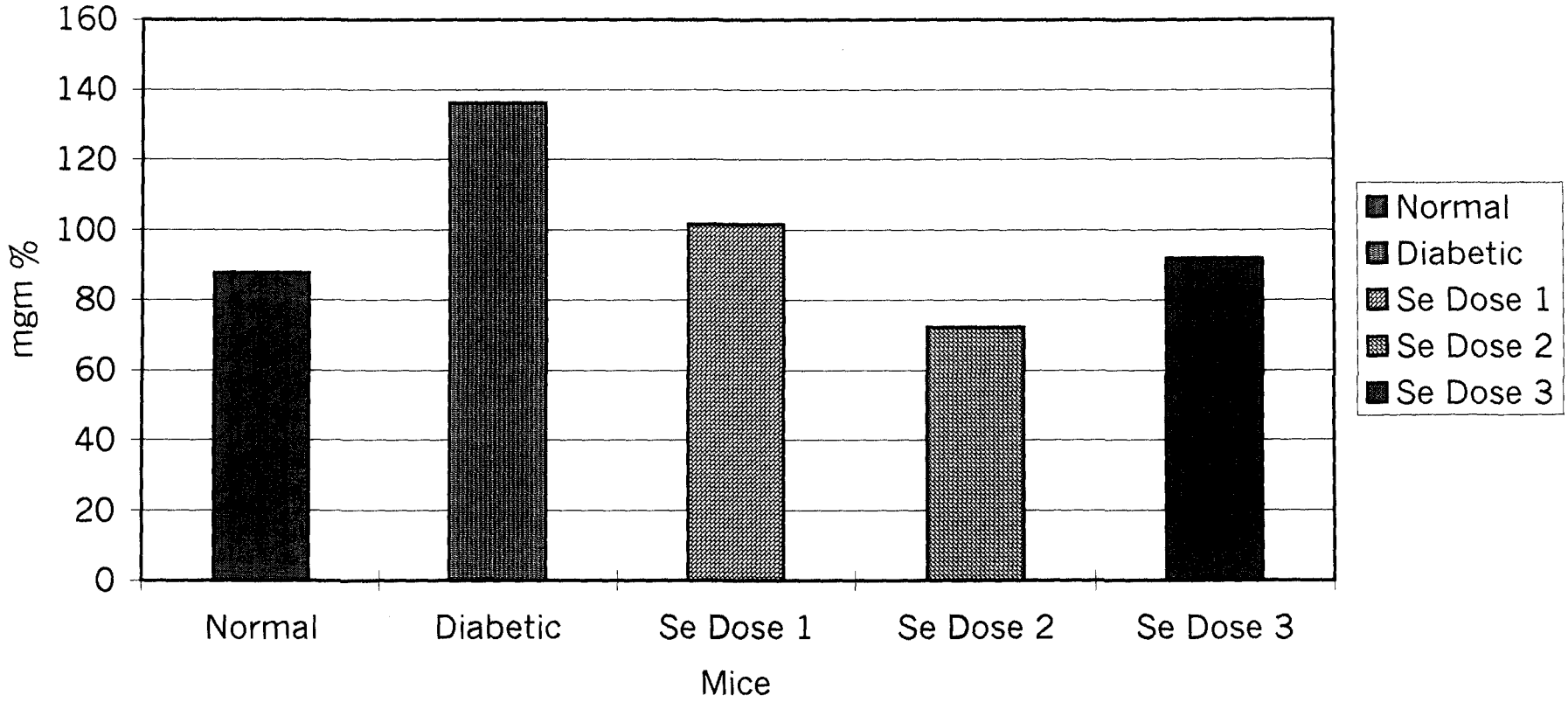


Fig. 9 : Changes of Lactic acid in blood on Selenium treatment.

5.1.6.2 Tissue :

Experiment 10 : Changes of Lactic acid in heart on Selenium treatment :

This experiment was part of the experiments to know the changes of Lactic acid in tissues following changes of Selenium doses in Streptozotocin induced diabetic mice. The normal level in adult mice heart was 101.38 ± 2.49 mgm%. The level in experimentally induced diabetic mice heart was 101.38 ± 2.38 mgm%. In Selenium fed diabetic mice in 3 different doses namely Dose-1, Dose-2 and Dose-3, the level of Lactic acid in heart were 101.38 ± 1.58 mgm%, 101.38 ± 2.16 mgm% and 101.38 ± 1.79 mgm% respectively (Table-10, Fig. 10).

Table 10 : Changes of Lactic acid in heart on Selenium treatment :

Treatment condition	Lactic acid level in Heart		p Value with respect to normal
	mgm%	± SD	
Normal adult mice	101.38	± 2.49	
Diabetic mice	101.38	± 2.38	
Se-Dose 1 fed diabetic mice	101.38	± 1.58	1.0
Se-Dose 2 fed diabetic mice	101.38	± 2.16	1.0
Se-Dose 3 fed diabetic mice	101.38	± 1.79	1.0

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The experiment showed that diabetes has no effect on the Lactic acid level in heart. Also the feeding of different doses of Selenium produced nil effect on the level of Lactic acid in heart.

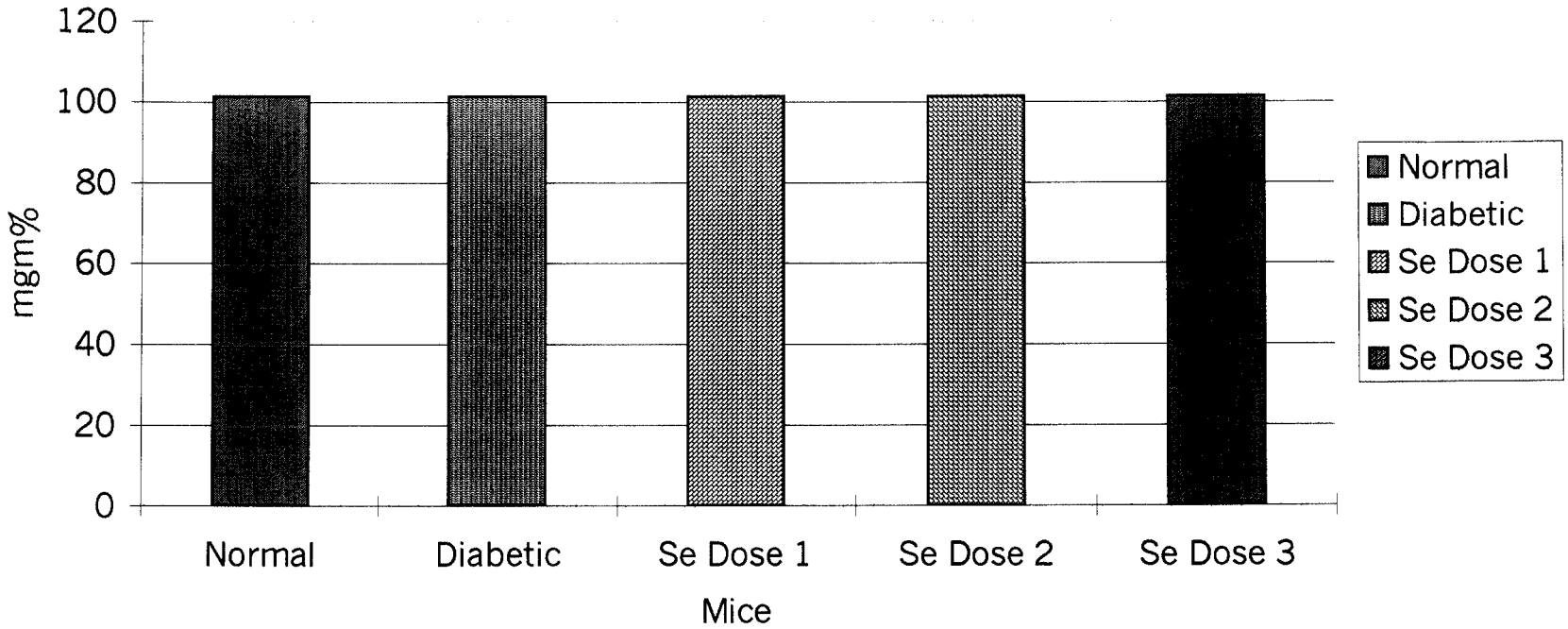


Fig. 10 : Changes of Lactic acid in heart on Selenium treatment.

Experiment 11 : Changes of Lactic acid in muscle on Selenium treatment :

This experiment was the second experiment to understand the changes of Lactic acid in tissues following changes of Selenium doses in experimentally induced diabetic mice. The normal Lactic acid level in muscle was 97.60 ± 3.40 mgm%. The level of Lactic acid in the muscle of Streptozotocin induced diabetic mice fell to 58.22 ± 4.54 mgm%. With Selenium feeding the level of Lactic acid in muscle rose. In Dose-1 fed the level was 67.17 ± 5.16 mgm%, in Dose-2 fed 85.74 ± 7.19 mgm% and in Dose-3 fed it was 70.93 ± 4.08 mgm% (Table-11, Fig.11).

Table 11 : Changes of Lactic acid in muscle on Selenium treatment :

Treatment condition	Lactic acid level in muscle		p Value with respect to normal
	mgm%	± SD	
Normal adult mice	97.60	± 3.40	
Diabetic mice	58.22	± 4.54	
Se-Dose 1 fed diabetic mice	67.17	± 5.16	0.0000
Se-Dose 2 fed diabetic mice	85.74	± 7.19	0.0374
Se-Dose 3 fed diabetic mice	70.93	± 4.08	0.0000

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The experiment showed that there was 40.3% diminution in the level of Lactic acid in muscle in diabetic mice. The feeding of Selenium increased the level of which Dose-2 rose to highest level of Lactic acid.

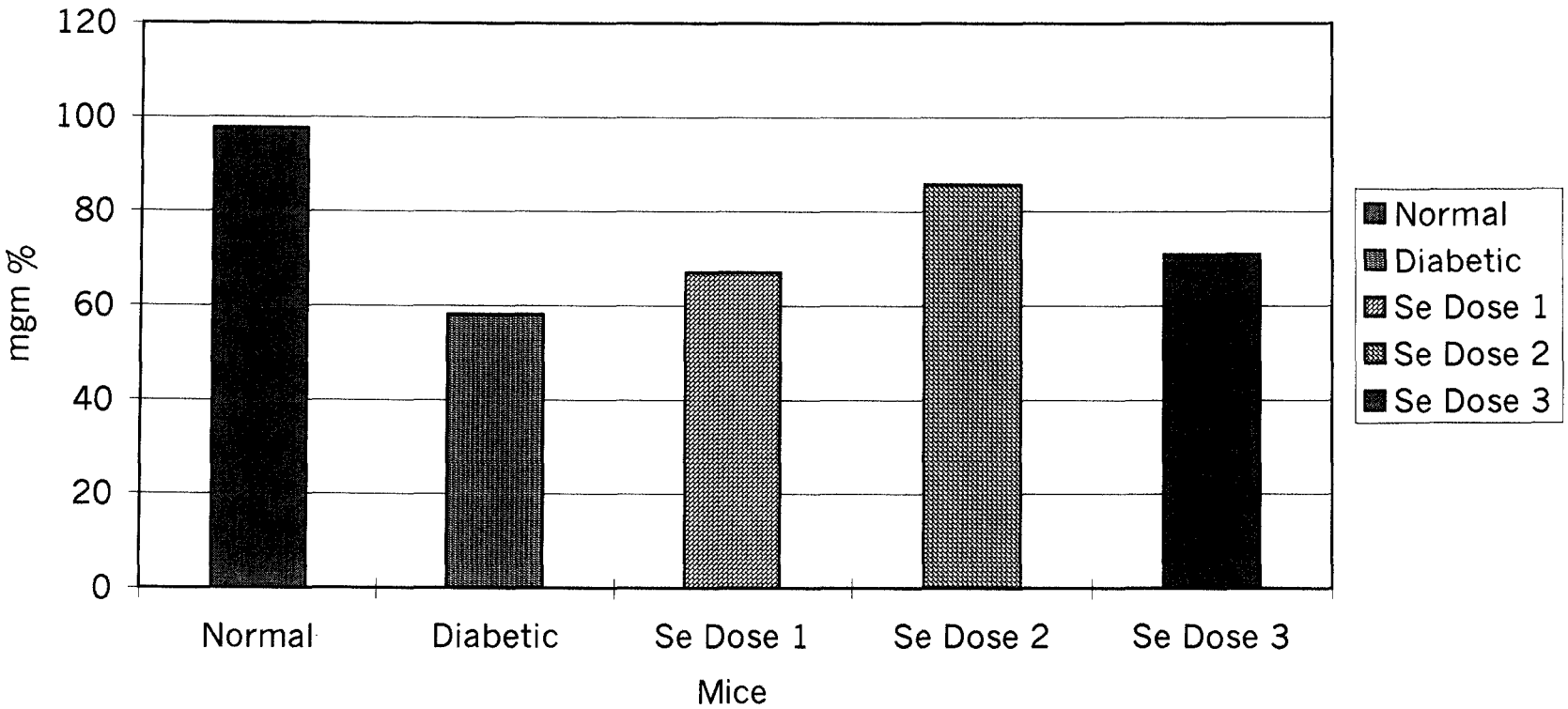


Fig. 11 : Changes of Lactic acid in muscle on Selenium treatment.

Experiment 12 : Changes of Lactic acid in liver on Selenium treatment :

This experiment was the last experiment to know the changes of Lactic acid in tissues following changes of Selenium doses in Streptozotocin induced diabetic mice. In normal adult mice liver the level of Lactic acid was 77.77 ± 2.17 mgm%. In diabetic mice the level of Lactic acid in liver was 49.96 ± 3.26 mgm%. In Dose-1 Selenium fed the level rose to 62.08 ± 3.26 mgm%. In Dose-2 fed the level was 74.14 ± 1.94 mgm% and in Dose-3 fed the level was 67.05 ± 2.63 mgm% (Table-12, Fig. 12).

Table 12 : Changes of Lactic acid in liver on Selenium treatment :

Treatment condition	Lactic acid level in liver		p Value with respect to normal
	mgm%	± SD	
Normal adult mice	77.77	± 2.17	
Diabetic mice	49.96	± 3.26	
Se-Dose 1 fed diabetic mice	62.08	± 3.26	0.0000
Se-Dose 2 fed diabetic mice	74.14	± 1.94	0.0239
Se-Dose 3 fed diabetic mice	67.05	± 2.63	0.0001

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Lactic acid in liver fell by 35.8% in diabetic mice. The level rose to almost normal in Dose-2 fed diabetic mice.

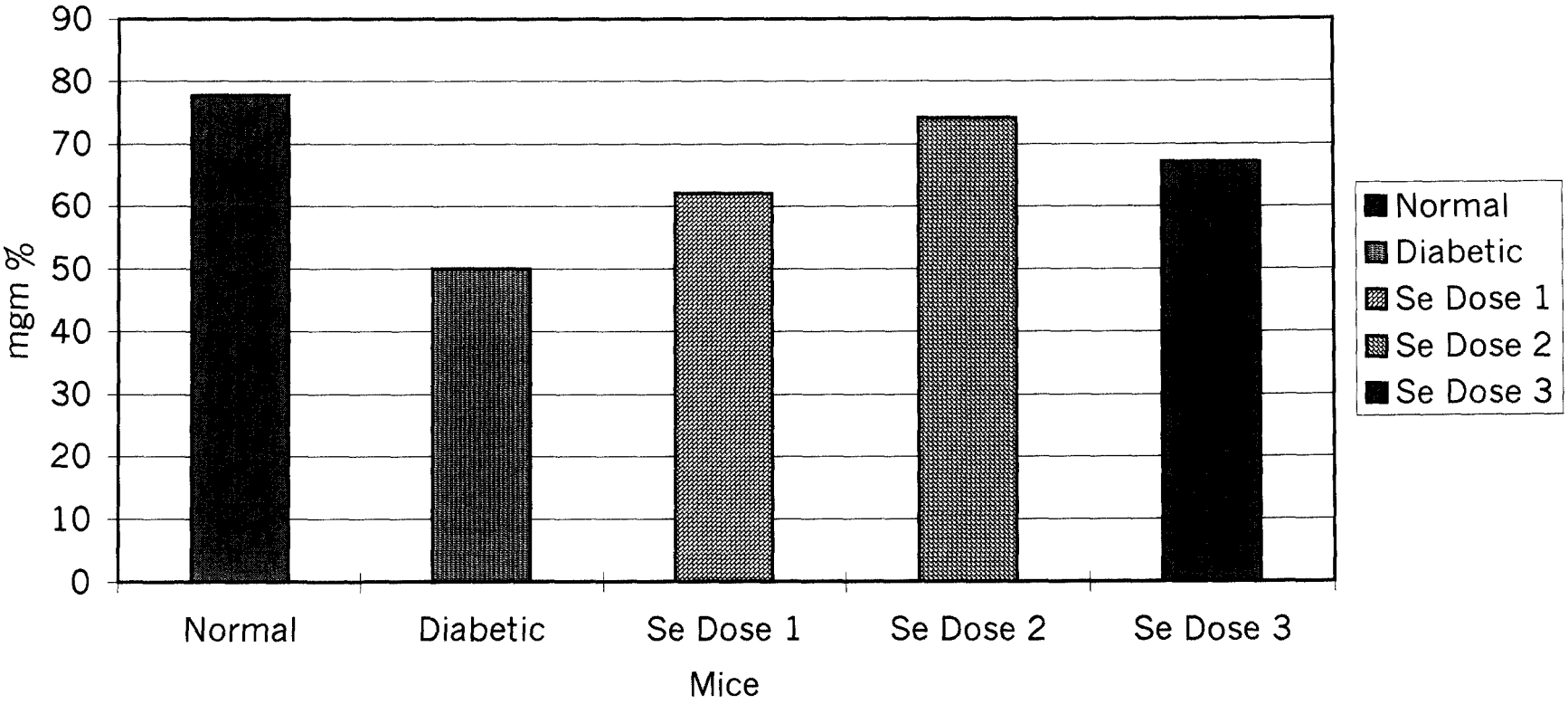


Fig. 12 : Changes of Lactic acid in liver on Selenium treatment.

5.1.7 Experiment related to Succinic dehydrogenase in liver :

Experiment 13 : Changes of Succinic dehydrogenase in liver on Selenium treatment :

This experiment was designed to note the changes of succinic dehydrogenase in liver with changes of doses of Selenium in Streptozotocin induced diabetic mice. All the values are expressed in multiples of 10^{-5} micromoles of Indophenol reduced/microgm of protein. The normal value in adult mice was 6.18 ± 0.42. In diabetic mice it was 7.78 ± 1.22. In Selenium Dose-1 fed mice the level of Succinic dehydrogenase in liver was 7.23 ± 0.59. In Dose-2 it was 6.64 ± 0.37 and in Dose-3 it was 6.97 ± 0.55 (Table-13, Fig. 13).

Table 13 : Changes of Succinic dehydrogenase in liver on Selenium treatment :

Treatment condition	Succinic dehydrogenase level in liver in multiple of 10^{-5} micromoles of Indophenol reduced/microgm of protein	± SD	p Value with respect to normal
Normal adult mice	6.18	± 0.42	
Diabetic mice	7.78	± 1.22	
Se-Dose 1 fed diabetic mice	7.23	± 0.59	0.0140
Se-Dose 2 fed diabetic mice	6.64	± 0.37	0.1059
Se-Dose 3 fed diabetic mice	6.97	± 0.55	0.0343

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Succinic dehydrogenase in liver was increased by 25.9% in diabetic mice. Following Selenium treatment the level fell. With Dose-2 fed mice the level came almost to normal.

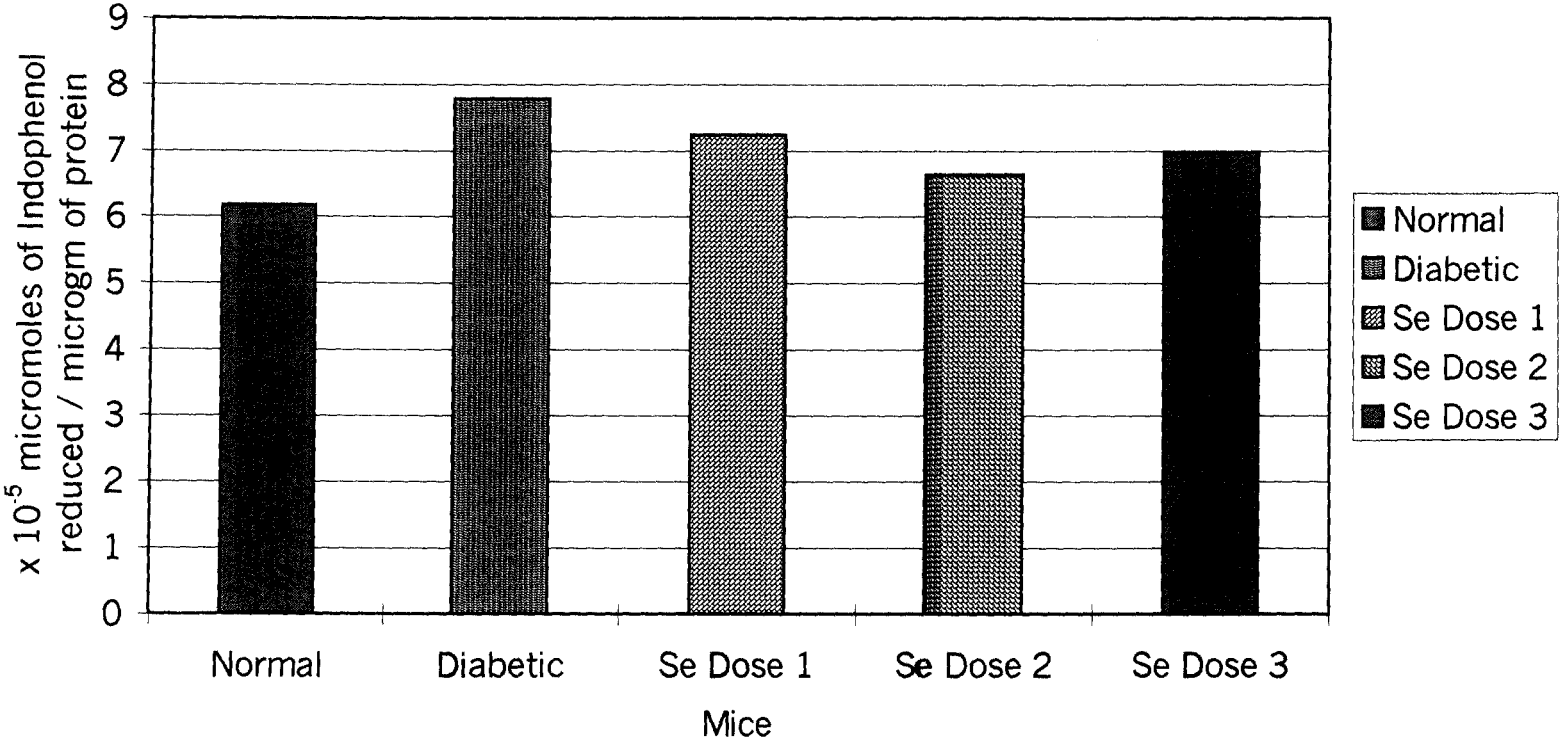


Fig. 13 : Changes of Succinic dehydrogenase in liver on Selenium treatment.

5.1.8 Experiment related to Lactate dehydrogenase in serum :

Experiment 14 : Changes of Lactate dehydrogenase in serum on Selenium treatment :

This experiment was designed to estimate the changes of Lactate dehydrogenase in serum of diabetic mice on changes of doses of Selenium feeding with respect to Selenium feeding in normal mice. In Dose-1 experiment both the normal group and the experimentally induced diabetic group were fed with 0.5 microgm/0.1 ml of Selenium. Changes were assayed at 2nd, 7th, 14th and 60th day. Similarly in Dose-2 experiment the amount of Selenium fed to both normal and diabetic group were 0.05 microgm/0.1 ml. In the 3rd group of experiments Selenium Dose-3 were fed to both normal and diabetic groups. The estimations were made at 2nd, 7th, 14th and 60th day as Dose-1 experiments. Following are the results (Table-14, Fig. 14).

Table 14 : Changes of lactate dehydrogenase in serum on Selenium treatment :

Treatment condition		Lactate dehydrogenase level in serum on different days in International unit			
		2nd day	7th day	14th day	60th day
Normal	- 1220				
Diabetic	- 1460				
Se-Dose 1 :	Normal	1260	1470	3400	6200
	Diabetic	2500	2900	7800	12,000
Se-Dose 2 :	Normal	1040	1200	4240	5200
	Diabetic	2080	2400	5120	10,000
Se-Dose 3 :	Normal	1030	1180	3400	4800
	Diabetic	1060	2400	5300	9500

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The table shows that the value in diabetic group becomes almost double the normal level. Only in 14th day of Dose-2 and Dose-3 the values raised less than the double.

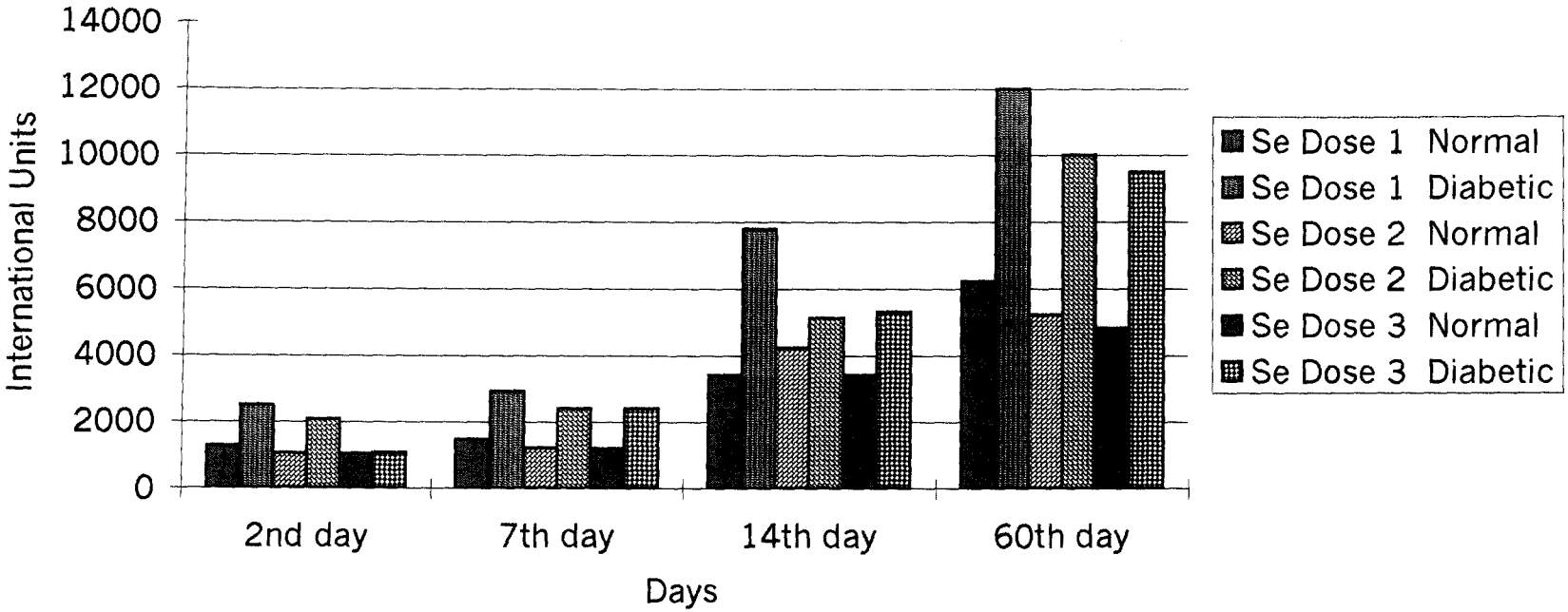


Fig. 14 : Changes of lactate dehydrogenase in serum on Selenium treatment.

5.2 Lipid Metabolism :

A couple of independent experiments were performed to find out the effect of various doses of Selenium treatment on different aspects of lipid metabolism in diabetic mice. The experimental results of each of the experiment thus described seperately below.

5.2.1 Experiments related to blood cholesterol :

Experiment 15 : Changes of blood cholesterol on Selenium treatment :

This experiment was done to asses the changes of blood cholesterol in experimentally induced diabetic mice with changes of doses of Selenium. In the normal adult mice the level of blood cholesterol was 86.97 ± 2.82 mgm%. In Streptozotocin induced diabetic mice the level was 103.47 ± 2.53 mgm%. In Selenium Dose-1 fed group the level fell to 97.79 ± 2.94 mgm%. In Dose-2 fed group the level fell even further to become 84.28 ± 2.04 mgm%. In the Dose-3 fed group the level was 91.95 ± 2.35 mgm% (Table-15, Fig. 15).

Table 15 : Changes of blood cholesterol on Selenium treatment :

Treatment Condition	Blood cholesterol level mgm%	± SD	p Value with respect to normal
Normal adult mice	86.97	± 2.82	
Diabetic mice	103.47	± 2.53	
Se-Dose 1 fed diabetic mice	97.79	± 2.94	0.0003
Se-Dose 2 fed diabetic mice	84.28	± 2.04	0.1251
Se-Dose 3 fed diabetic mice	91.95	± 2.35	0.0166

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The blood cholesterol level rose to 18.9% in diabetic mice in comparison to normal mice. With Selenium treatment the level fell. In Dose-2 fed group the level came to normal level.

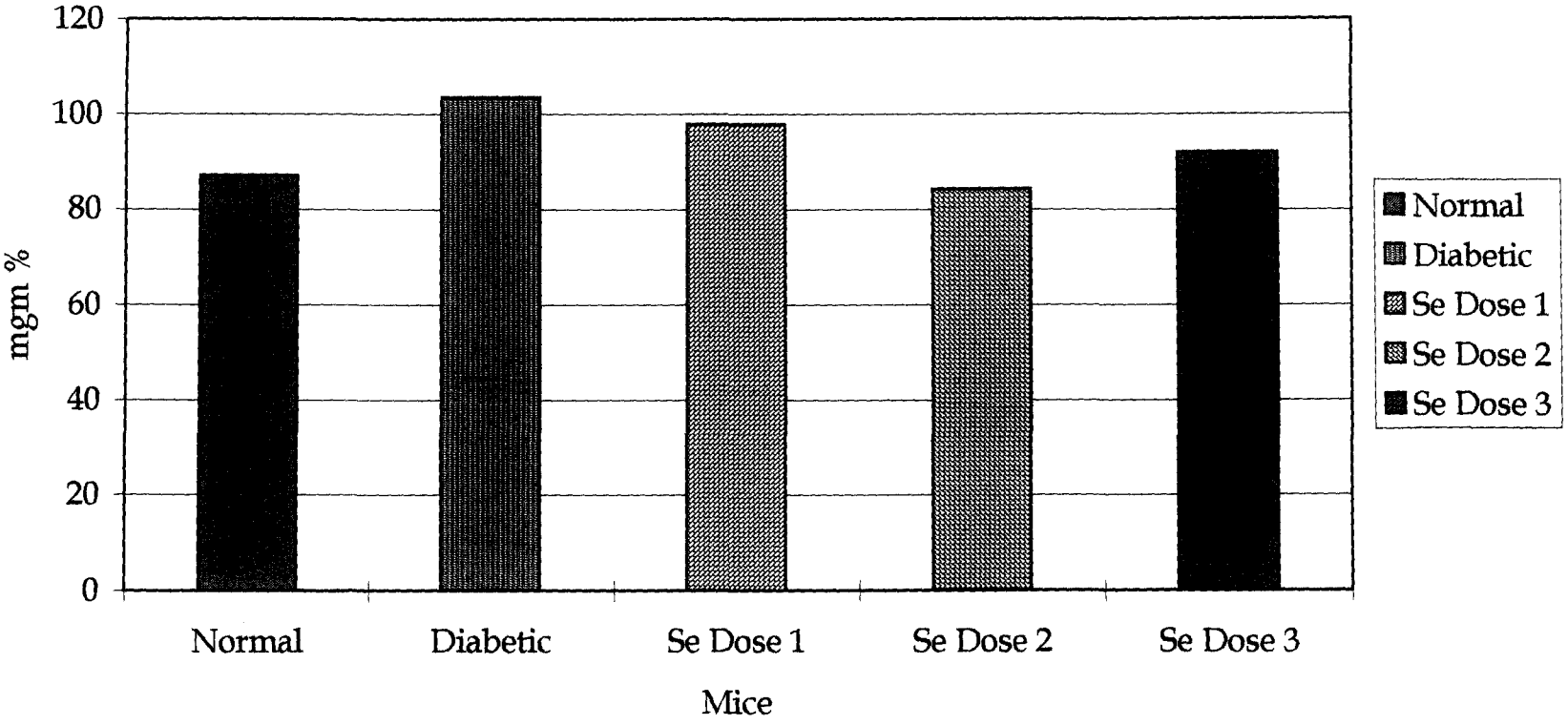


Fig. 15 : Changes of blood cholesterol on Seleninm treatment.

5.2.2 Experiment related to Serum lipoproteins :

5.2.2.1 LDL :

Experiment 16 : Changes of Serum LDL level on Selenium treatment :

This experiment was done to know the changes of serum LDL level with changes of doses of Selenium in Streptozotocin induced diabetic mice. The normal serum LDL level was 28 ± 3.16 mgm%. With induction of diabetes the level rose to 61.25 ± 4.79 mgm%. In Dose-1 fed animals the dose fell to 43.6 ± 3.36 mgm%. In Dose-2 fed mice the dose was 31.2 ± 2.28 mgm% and in Dose-3 fed mice group the level of serum LDL was 39.2 ± 2.28 mgm%. (Table-16, Fig. 16).

Table 16 : Changes of Serum LDL level on Selenium treatment :

Treatment Condition	Serum LDL level		p Value with respect to normal
	mgm%	± SD	
Normal adult mice	28	± 3.16	
Diabetic mice	61.25	± 4.79	
Se-Dose 1 fed diabetic mice	43.6	± 3.36	0.0001
Se-Dose 2 fed diabetic mice	31.2	± 2.28	0.1075
Se-Dose 3 fed diabetic mice	39.2	± 2.28	0.0003

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Srum LDL rose more than twice the normal value in experimentally induced diabetic mice. With Selenium feeding the LDL level gradually came down. In Dose-2 fed animals the LDL level reached almost normal level.

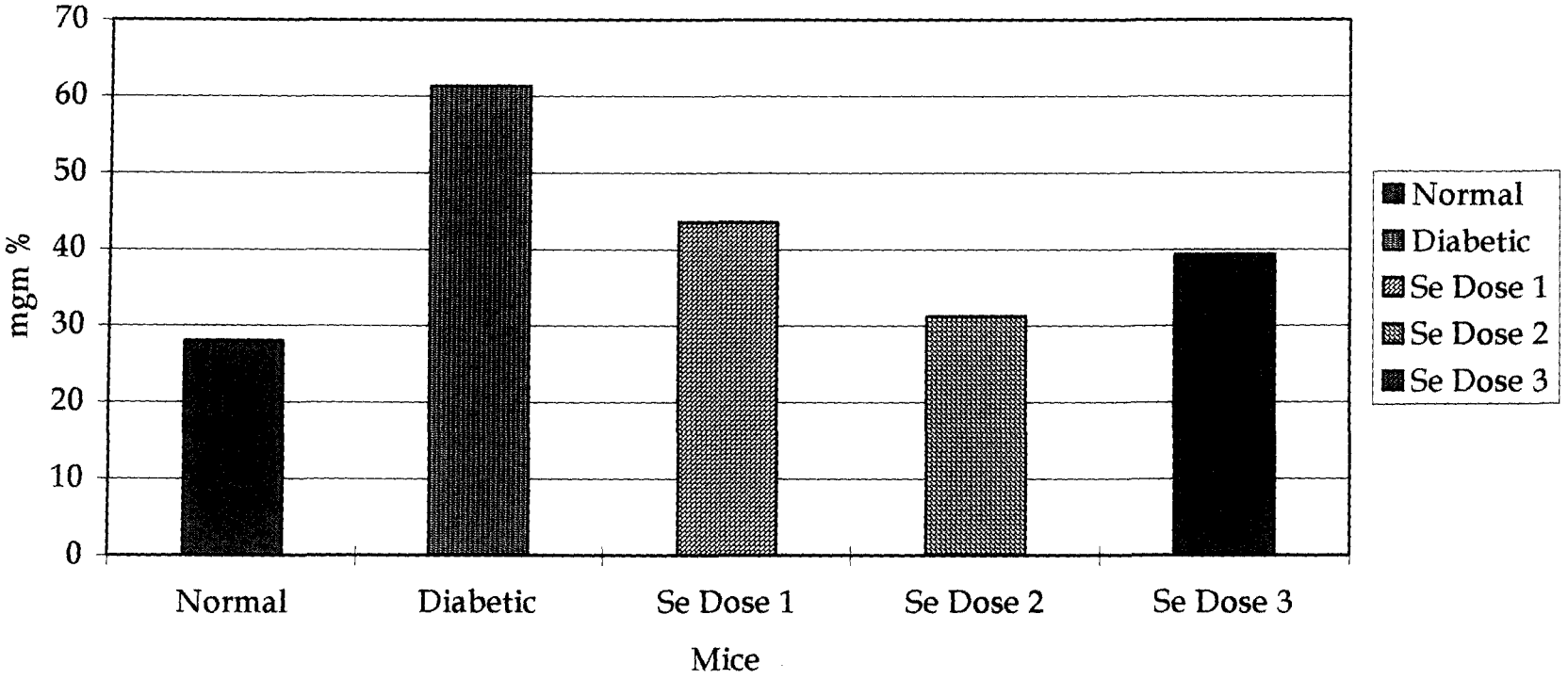


Fig. 16 : Changes of Serum LDL level on Selenium treatment.

5.2.2.2 VLDL :

Experiment 17 : Changes of Serum VLDL level on Selenium treatment :

The experiment was done to know the changes of serum VLDL level with changes of Selenium doses in diabetic mice. The value in normal adult mice was 28.8 ± 2.59 mgm%. With induction of diabetes by intraperitoneal injection of Streptozotocin the level rose to 41.6 ± 3.78 mgm%. In Dose-1 fed animals the level of VLDL was 35.4 ± 2.30 mgm%. In Dose-2 fed mice the level was 30.2 ± 2.68 mgm% and in Dose-3 fed mice the level was 33.4 ± 3.36 mgm% (Table-17, Fig. 17).

Table 17 : Changes of Serum VLDL level on selenium treatment :

Treatment Condition	Serum VLDL level mgm%	± SD	p Value with respect to normal
Normal adult mice	28.8	± 2.59	
Diabetic mice	41.6	± 3.78	
Se-Dose 1 fed diabetic mice	35.4	± 2.30	0.0028
Se-Dose 2 fed diabetic mice	30.2	± 2.68	0.4255
Se-Dose 3 fed diabetic mice	33.4	± 3.36	0.0435

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The Serum VLDL level rose 44.4% than the normal value with production of diabetes. With Selenium treatment the level of VLDL fell and in Dose-2 fed animals the level was almost normal.

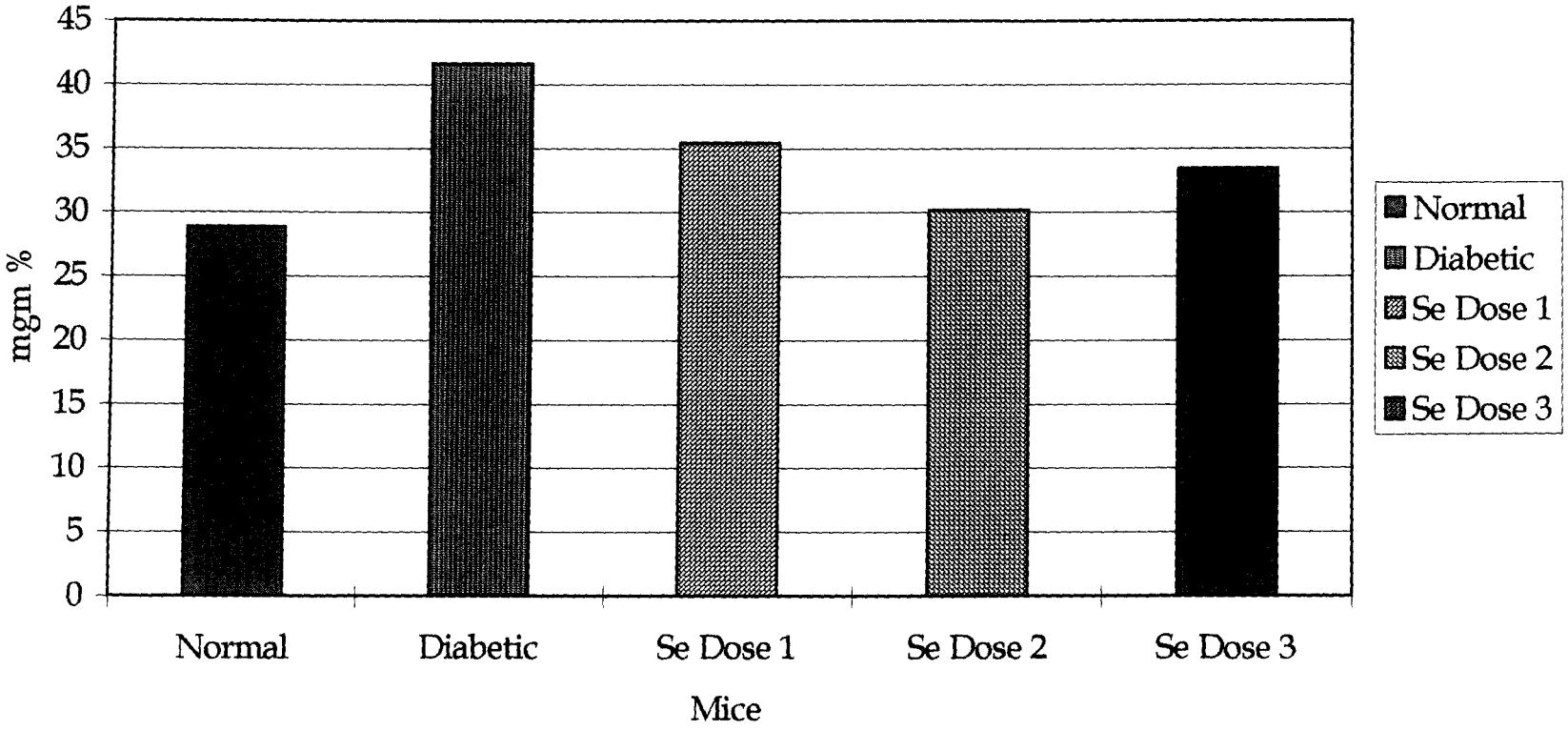


Fig. 17 : Changes of Serum VLDL level on selenium treatment.

5.2.2.3 HDL :

Experiment 18 : Changes of Serum HDL level on Selenium treatment :

This experiment was done to assess the changes of Serum HDL level on changing the 3 different doses of Selenium in experimentally induced diabetic mice. The normal HDL level in adult mice was 53.8 ± 1.79 mgm%. In diabetic mice the level fell to 26 ± 3.54 mgm%. In Dose-1 Selenium fed mice the level was 38.2 ± 2.77 mgm%. In Dose-2 the level was 51.8 ± 2.39 mgm% and in Dose-3 the level was 46 ± 1.22 mgm% (Table-18, Fig. 18).

Table 18 : Changes of Serum HDL level on Selenium treatment :

Treatment Condition	Serum HDL level		p Value with respect to normal
	mgm%	± SD	
Normal adult mice	53.8	± 1.79	
Diabetic mice	26	± 3.54	
Se-Dose 1 fed diabetic mice	38.2	± 2.77	0.0000
Se-Dose 2 fed diabetic mice	51.8	± 2.39	0.1752
Se-Dose 3 fed diabetic mice	46	± 1.22	0.0001

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Serum HDL was halved by induction of diabetes. With Selenium treatment the level rose and in Dose-2 fed animals the level approched near normal value.

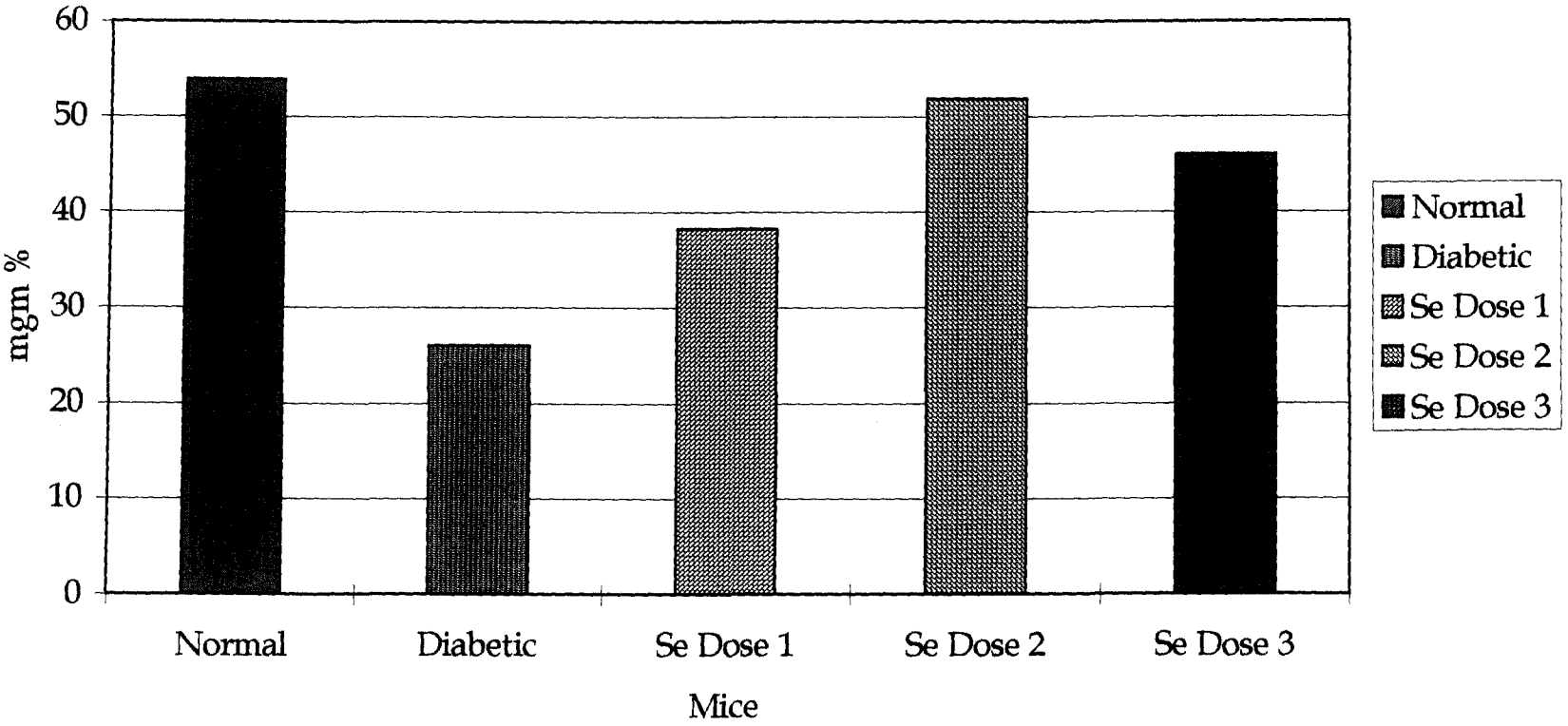


Fig. 18 : Changes of Serum HDL level on Selenium treatment.

5.2.3 Experiments related to triglyceride :

5.2.3.1 Serum :

Experiment 19 : Changes of Serum triglyceride level on Selenium treatment :

This experiment was done to understand the changes of serum triglyceride level in diabetic mice on changing the doses of Selenium. The normal level in adult mice was 200 ± 14.14 mgm%. The level rose to 1425 ± 64.55 mgm% in Streptozotocin induced diabetic mice. With Selenium feeding the level started falling. In Dose-1 the level was 917.5 ± 66.52 mgm% and in Dose-2 the level was 450 ± 34.64 mgm% and in Dose-3 the level of serum triglyceride was 657.5 ± 80.98 mgm% (Table-19, Fig. 19).

Table 19 : Changes of Serum triglyceride level on Selenium treatment :

Treatment Condition	Serum triglyceride level mgm%	± SD	p Value with respect to normal
Normal adult mice	200	± 14.14	
Diabetic mice	1425	± 64.55	
Se-Dose 1 fed diabetic mice	917.5	± 66.52	0.0001
Se-Dose 2 fed diabetic mice	450	± 34.64	0.0002
Se-Dose 3 fed diabetic mice	657.5	± 80.98	0.0012

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Serum triglyceride was increased by 7 times in diabetic mice. Selenium feeding lowered the level and Dose-2 was the most effective in reducing the level of serum triglyceride.

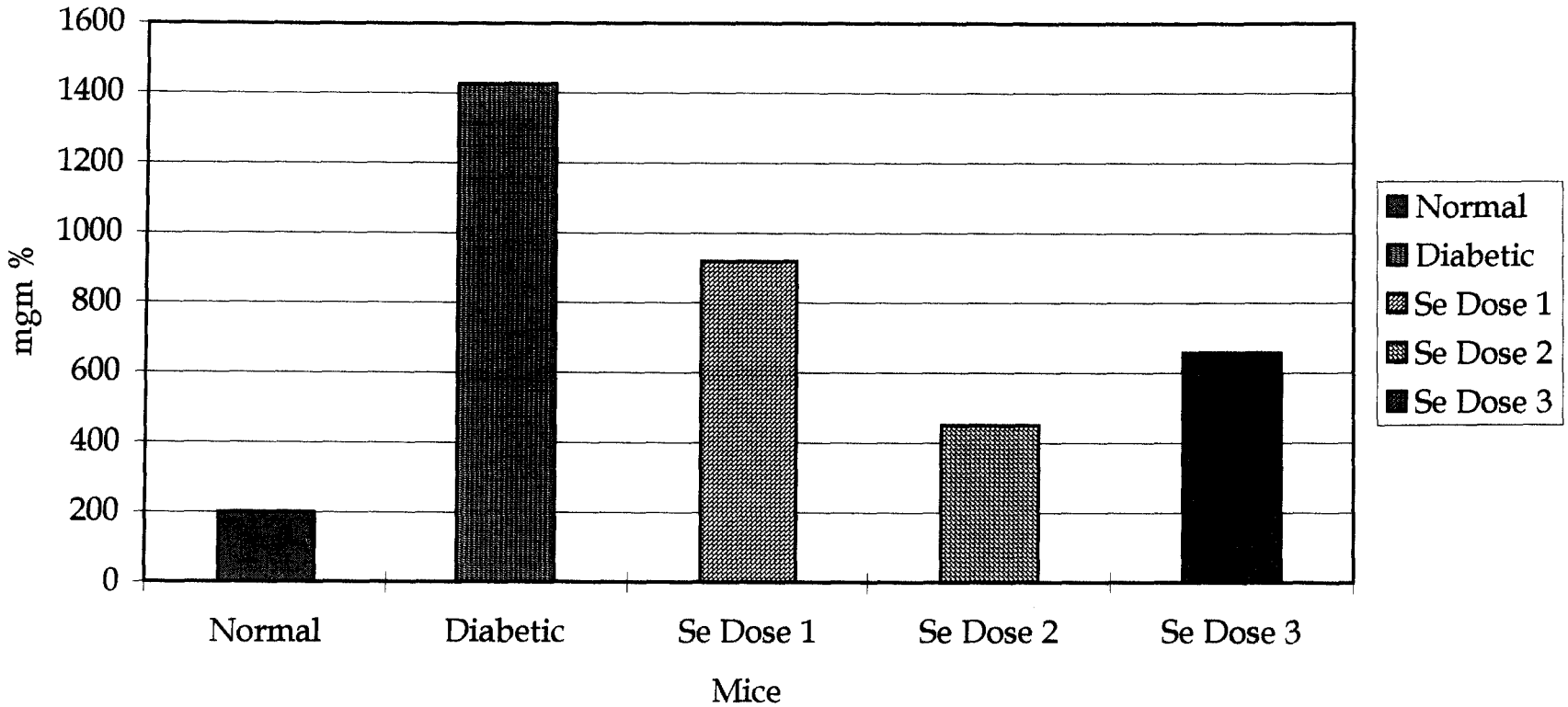


Fig. 19 : Changes of Serum triglyceride level on Selenium treatment.

5.2.3.2 Liver :

Experiment 20 : Changes of liver triglyceride level on Selenium treatment :

This experiment was done to evaluate the changes of liver triglyceride level in Streptozotocin induced diabetic mice with changes of doses of Selenium. The normal value was 810 ± 41.63 mgm%. With induction of diabetes the level fell to 115 ± 19.15 mgm%. With Selenium feeding the level rose. In Dose-1 fed mice the level was 522.5 ± 33.04 mgm%. In Dose-2 fed mice the level was 717.5 ± 23.63 mgm% and in Dose-3 fed mice the level was 630 ± 35.59 mgm% (Table-20, Fig. 20).

Table 20 : Changes of liver triglyceride level on Selenium treatment :

Treatment Condition	Liver triglyceride level mgm%	± SD	p Value with respect to normal
Normal adult mice	810	± 41.63	
Diabetic mice	115	± 19.15	
Se-Dose 1 fed diabetic mice	522.5	± 33.04	0.0001
Se-Dose 2 fed diabetic mice	717.5	± 23.63	0.0130
Se-Dose 3 fed diabetic mice	630	± 35.59	0.0007

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of liver triglyceride was diminished by 7 times on induction of diabetes. With Selenium treatment the level started rising and in Dose-2 fed animals the level of liver triglyceride reached almost normal level.

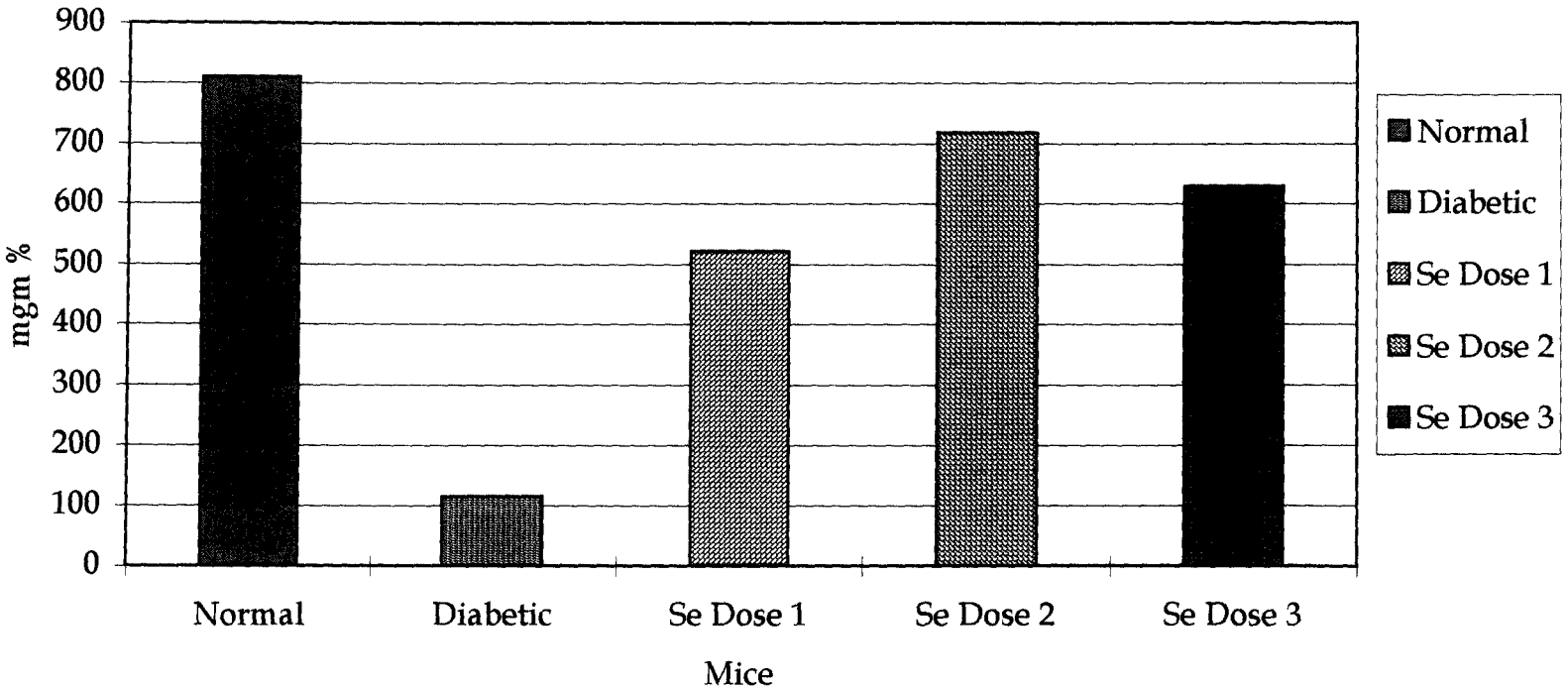


Fig. 20 : Changes of liver triglyceride level on Selenium treatment.

5.2.3.3 Vanadium and Selenium treatment :

Experiment 21 : Changes of serum triglyceride level on Vanadium and Selenium feeding :

This experiment was designed to understand the changes of serum triglyceride level on Vanadium and Selenium feeding in Streptozotocin induced diabetic mice. The normal serum triglyceride level in adult mice was 200 ± 15.81 mgm%. The level in mice fed with Vanadium was 101 ± 7.42 mgm%. Vanadium was fed to a group of mice made diabetic by injection Streptozotocin. The level of serum triglyceride was 2190 ± 151.66 mgm%. Selenium was fed to normal and diabetic mice. The levels were 99 ± 7.42 and 386 ± 25.10 mgm% respectively. Similarly to another two groups of normal and diabetic mice both Vanadium and Selenium were fed. The levels of serum triglyceride were 100 ± 7.91 and 900 ± 79.06 mgm% respectively (Table-21, Fig. 21).

Table 21 : Changes of serum triglyceride level on Vanadium and Selenium feeding :

Treatment Condition		Serum triglyceride level mgm%	± SD	p Value with respect to normal
Normal adult mice		200	± 15.81	
Vanadium Fed :	Normal	101	± 7.42	0.00002
	Diabetic	2190	± 151.66	0.00001
Selenium Fed :	Normal	99	± 7.42	0.00002
	Diabetic	386	± 25.10	0.00000
Vanadium & Selenium Fed :	Normal	100	± 7.91	0.00002
	Diabetic	900	± 79.06	0.00002

Se Dose 2 = 0.05 micro gm/0.1 ml

Vanadium dose = 0.05 micro gm/0.1 ml

This experiment shows that in 3 groups of Vanadium, Selenium and Vanadium & Selenium fed normal mice the serum triglyceride level falls to half the normal level. But in diabetic groups Vanadium alone failed to reduce the triglyceride level. Selenium alone with Dose-2 is most effective in reducing the level. Also in the Vanadium and Selenium fed group the level is not satisfactorily reduced.

So Selenium Dose-2 is more effective insulin mimetic agent than Vanadium in respect to lowering of serum triglyceride level.

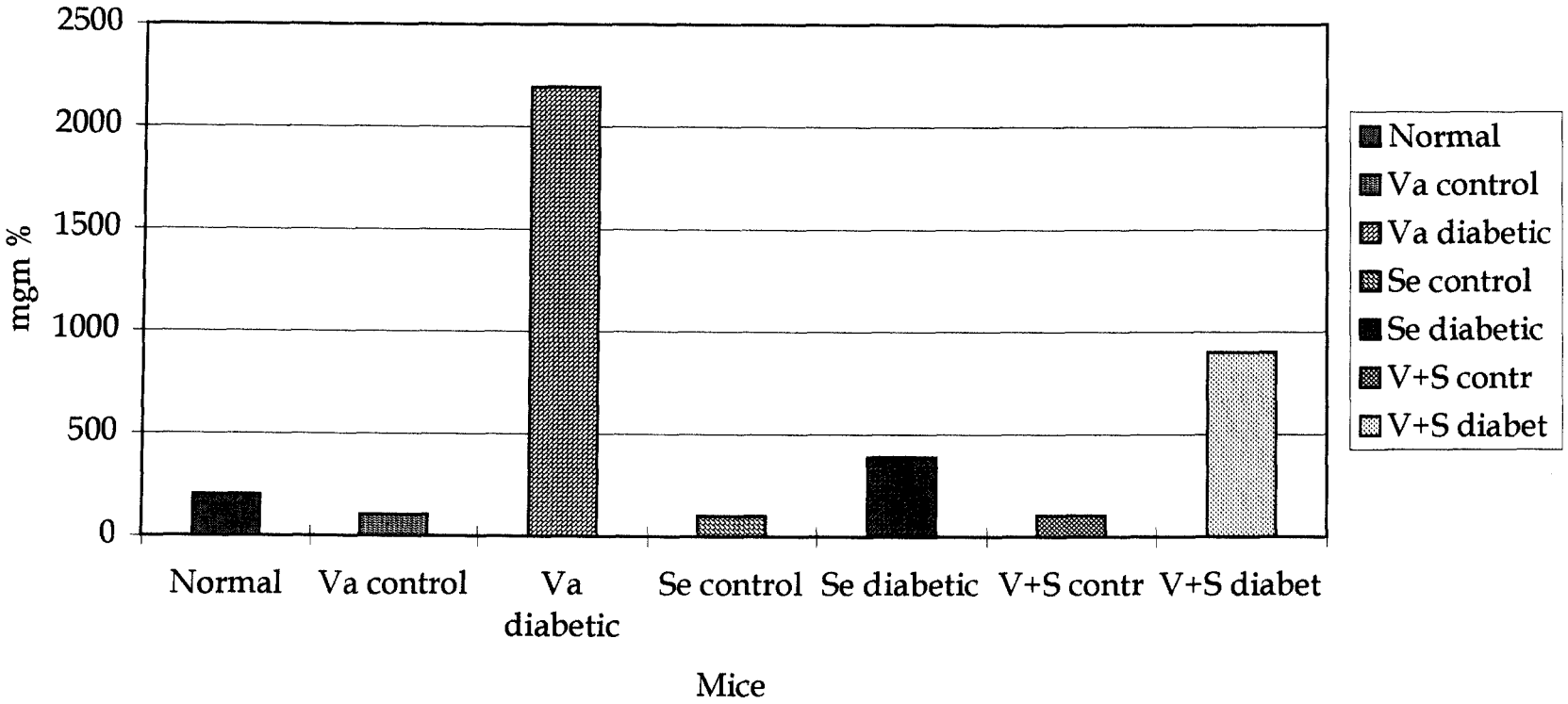


Fig. 21 : Changes of serum triglyceride level on Vanadium and Selenium feeding.

5.2.4 Experiment related to HMG-CoA reductase activity in liver :

Experiment 22 : Changes of HMG-CoA reductase activity in liver on Selenium treatment :

This experiment was done to assess the changes of HMG-CoA reductase activity in liver in experimentally induced diabetic mice with changes of doses of Selenium feeding. The values were expressed as activity in p moles/min/mgm of protein. The activity in normal adult mice was 123 ± 5.29. The activity fell to 95 ± 4.47 in diabetic mice. In Selenium Dose-1 fed mice the activity was 100 ± 5.83. In Dose-2 fed animals the activity was 116.4 ± 6.39 and in Dose-3 fed mice the acitvity was 108.4 ± 6.54 (Table-22, Fig. 22).

Table 22 : Changes of HMG-CoA reductase activity in liver on Selenium treatment :

Treatment Condition	Liver triglyceride level		p Value with respect to normal
	Activity in p moles/ min/mgm of protein	± SD	
Normal adult mice	123	± 5.29	
Diabetic mice	95	± 4.47	
Se-Dose 1 fed diabetic mice	100	± 5.83	0.0002
Se-Dose 2 fed diabetic mice	116.4	± 6.39	0.1144
Se-Dose 3 fed diabetic mice	108.4	± 6.54	0.0051

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The activity of HMG-CoA reductase in liver was reduced by 22.8% in diabetic mice. With Selenium feeding the activity started rising. In Dose-2 fed animals the activity neared the normal activity.

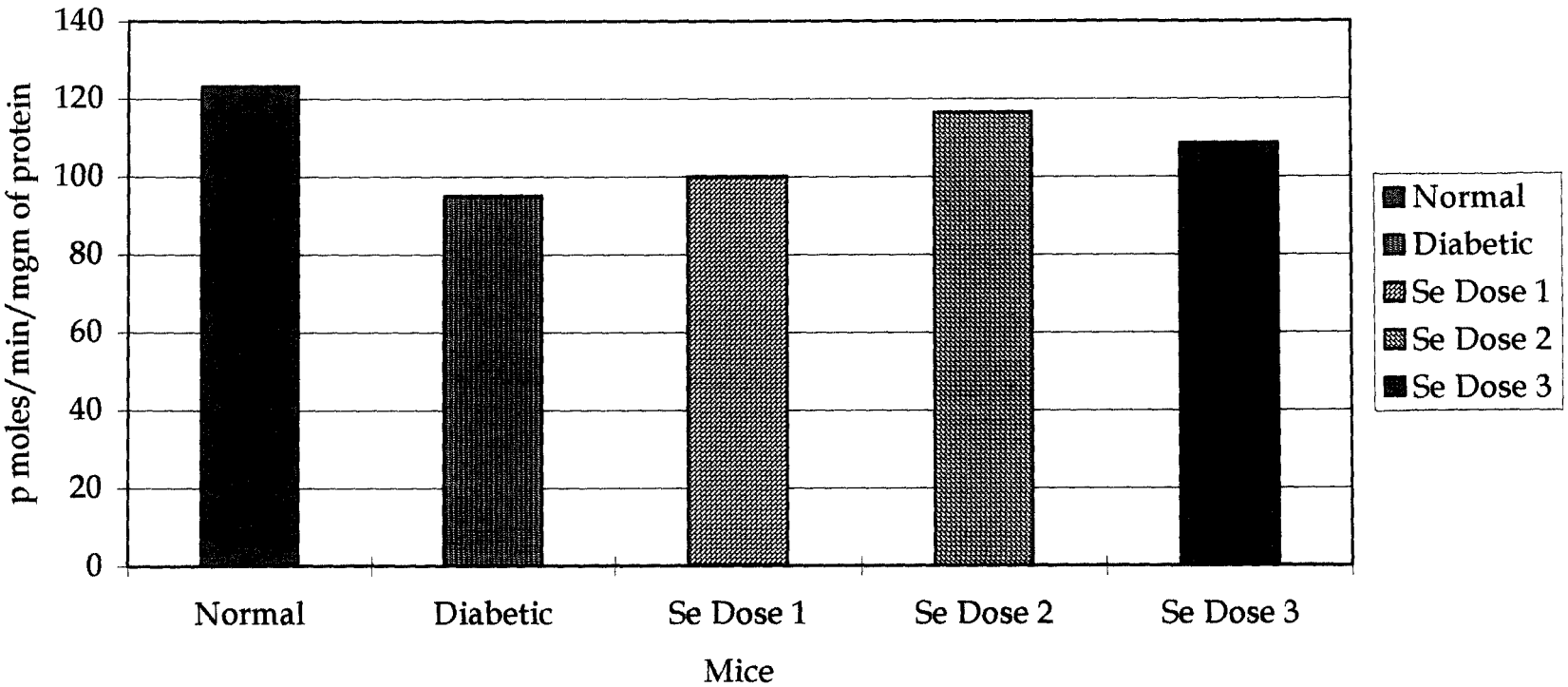


Fig. 22 : Changes of HMG-CoA reductase activity in liver on Selenium treatment.

5.3 Detoxicating microsomal enzymes :

A couple of independent experiments were performed to find out the effect of various doses of Selenium treatment on different detoxicating microsomal enzymes in diabetic mice. The experimental results of each of the experiment thus described seperately below.

5.3.1 Experiments related to Glutathione :

5.3.1.1 Liver :

Experiment 23 : Changes of Glutathione level in liver on Selenium treatment :

This experiment was designed to understand the changes of Glutathione level in liver in diabetic mice with changing the different doses of Selenium feeding. The normal level was 37.75 ± 6.24 microgm/ml. The level in Streptozotocin induced diabetic mice was 52.5 ± 9.98 microgm/ml. In Dose-1 fed mice group the level was 49.75 ± 9.64 microgm/ml. In Dose-2 fed group the level was 35.38 ± 2.87 microgm/ml and in Dose-3 fed group the level was 39.75 ± 6.24 microgm/ml (Table-23, Fig. 23)

Table 23 : Changes of Glutathione level in liver on Selenium treatment :

Treatment Condition	Glutathione level in liver		p Value with respect to normal
	microgm/ml	± SD	
Normal adult mice	37.75	± 6.24	
Diabetic mice	52.5	± 9.98	
Se-Dose 1 fed diabetic mice	49.75	± 9.64	0.0894
Se-Dose 2 fed diabetic mice	35.38	± 2.87	0.5253
Se-Dose 3 fed diabetic mice	39.75	± 6.24	0.6662

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Glutathione in liver was raised by 39.1% in diabetic mice. With Selenium feeding the level started falling and in Dose-2 fed group the level was almost normal.

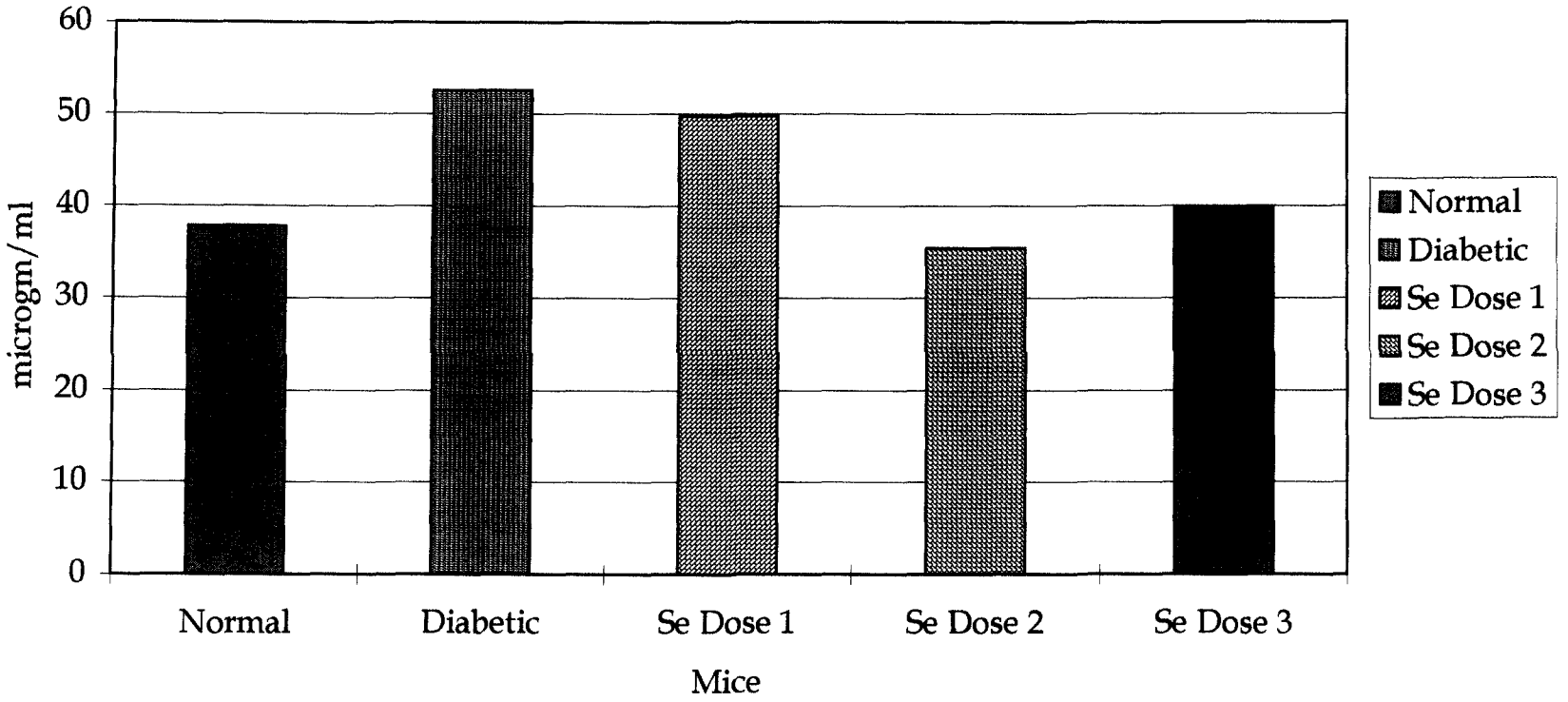


Fig. 23 : Changes of Glutathione level in liver on Selenium treatment.

5.3.1.2 Blood :

Experiment 24 : Changes of Glutathione level in blood on Selenium treatment :

This experiment was done to evaluate the changes of glutathione level in blood in Streptozotocin induced diabetic mice on changes with the doses of Selenium feeding. The normal level in adult mice was 22.25 ± 8.42 microgm/ml. The level in diabetic mice was 12.5 ± 5.80 microgm/ml. The level in Dose-1 fed group was 15.5 ± 7 microgm/ml. The level in Dose-2 fed group was 20.5 ± 8.54 microgm/ml. and in Dose-3 fed group the level of Glutathione was 17.25 ± 7.37 microgm/ml. (Table-24, Fig. 24).

Table 24 : Changes of Glutathione level in blood on Selenium treatment :

Treatment Condition	Glutathione level in blood microgm/ml	± SD	p Value with respect to normal
Normal adult mice	22.25	± 8.42	
Diabetic mice	12.5	± 5.80	
Se-Dose 1 fed diabetic mice	15.5	± 7	0.2652
Se-Dose 2 fed diabetic mice	20.5	± 8.54	0.7803
Se-Dose 3 fed diabetic mice	17.25	± 7.37	0.4064

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Glutathione in blood in diabetic mice fell to almost half the normal level. With Selenium feeding the level started rising and in Dose-2 fed group it was almost normal.

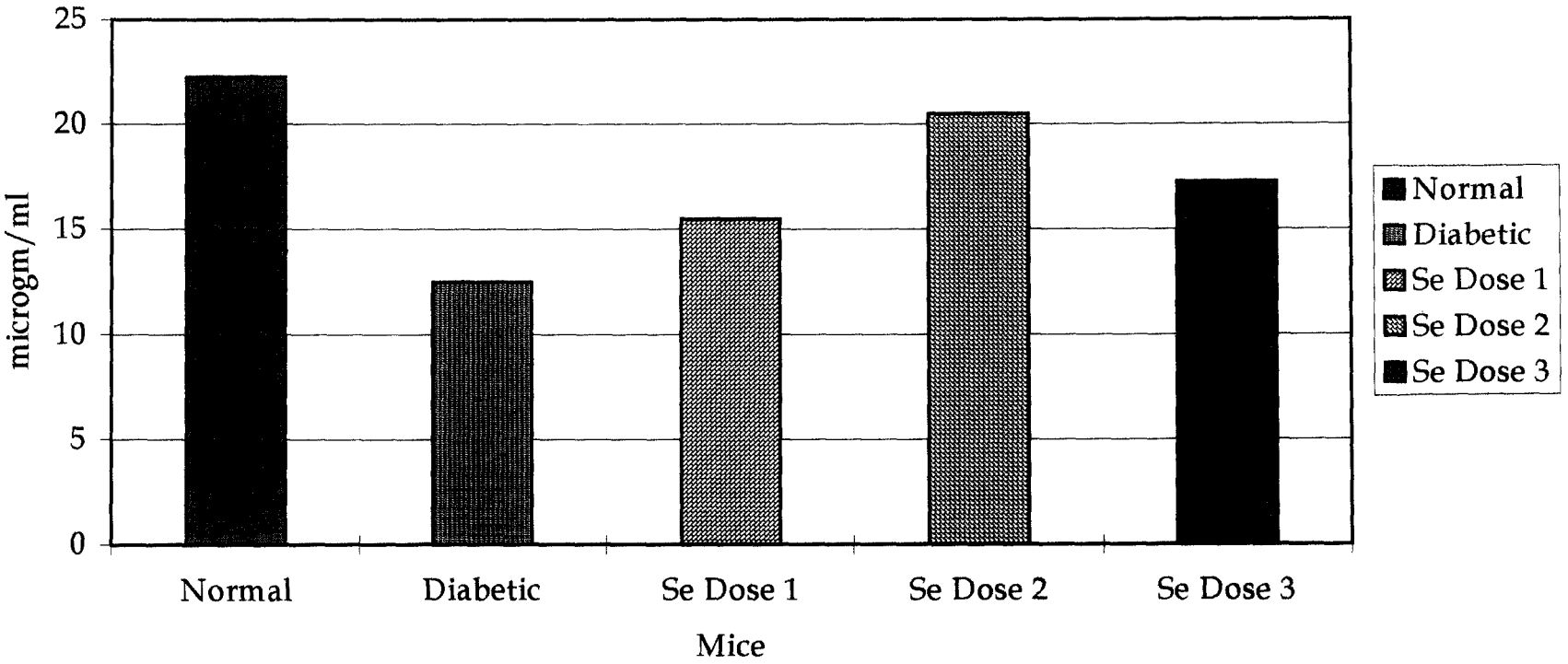


Fig. 24 : Changes of Glutathione level in blood on Selenium treatment.

5.3.2 Experiment related to Glutathione reductase in liver :

Experiment 25 : Changes of Glutathione reductase level in liver on Selenium treatment :

This experiment was done to assess the changes of Glutathione reductase in liver on Selenium treatment with variation of doses in Streptozotocin induced diabetic mice. The normal level of Glutathione reductase was expressed in micromol NADPH oxidised/min/mgm of protein. The normal level was 42.08 ± 1.93. The level in diabetic mice was 33.3 ± 2.05. The level in Dose-1 fed group was 36 ± 2.35. In Dose-2 fed group the level was 41.5 ± 1.87 and in Dose-3 fed group the level was 38 ± 2 (Table-25, Fig. 25).

Table 25 : Changes of Glutathione reductase level in liver on Selenium treatment :

Treatment Condition	Glutathione reductase in liver		p Value with respect to normal
	micromol NADPH oxidised/ min/mgm of protein	± SD	
Normal adult mice	42.08	± 1.93	
Diabetic mice	33.3	± 2.05	
Se-Dose 1 fed diabetic mice	36	± 2.35	0.0023
Se-Dose 2 fed diabetic mice	41.5	± 1.87	0.6420
Se-Dose 3 fed diabetic mice	38	± 2	0.0111

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Glutathione reductase in liver fell by 20.9% in diabetic mice. With Selenium feeding the level rose. In Dose-2 fed animals the level approached the normal level.

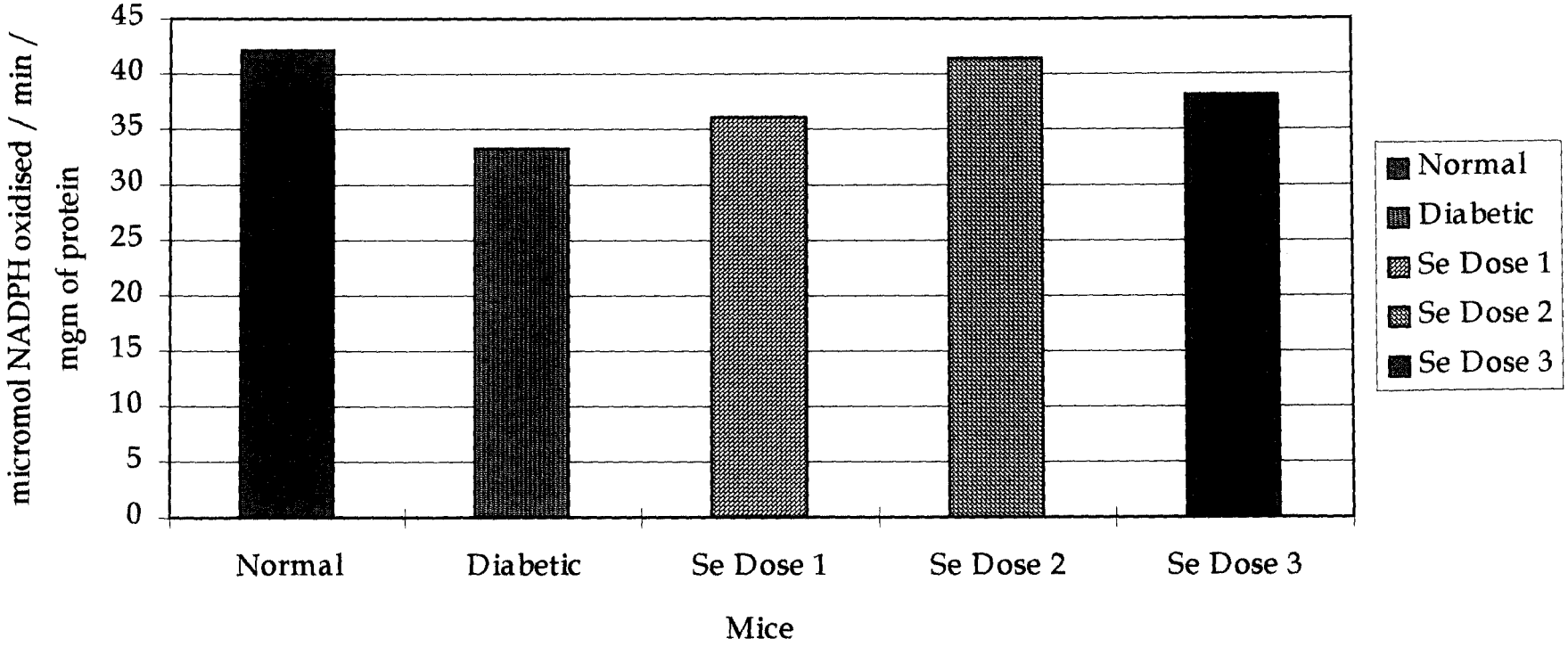


Fig. 25 : Changes of Glutathione reductase level in liver on Selenium treatment.

5.3.3 Experiment related to Glutathione-s-transferase in liver :

Experiment 26 : Changes of Glutathione-s-transferase level in liver on Selenium treatment :

This experiment was designed to understand the changes of Glutathione-s-transferase level in liver in Streptozotocin induced diabetic mice with changes of doses of Selenium feeding. The level of Glutathione-s-transferase was expressed in Unit activity of enzyme/mgm of protein/ minute. The normal level was 0.356 ± 0.12. The level in diabetic mice was 0.43 ± 0.16. The level in Dose-1, 2 & 3 fed groups were 0.464 ± 0.16, 0.5 ± 0.16 and 0.53 ± 0.15 respectively (Table-26, Fig. 26).

Table 26 : Changes of Glutathione-s-transferase level in liver on Selenium treatment :

Treatment Condition	Glutathione-s-transferase level in liver		p Value with respect to normal
	Unit activity of enzyme/ mgm of protein/min	± SD	
Normal adult mice	0.356	± 0.12	
Diabetic mice	0.43	± 0.16	
Se-Dose 1 fed diabetic mice	0.464	± 0.16	0.2657
Se-Dose 2 fed diabetic mice	0.5	± 0.16	0.1486
Se-Dose 3 fed diabetic mice	0.53	± 0.15	0.0839

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Glutathione-s-transferase in liver rose with production of diabetes and with Selenium treatment the level continued to rise.

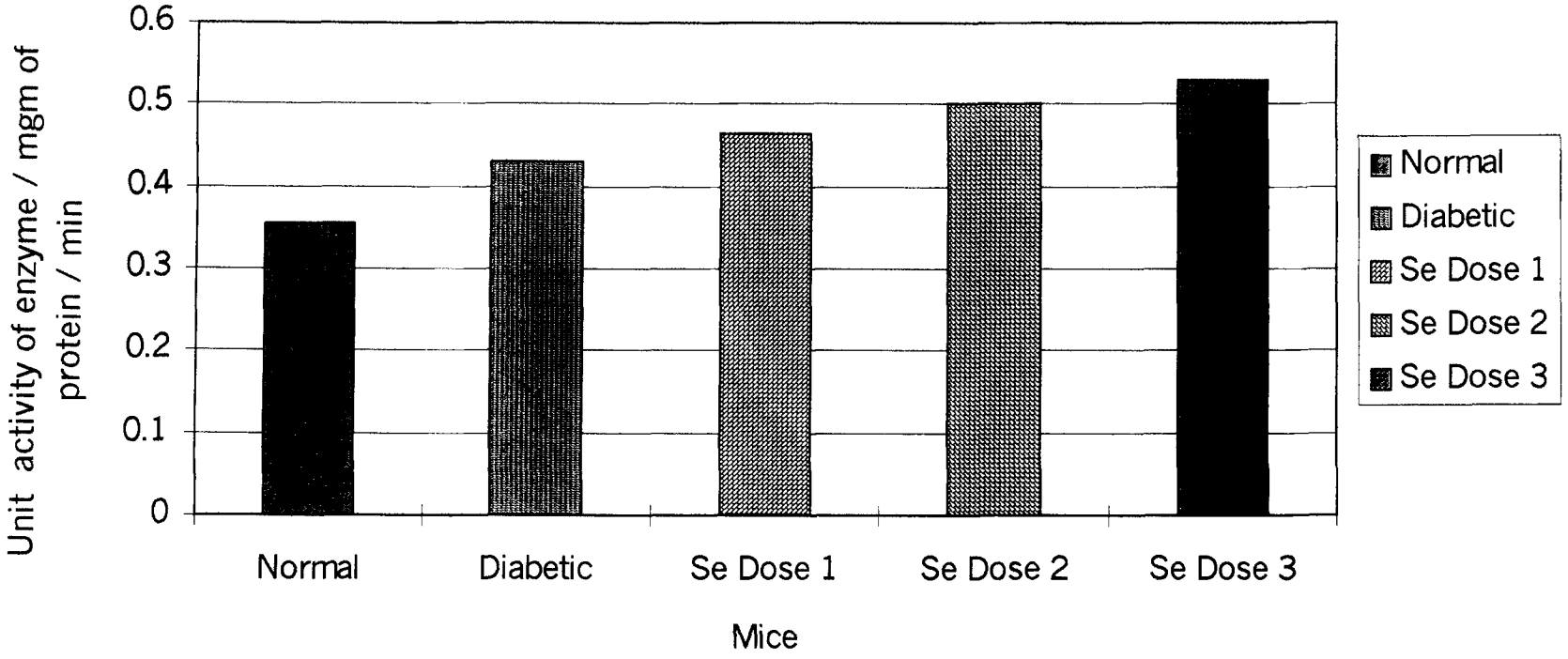


Fig. 26 : Changes of Glutathione-s-transferase level in liver on Selenium treatment.

5.3.4 Experiment related to hepatic UDP-Glucoronyl transferase activity :

Experiment 27 : Changes of hepatic UDP-Glucoronyl transferase activity on Selenium treatment :

This experiment was done to understand the changes of hepatic UDP-Glucoronyl transferase activity in diabetic mice on varying the doses of Selenium treatment. The activity of UDP-Glucoronyl transferase was expressed in Unit of activity/mgm of protein. The normal level was 2.36 ± 0.21. The level in diabetic group was 2.7 ± 0.29. The level in Selenium Dose-1 fed group was 2.8 ± 0.29. The level in Dose-2 fed group was 2.94 ± 0.29 and the level in Dose-3 fed group of mice the level was 3.06 ± 0.27 (Table-27, Fig. 27).

Table 27 : Changes of hepatic UDP-Glucoronyl transferase activity on Selenium treatment :

Treatment Condition	Hepatic UDP-Glucoronyl transferse level		p Value with respect to normal
	Unit of activity/mgm of protein	± SD	
Normal adult mice	2.36	± 0.21	
Diabetic mice	2.7	± 0.29	
Se-Dose 1 fed diabetic mice	2.8	± 0.29	0.0276
Se-Dose 2 fed diabetic mice	2.94	± 0.29	0.0076
Se-Dose 3 fed diabetic mice	3.06	± 0.27	0.0021

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of hepatic UDP-Glucoronyl transferase rose on induction of diabetes and on Selenium treatment went on rising.

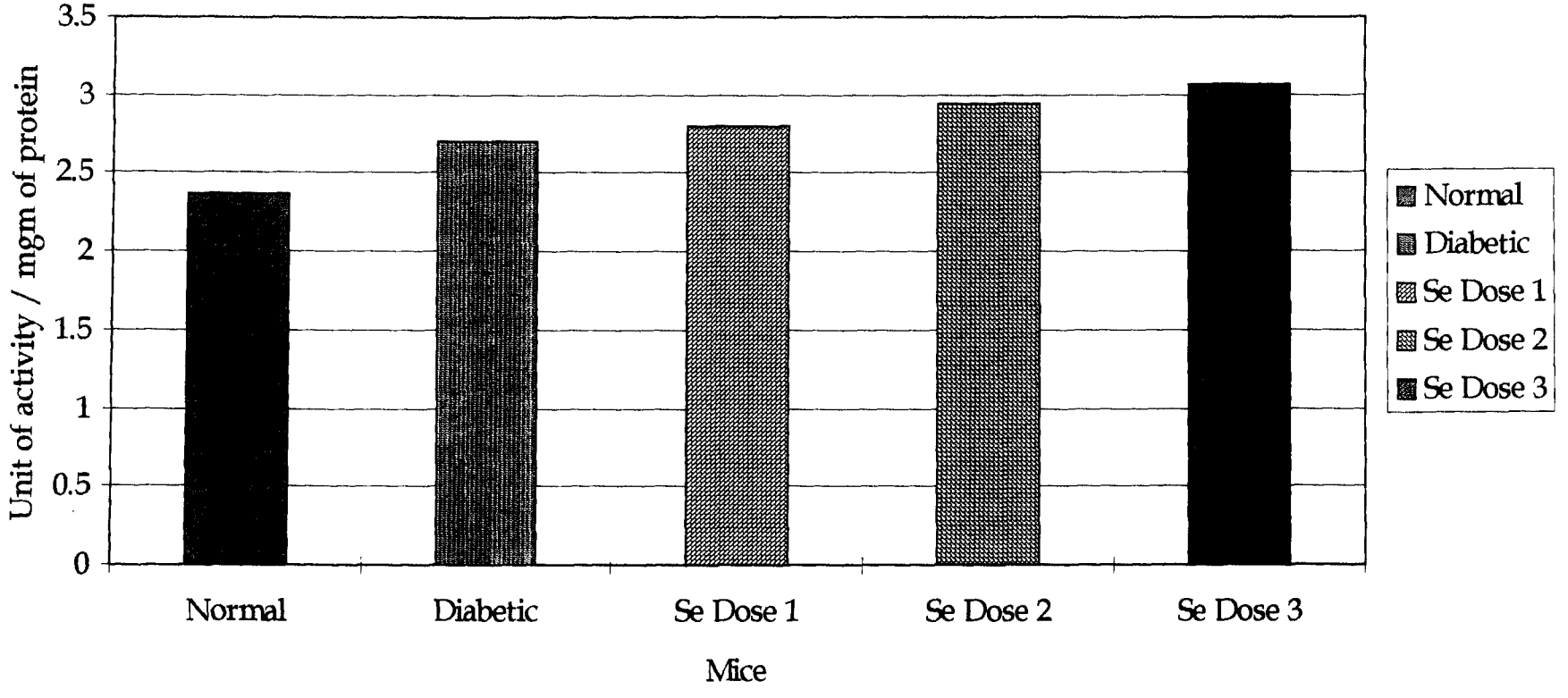


Fig. 27 : Changes of hepatic UDP-Glucoronyl transferase activity on Selenium treatment.

5.4 Peroxidation :

A couple of independent experiments were performed to find out the effect of various doses of Selenium treatment on different aspects of peroxidation in diabetic mice. The experimental results of each of the experiment thus described seperately below.

5.4.1 Experiments related to lipid peroxidation :

5.4.1.1 Liver :

Experiment 28 : Changes of level of lipid peroxidation in liver on Selenium treatment :

This experiment was done to understand the changes of level of lipid peroxidation in liver in diabetic mice on changing the doses of Selenium treatment. Lipid peroxidation was expressed in terms of nmoles of Malondialdehyde per gm of tissue. The normal level was 8.84 ± 2.72. The level in diabetic mice was 49.58 ± 2.06. The level in Dose-1, Dose-2 and Dose-3 fed mice groups were 36.02 ± 1.91, 13.58 ± 3.19 and 26.58 ± 2.43 respectively (Table-28, Fig. 28).

Table 28 : Changes of level of lipid peroxidation in liver on Selenium treatment :

Treatment Condition	Lipid peroxidation level in liver nmoles of Malondialdehyde/ gm of tissue	± SD	p Value with respect to normal
Normal adult mice	8.84	± 2.72	
Diabetic mice	49.58	± 2.06	
Se-Dose 1 fed diabetic mice	36.02	± 1.91	0.0000
Se-Dose 2 fed diabetic mice	13.58	± 3.19	0.0360
Se-Dose 3 fed diabetic mice	26.58	± 2.43	0.0000

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of lipid peroxidation in liver rose by six times in diabetic mice. With Selenium treatment the level started falling and in Dose-2 fed group it approached the normal level.

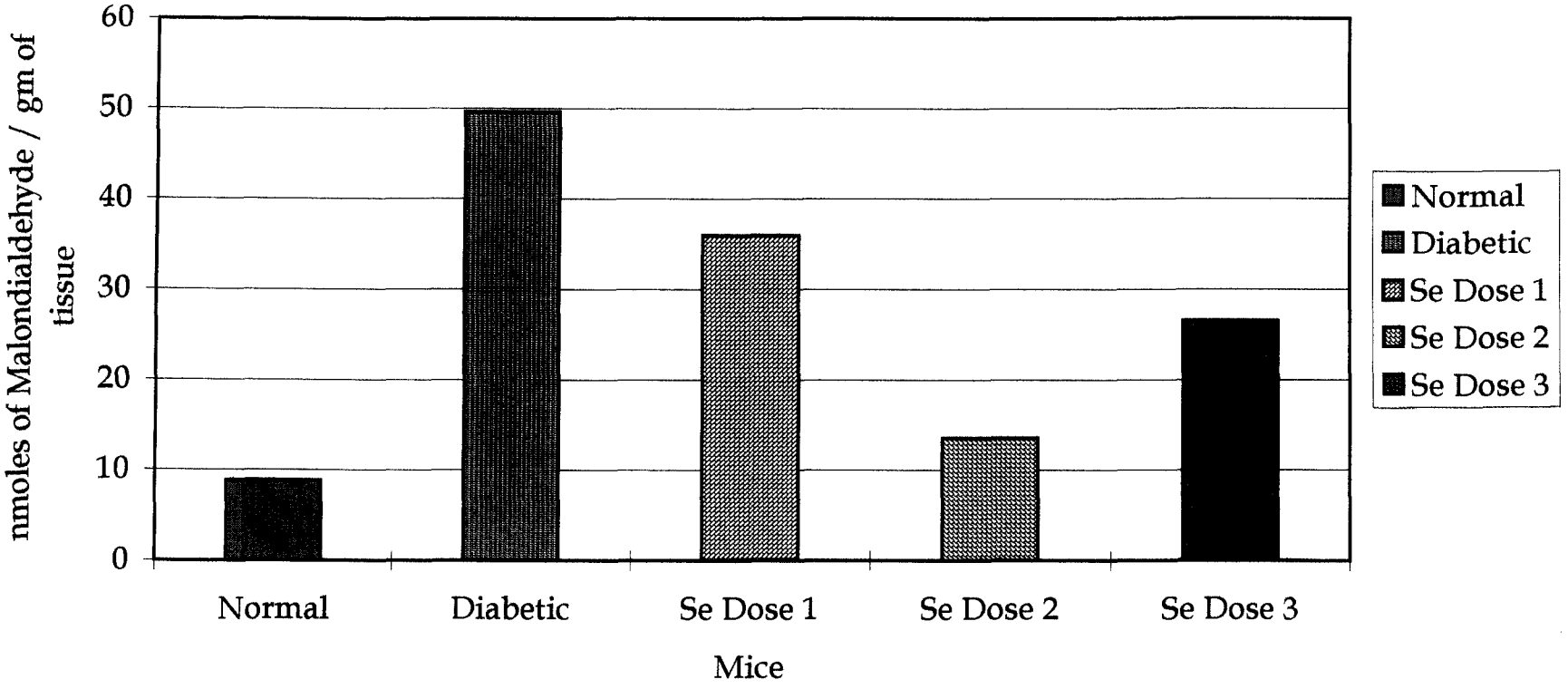


Fig. 28 : Changes of level of lipid peroxidation in liver on Selenium treatment.

5.4.1.2 Brain :

Experiment 29 : Changes of level of lipid peroxidation in brain on Selenium treatment :

This experiment was done to note the changes of level of lipid peroxidation in brain in diabetic mice on changing the doses of Selenium feeding. Lipid peroxidation was expressed in terms of nmoles of Malondialdehyde/gm of tissue. The normal level in adult mice was 13.32 ± 0.74. In diabetic mice the level was 51.46 ± 1.43. In Dose-1 fed group the level was 37.86 ± 1.84. In Dose-2 fed mice the level was 18.88 ± 0.88 and in Dose-3 fed group the level was 28.12 ± 2.04 (Table-29, Fig. 29).

Table 29 : Changes of level of lipid peroxidation in brain on Selenium treatment :

Treatment Condition	Lipid peroxidation level in brain		p Value with respect to normal
	nmoles of Malondialdehyde/ gm of tissue	± SD	
Normal adult mice	13.32	± 0.74	
Diabetic mice	51.46	± 1.43	
Se-Dose 1 fed diabetic mice	37.86	± 1.84	0.0000
Se-Dose 2 fed diabetic mice	18.88	± 0.88	0.0000
Se-Dose 3 fed diabetic mice	28.12	± 2.04	0.0000

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of lipid peroxidation in brain rose by four fold in diabetic mice. With Selenium feeding the level fell and with Dose-2 treatment the level fell almost to normal value.

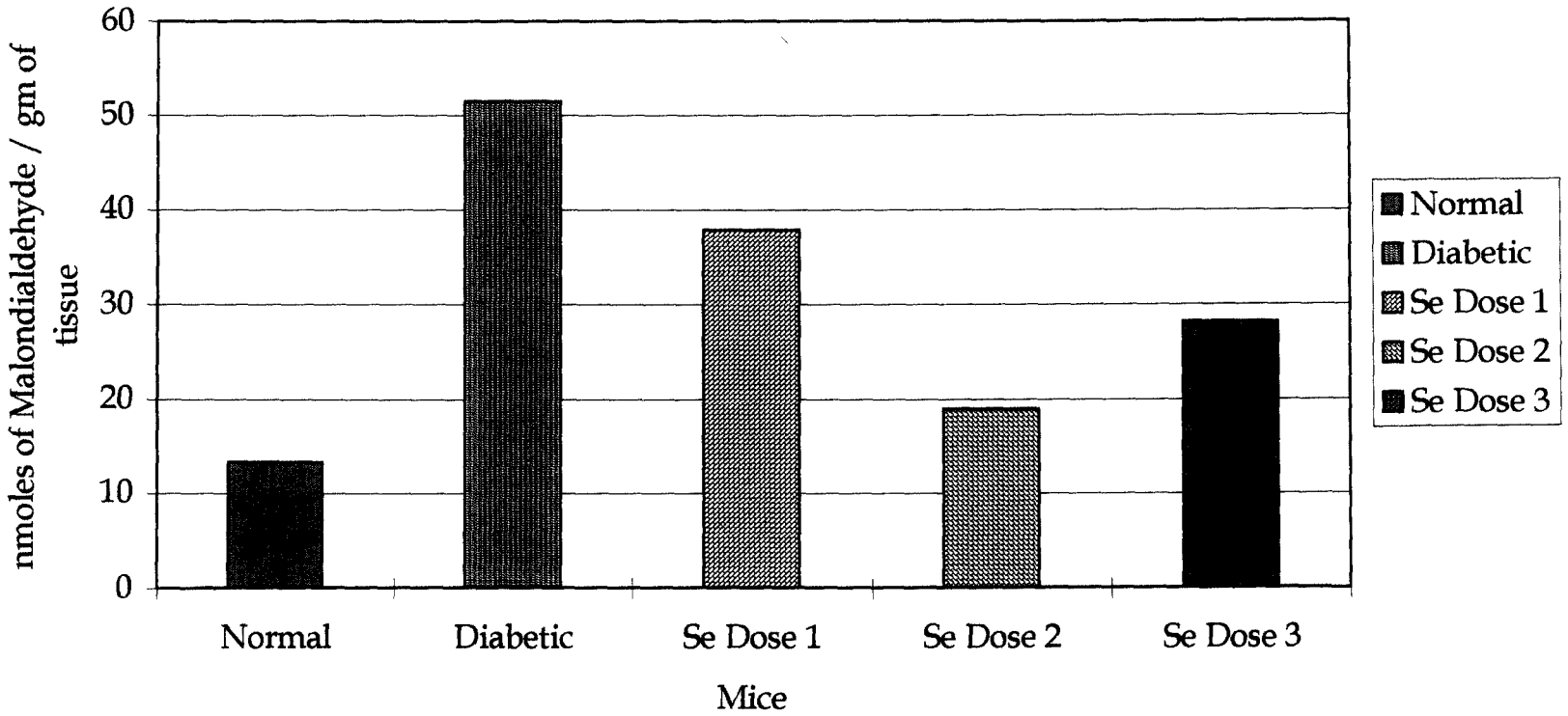


Fig. 29 : Changes of level of lipid peroxidation in brain on Selenium treatment.

5.4.1.3 Kidney :

Experiment 30 : Changes of level of lipid peroxidation in kidney on Selenium treatment :

This experiment was done to understand the changes of level of lipid peroxidation in kidney in experimentally induced diabetic mice on changing the doses of Selenium treatment. Lipid peroxidation was expressed in terms of nmoles of Malondialdehyde/gm of tissue. The normal level was 11.08 ± 1.53. The level in diabetic mice was 62.98 ± 3.57. The level in Dose-1 fed mice group was 42.94 ± 1.71. The level in Dose-2 fed group was 13.88 ± 0.93 and the level in Dose-3 fed group was 24.225 ± 1.33 (Table-30, Fig. 30)

Table 30 : Changes of level of lipid peroxidation in kidney on Selenium treatment :

Treatment Condition	Lipid peroxidation level in kidney		p Value with respect to normal
	nmoles of Malondialdehyde/ gm of tissue	± SD	
Normal adult mice	11.08	± 1.53	
Diabetic mice	62.98	± 3.57	
Se-Dose 1 fed diabetic mice	42.94	± 1.71	0.0000
Se-Dose 2 fed diabetic mice	13.88	± 0.93	0.0109
Se-Dose 3 fed diabetic mice	24.225	± 1.33	0.0000

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of lipid peroxidation in kidney rose by five and half times in diabetic mice. With Selenium treatment the level started falling . With Dose-2 fed group the level was almost normal.

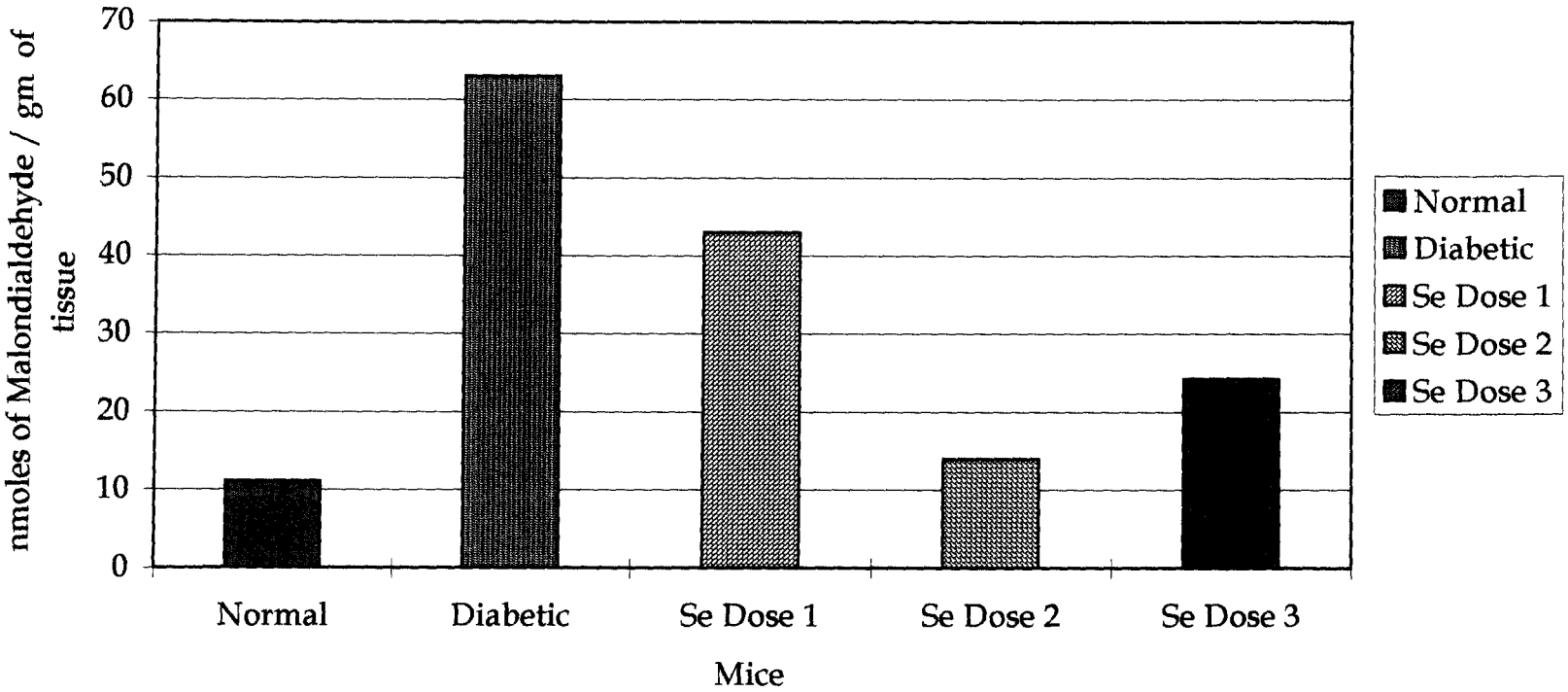


Fig. 30 : Changes of level of lipid peroxidation in kidney on Selenium treatment.

5.4.1.4 Vanadium and Selenium treatment :

Experiment 31 : Changes of liver lipid peroxidation level on Vanadium and Selenium feeding :

This experiment was designed to understand the changes of liver lipid peroxidation level on Vanadium and Selenium feeding in Streptozotocin induced diabetic mice. The level of lipid peroxidation was expressed in terms of nmoles of Malondialdehyde/gm of tissue. The normal liver lipid peroxidation level in adult mice was 48.02 ± 9.29. The level in mice fed with Vanadium was 40.6 ± 7.48. Vanadium fed to experimentally induced diabetic group had the level as 34.32 ± 4.12. Selenium was fed to normal and diabetic mice. The levels were 39.88 ± 5.32 and 35.7 ± 3.36 respectively. Similarly to another two groups of normal and diabetic mice both Vanadium and Selenium were fed. The levels of liver lipid peroxidation were 45.82 ± 8.77 and 48.24 ± 9.42 respectively (Table-31, Fig. 31).

Table 31 : Changes of liver lipid peroxidation level on Vanadium & Selenium feeding :

Treatment Condition		Liver lipid peroxidation level		p Value with respect to normal
		nmoles of Malondialdehyde/ gm of tissue	± SD	
Normal adult mice		48.02	± 9.29	
Vanadium Fed :	Normal	40.6	± 7.48	0.2035
	Diabetic	34.32	± 4.12	0.0262
Selenium Fed :	Normal	39.88	± 5.32	0.1373
	Diabetic	35.7	± 3.36	0.0383
Vanadium & Selenium Fed :	Normal	45.82	± 8.77	0.7103
	Diabetic	48.24	± 9.42	0.9713

Se Dose 2 = 0.05 micro gm/0.1 ml

Vanadium dose = 0.05 micro gm/0.1 ml

This experiment showed that the level of liver lipid peroxidation fell by one sixth part the normal value in both the Vanadium and Selenium fed normal mice groups. In both the Vanadium and Selenium fed deabetic groups the level fell by another one sixth part. Contrary to this result the level in Vanadium plus Selenium fed normal mice group only fell by a negligible amount and the level of liver lipid peroxidation came to normal in diabetic mice group.

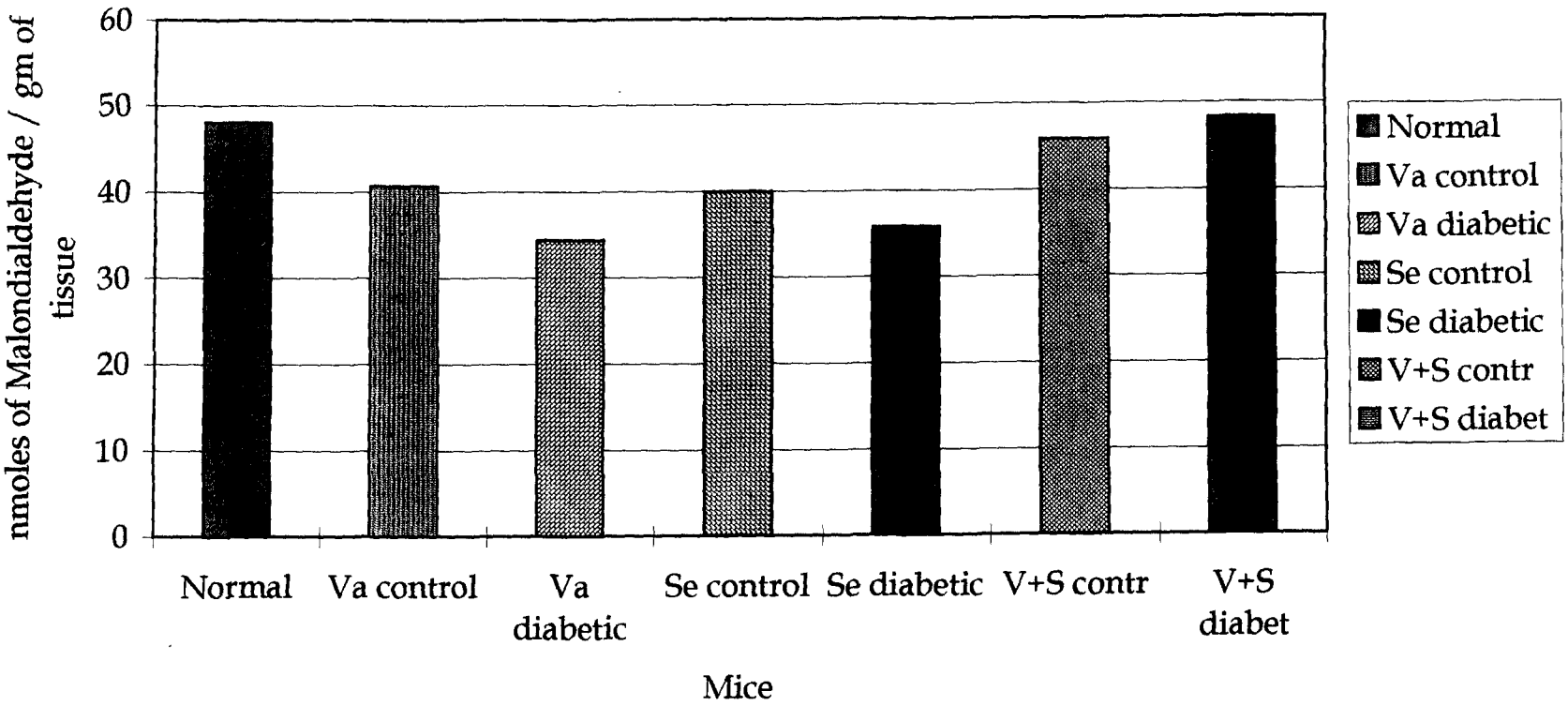


Fig. 31 : Changes of liver lipid peroxidation level on Vanadium & Selenium feeding.

5.4.2 Experiment related to catalase in liver :

Experiment 32 : Changes of catalase level in liver on Selenium treatment :

This experiment was done to note the changes of catalase level in liver in Streptozotocin induced diabetic mice with changes of doses of Selenium treatment. Catalase was expressed in 1st order reaction rate constant per minute x 10^{-3}. It was observed that the level in all the groups i.e., normal adult mice, diabetic group and the 3 different selenium fed groups had the same reading 5.06 ± 0.17 (Table-32, Fig. 32).

Table 32 : Changes of catalase level in liver on Selenium treatment :

Treatment Condition	Catalase level in liver		p Value with respect to normal
	1st order reaction rate constant/min x 10^{-3}	± SD	
Normal adult mice	5.06	± 0.17	
Diabetic mice	5.06	± 0.17	
Se-Dose 1 fed diabetic mice	5.06	± 0.17	1.0
Se-Dose 2 fed diabetic mice	5.06	± 0.17	1.0
Se-Dose 3 fed diabetic mice	5.06	± 0.17	1.0

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

This experiment showed diabetic and Selenium feeding had no effect on catalase level.

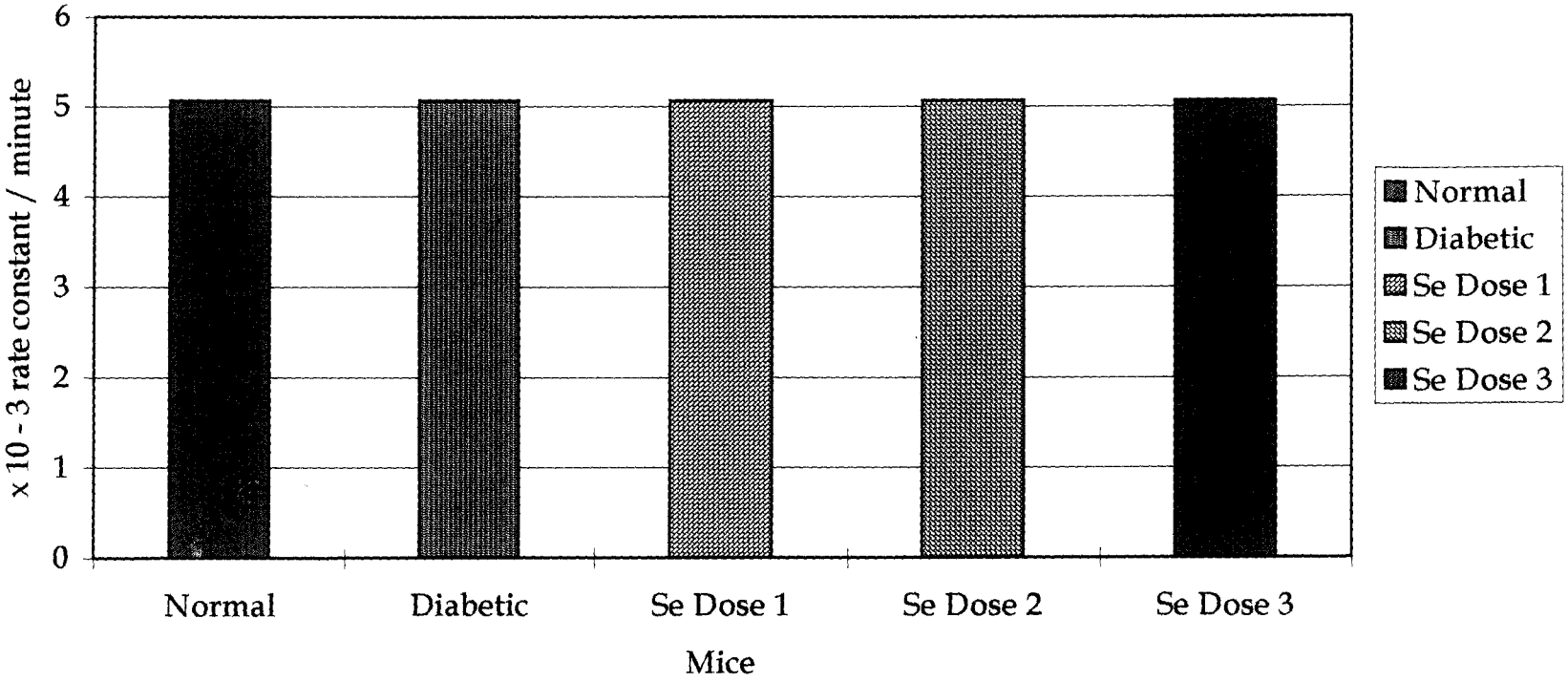


Fig. 32 : Changes of catalase level in liver on Selenium treatment.

5.4.3 Experiment related to Cyt P-450 monoxygenase in liver :

Experiment 33 : Changes of Cyt P-450 level in liver on Selenium treatment :

This experiment was done to know the effect on the changes of Cyt P-450 level in liver in experimentally induced diabetic mice on changing the doses of Selenium treatment. Cyt P-450 was expressed in micromole/mgm of protein. The level in normal adult mice was 5678.46 ± 149.17. The level in diabetic group was 2845.9 ± 207.11. The level in Dose-1 fed group was 2553.02 ± 110.52. The level in Dose-2 fed group was 2374 ± 131.56 and the level in Dose-3 fed group was 2480.46 ± 105.70 (Table-33, Fig. 33).

Table 33 : Changes of Cyt P-450 level in liver on Selenium treatment :

Treatment Condition	Cyt P-450 level in liver micromole/mgm of protein	± SD	p Value with respect to normal
Normal adult mice	5678.46	± 149.17	
Diabetic mice	2845.9	± 207.11	
Se-Dose 1 fed diabetic mice	2553.02	± 110.52	0.0000
Se-Dose 2 fed diabetic mice	2374	± 131.56	0.0000
Se-Dose 3 fed diabetic mice	2480.46	± 105.70	0.0000

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

The level of Cyt P-450 in liver fell by half in the diabetic group. With Selenium feeding the level was more decreased and in Dose-2 fed group the reading was least.

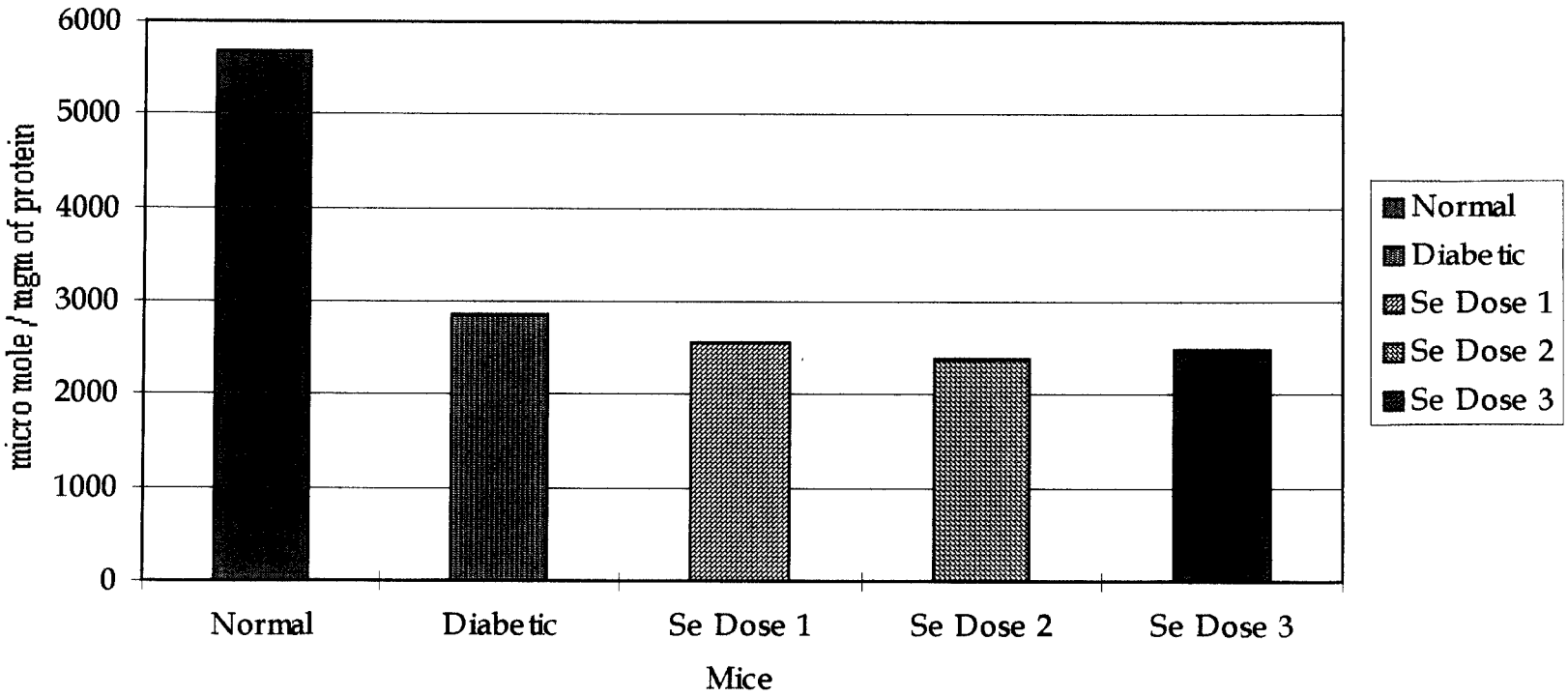


Fig. 33 : Changes of Cyt P-450 level in liver on Seleninm treatment.

5.5 Miscellaneous :

5.5.1 Experiments related to Fibrinogen in blood :

Experiment 34 : Changes of Fibrinogen level in blood on Selenium treatment :

This experiment was done to note the changes of Fibrinogen level in blood in diabetic mice on changing the different doses of Selenium feeding. The normal level in adult mice was 33.22 ± 2.80 mgm%. On production of experimentally induced diabetes the level rose to 100.23 ± 7.81 mgm%. On Selenium Dose-1 fed mice group the level was 95.75 ± 9.97 mgm%. On Dose-2 fed group the level was 89.95 ± 10.39 mgm%. On Dose-3 fed mice the level of fibrinogen in blood was 91.45 ± 9.69 mgm% (Table-34, Fig. 34).

Table 34 : Changes of Fibrinogen level in blood on Selenium treatment :

Treatment Condition	Fibrinogen level in blood mgm%	± SD	p Value with respect to normal
Normal adult mice	33.22	± 2.80	
Diabetic mice	100.23	± 7.81	
Se-Dose 1 fed diabetic mice	95.75	± 9.97	0.0550
Se-Dose 2 fed diabetic mice	89.95	± 10.39	0.0656
Se-Dose 3 fed diabetic mice	91.45	± 9.69	0.0569

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

This experiment showed that the level of fibrinogen in blood rose to 3 times in diabetic group. With Selenium treatment the level fell and in Dose-2 the level of fibrinogen fell most.

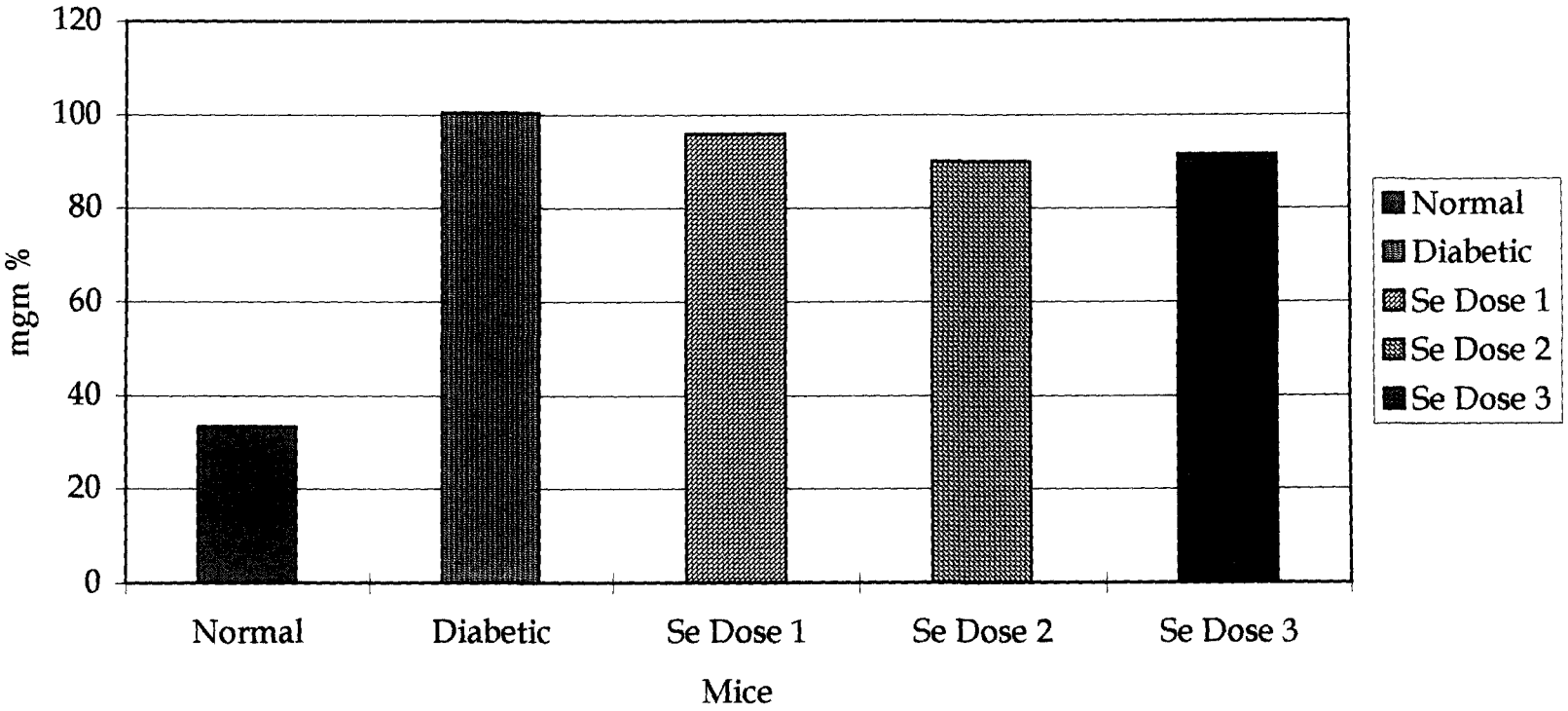


Fig. 34 : Changes of Fibrinogen level in blood on Selenium treatment.

5.5.2 Experiment related to Monoamine oxidase in serum :

Experiment 35 : Changes of Monoamine oxidase level in serum on Selenium treatment :

This experiment was done to assess the changes of Monoamine oxidase level in serum on varying the doses of Selenium feeding in diabetic mice. The level of Monoamine oxidase was expressed as Units of enzyme/mgm of protein. The normal level in adult mice was 6.28 ± 0.94. The level in diabetic mice was 9.04 ± 1.76. The level in Dose-1 fed group was 7.84 ± 1.22. The level in Dose-2 fed group was 6.9 ± 0.79 and the level in Dose-3 fed mice was 7.5 ± 0.75 (Table-35, Fig. 35).

Table 35 : Changes of Monoamine oxidase level in serum on Selenium treatment :

Treatment Condition	Monoamine oxidase level in serum		p Value with respect to normal
	Units of enzyme/ mgm of protein	± SD	
Normal adult mice	6.28	± 0.94	
Diabetic mice	9.04	± 1.76	
Se-Dose 1 fed diabetic mice	7.84	± 1.22	0.0559
Se-Dose 2 fed diabetic mice	6.9	± 0.79	0.2939
Se-Dose 3 fed diabetic mice	7.5	± 0.75	0.0553

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

This experiment showed that the level rose by 43.9% in diabetic mice. With Selenium feeding the level of Monoamine oxidase in serum fell. In Dose-2 fed group the level came almost to normal level.

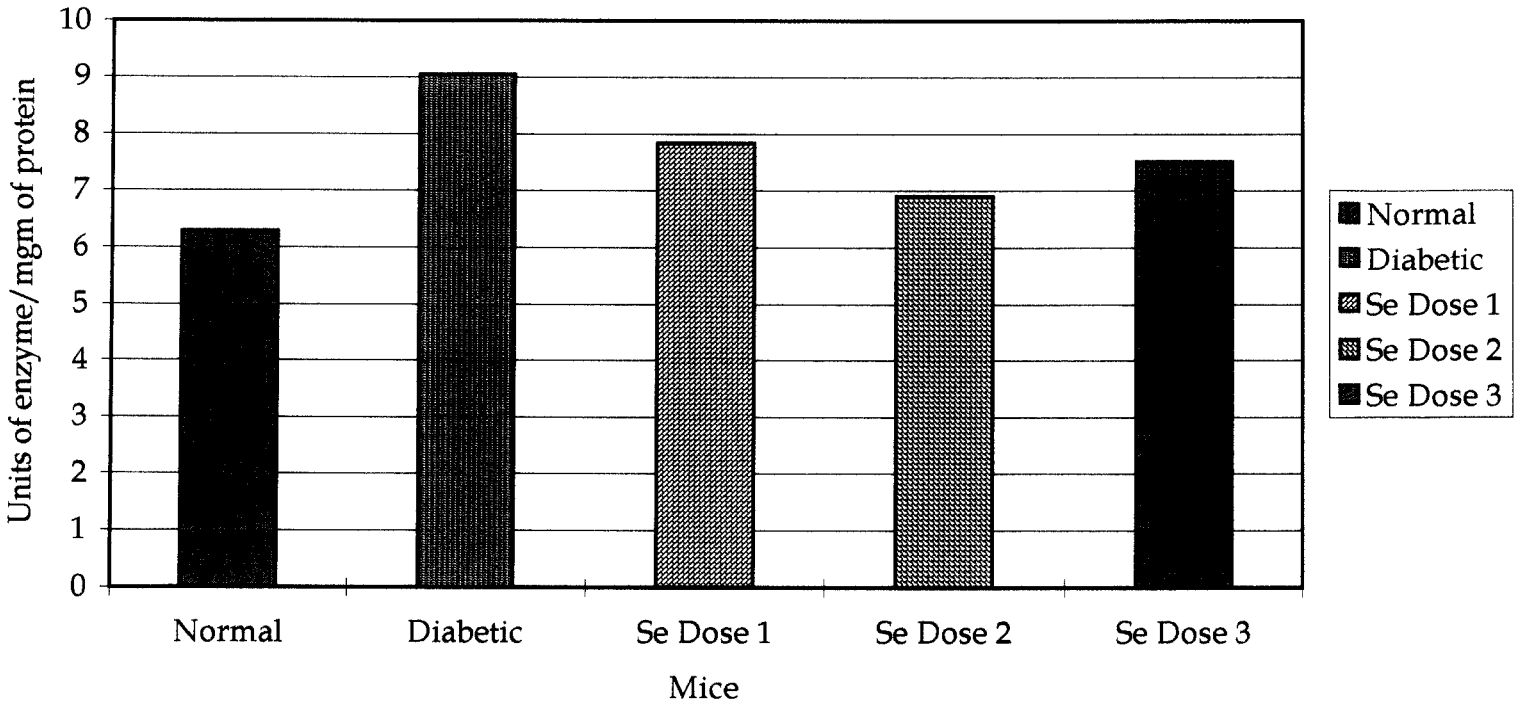


Fig. 35 : Changes of Monoamine oxidase level in serum on Selenium treatment.

5.5.3 Experiment related to Acetyl choline esterase in brain :

Experiment 36 : Changes of acetyl choline esterase level in brain on Selenium treatment :

This experiment was designed to understand the changes of Acetyl choline esterase level in brain in diabetic mice on changing the different doses of Selenium feeding. The normal level in adult mice was 169.125 ± 2.68 units/ml. The level in diabetic mice was 13.02 ± 2.81 units/ml. The level in Dose-1 fed group was 24.88 ± 3.99 units/ml. The level in Dose-2 fed group was 44.85 ± 6.42 units/ml. and the level of Acetyl choline esterase in brain in Dose-3 fed group was 30.02 ± 4.48 units/ml. (Table-36, Fig. 36).

Table 36 : Changes of Acetyl choline esterase level in brain on Selenium treatment :

Treatment Condition	Acetyl choline esterase level in brain		p Value with respect to normal
	Units /ml.	± SD	
Normal adult mice	169.125	± 2.68	
Diabetic mice	13.02	± 2.81	
Se-Dose 1 fed diabetic mice	24.88	± 3.99	0.0000
Se-Dose 2 fed diabetic mice	44.85	± 6.42	0.0000
Se-Dose 3 fed diabetic mice	30.02	± 4.48	0.0000

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

This experiment showed that the level of Acetyl choline esterase in brain in diabetic group fell by 13 times. With Selenium feeding the level rose. Amongst the readings the Dose-2 fed group the level rose more than 3 times the diabetic level.

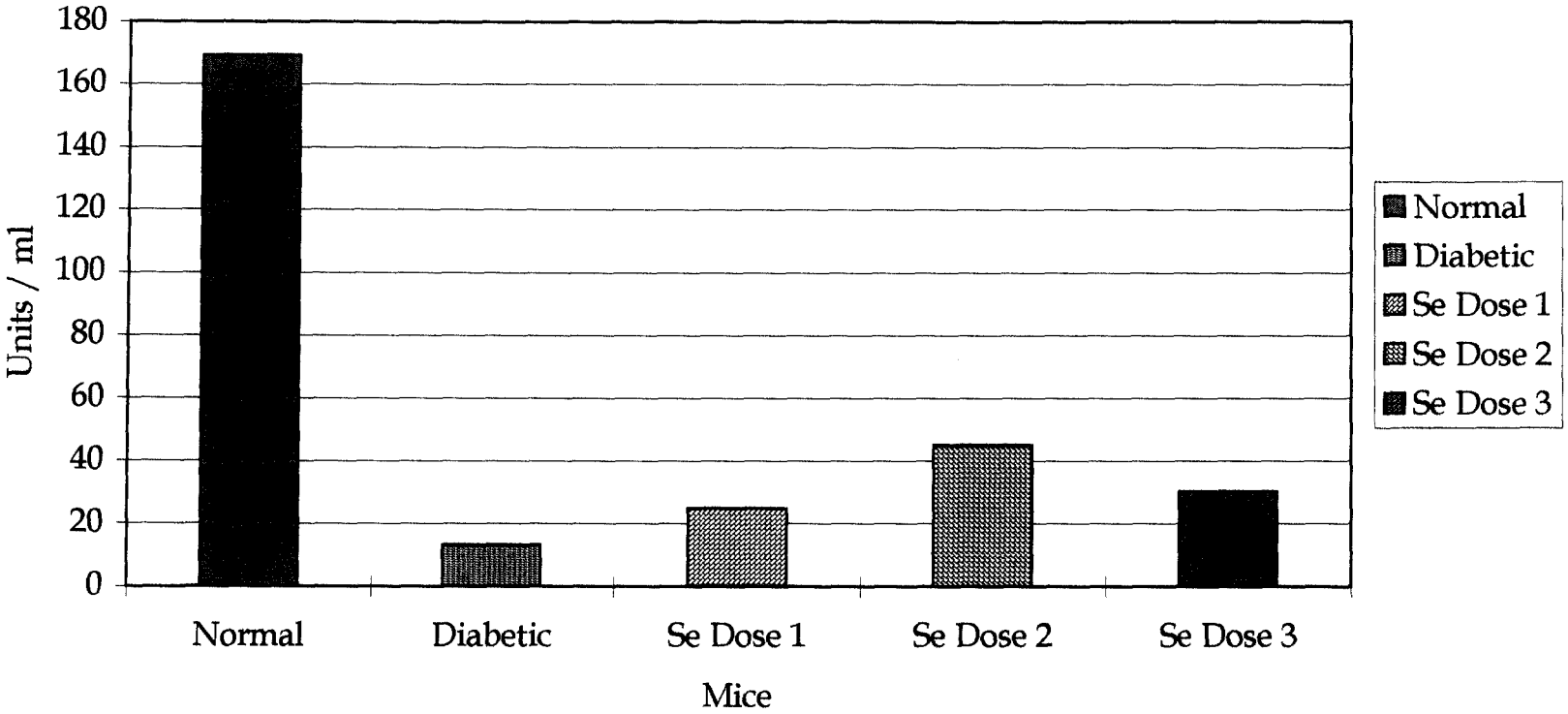


Fig. 36 : Changes of Acetyl choline esterase level in brain on Seleninm treatment.

5.5.4 Experiment related to Urea in blood :

Experiment 37 : Changes of Urea level in blood on Selenium treatment :

This experiment was done to show the changes of level of urea in blood in diabetic mice on changing the doses of Selenium feeding. The normal level in adult mice was 16.906 ± 3.64 mgm%. The level in Streptozotocin induced diabetic mice was 20.352 ± 4.44 mgm%. The level in Dose-1 fed group was 27.82 ± 6.65 mgm%. The level in Dose-2 fed group was 39.42 ± 8.78 mgm% and the level in Dose-3 fed group was 34.4 ± 6.75 mgm% (Table-37, Fig. 37).

Table 37 : Changes of Urea level in blood on Selenium treatment :

Treatment Condition	Urea level in blood mgm%	± SD	p Value with respect to normal
Normal adult mice	16.906	± 3.64	
Diabetic mice	20.352	± 4.44	
Se-Dose 1 fed diabetic mice	27.82	± 6.65	0.0173
Se-Dose 2 fed diabetic mice	39.42	± 8.78	0.0026
Se-Dose 3 fed diabetic mice	34.4	± 6.75	0.0021

Se Dose 1 = 0.5 micro gm/0.1 ml

Se Dose 2 = 0.05 micro gm/0.1 ml

Se Dose 3 = 0.0005 micro gm/0.1 ml

This experiment showed that the blood urea level rose by 20.4% in diabetic mice. With Selenium treatment the levels rose and in Dose-2 fed group the level rose more than twice.

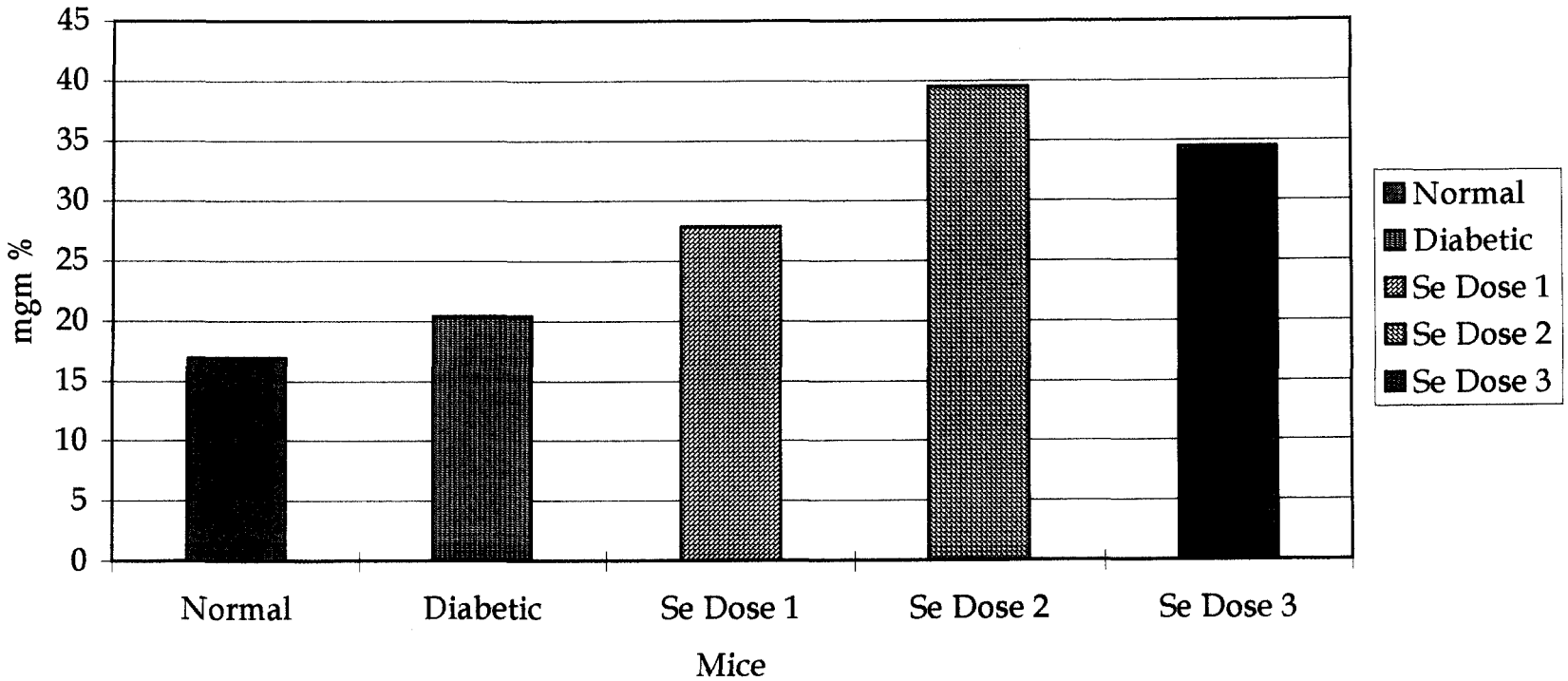


Fig. 37 : Changes of Urea level in blood on Selenium treatment.

5.5.5 Experiments related to Selenium level in Pancreas, Brain, Liver and Blood :

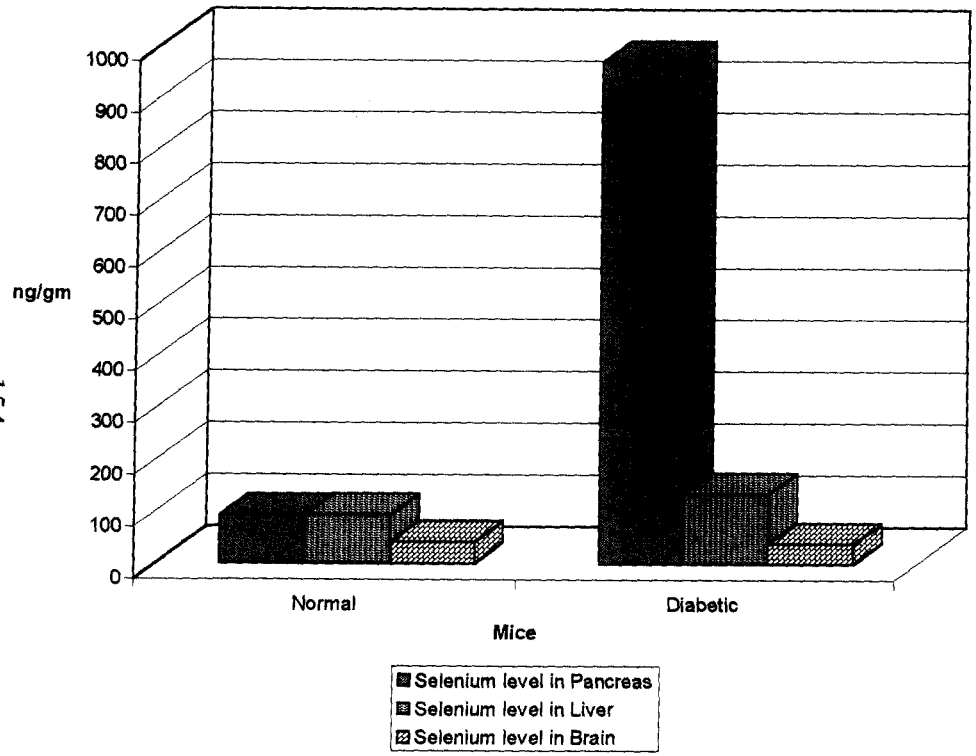
Experiment 38 : Changes of level of Selenium in different tissues and blood on induction of diabetes :

This experiment was designed to understand the changes of level of Selenium content in pancreas, brain, liver and blood of mice after Streptozotocin induced diabetes. Selenium level was assayed in normal tissue and compared with diabetic mice. The level in normal pancreas was 92.46 ng/gm. On production of diabetes the level went to 970.23 ng/gm. Selenium level in normal liver was 93.28 ng/gm and in diabetic liver was 134.18 ng/gm. Similarly the Selenium levels in normal and diabetic brain were 41.41 ng/gm and 38.86 ng/gm respectively. The Selenium level in blood of normal and diabetic groups were 1.46 and 1.34 μg/gm respectively (Table-38, Fig. 38).

Table 38 : Changes of level of Selenium in different tissues and blood on induction of diabetes :

Treatment Condition	Selenium level in pancreas ng/gm	± SD
Normal	92.46	± 3.64
Diabetic	970.23	± 27.47
	Selenium level in liver	
	ng/gm	**± SD**
Normal	93.28	± 4.72
Diabetic	134.18	± 5.22
	Selenium level in brain	
	ng/gm	**± SD**
Normal	41.41	± 4.77
Diabetic	38.86	± 3.25
	Selenium level in blood	
	μg/gm	**± SD**
Normal	1.46	± 0.22
Diabetic	1.34	± 0.18

This experiment showed the variation in the level of Selenium content on production of experimental diabetes. The level in pancreas was increased by more than 10 times the normal value. The level in liver rose by one and half times in diabetic mice. In brain there is marginal lowering of Selenium content and in blood also there is lowering of the level.



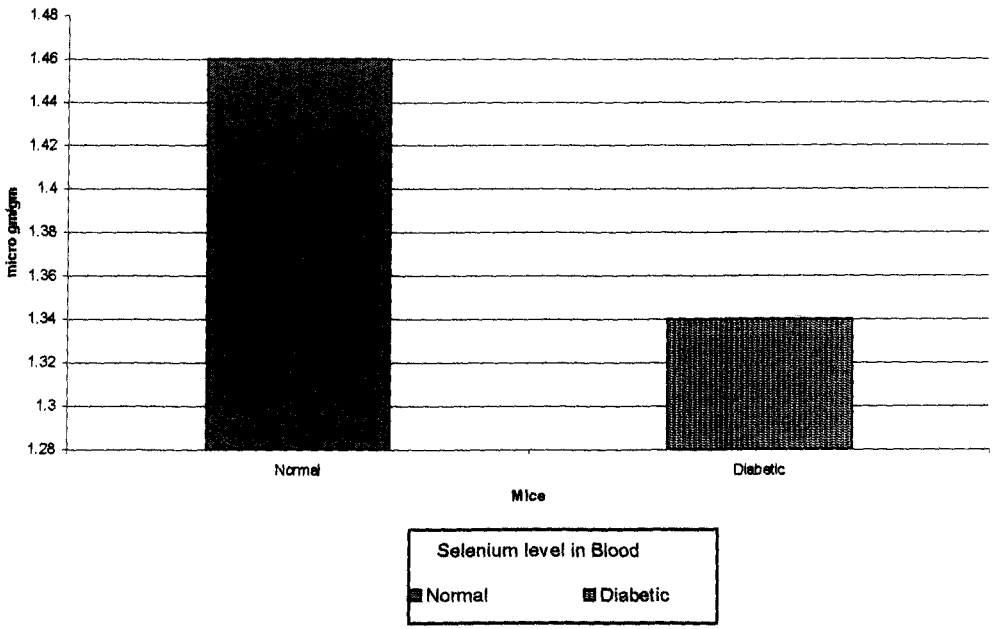
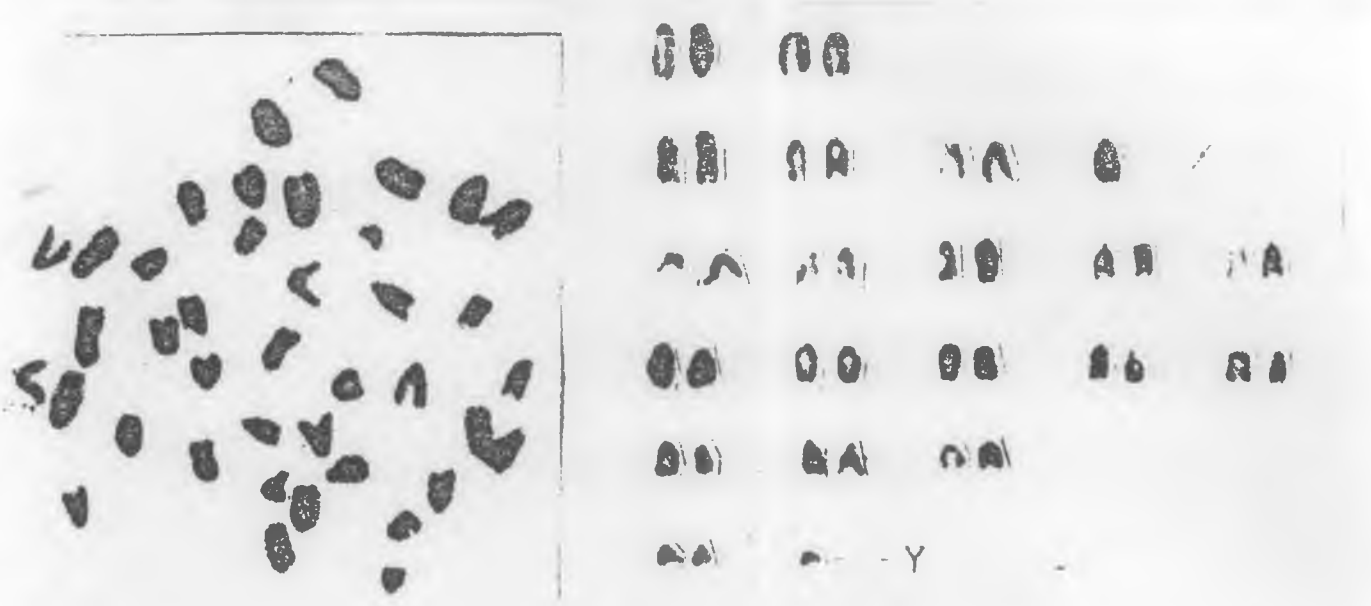


Fig. 38 : Changes of level of Selenium in different tissues and blood on induction of diabetes.

5.6 Chromosome Preparation :

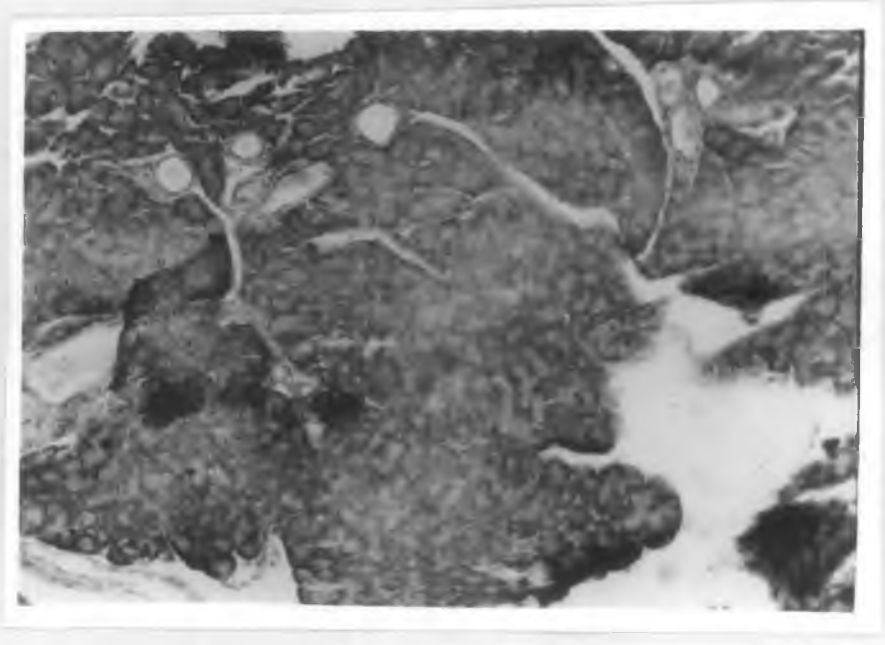


(A) Normal mice

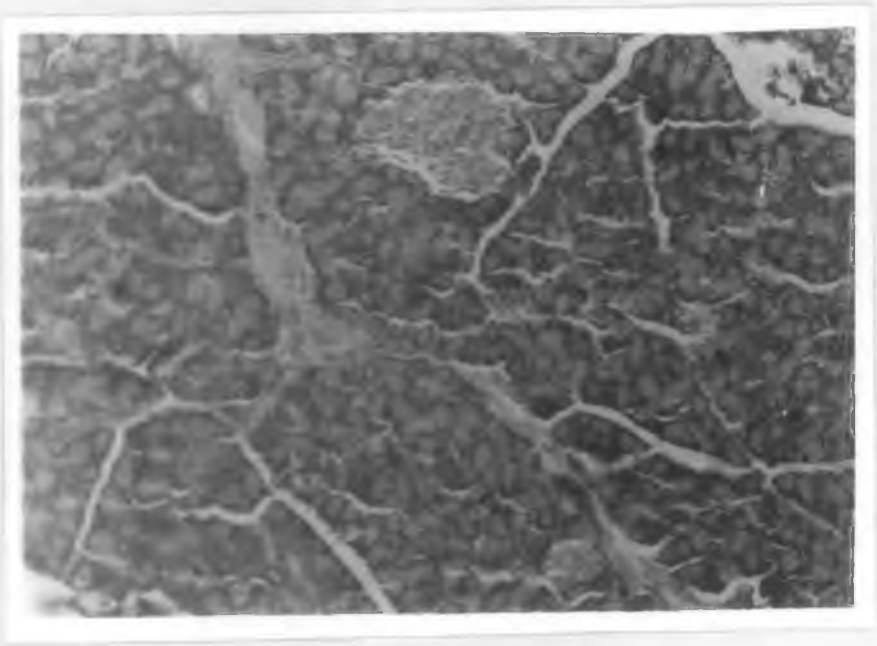
(B) Diabetic mice

(C) Selenium Dose-2 fed diabetic mice.

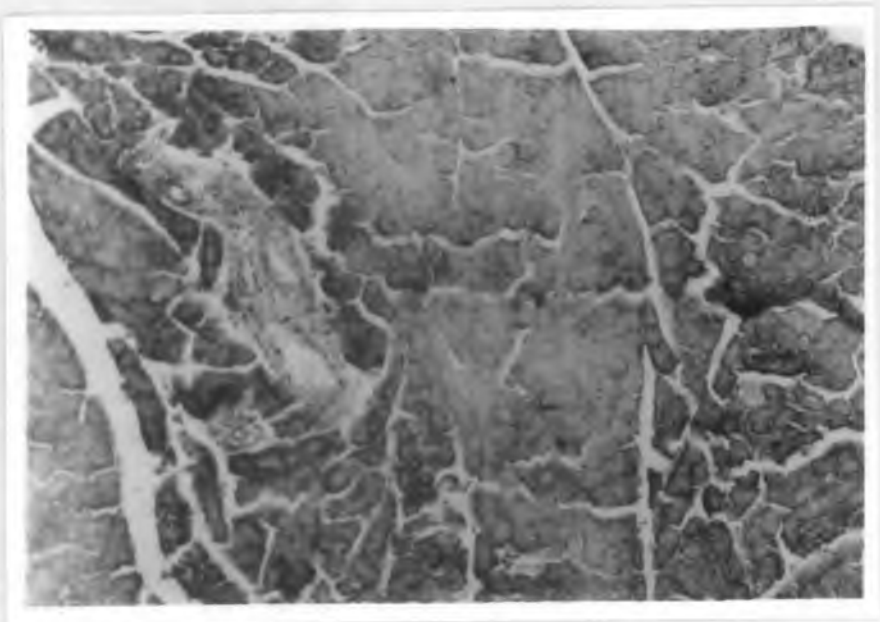
5.7 Histopathology of Pancreas :



(A) Normal mice



(B) Diabetic mice



C) Selenium Dose-2 fed diabetic mice.

DISCUSSION

6.0 Discussion :

Diabetes has reached an alarming state and is found to be a powerful predisposing factor for many diseases in general. The identification of the independent unique effect in diabetes still remains to be a chalanging problem and have implecated the needs for continued research.

The sugar glucose is the most important carbohydrate and it is as glucose that the bulk of dietary carbohydrate is absorbed into the blood-stream or into which it is converted in the liver and it is from glucose that all other carbohydrates in the body can be formed [177]. In diabetes as glucose is not utilised in the cell the blood sugar level rises.

Chemical toxins such as streptozotocin have been shown to cause abrupt onset of diabetes in a variety of animals including rat, mouse, Chinese hamster, dog, sheep, rabbit and monkey.

Streptozotocin-2-Deoxy-2-[{(methylnitrosoamino)-carbonyl} amino]-D-glucopyranose; or 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose.

$C_8H_{15}N_3O_7$	-	molecular weight 265.22
LD_{50} in female mice	-	360 mgm/Kg intraperitoneal 275 mgm/Kg intravenous [178].

Damaged β cells initially exhibit a vacuolated cytoplasm followed by shrinkage of the nuclei as the cells detach from one another. As cellular destruction proceeds nuclear damage and disintegration of the nuclear membrane become obvious. Generally β cell destruction is complete by 24 hours after the toxin is given. Following significant β cell loss, the islets in diabetic animals are small. The islets cells are arranged in small cords that are surrounded by fibrous tissue. Using immunocytochemical techniques, it has been demonstrated that these cords are composed of two thirds glucagon containing α-cells and one third somatostatin containing δ cells. Insulin containing β cells are almost totally absent in these islets.

There is no effective regeneration of β cells in the islets of adult animals treated with islet cell toxins; however, in neonates there is marked regenaration of β cells following a toxic insult. Neonatal rats treated with a high dose of streptozotocin show a dramatic increase in β cell mass following initial β cell destruction. This results from both replication of surviving β cells and the budding of new islets from ducts. However, the regenarative process is not complete because these rats are glucose-intolerant in adult life. From these experiments with streptozotocin apparently a toxic insult to the β cells early in life can have dire consequences much later.

Streptozotocin apparently does trigger an autoimmune response against β cells in mice. Repeated subdiabetogenic doses of streptozotocin, administered to certain strains of mice can cause diabetes associated with islet inflammation and β cells destruction [179].

Our study corroborates the previous finding of blood sugar changes following streptozotocin injection. Following intraperitoneal injection of streptozotocin the blood sugar level started rising and the animal became diabetic and this increased blood sugar level was sustained till the death of the animal.

The idea of finding an element which may normalise the increased blood sugar level in streptozotocin induced experimental mice came from the works on Vanadium in lowering the increased blood sugar in diabetic mice.

Vanadium has been considered to be a potent environmental contaminant and extensive work has already been carried out on the toxic properties ascribed to vanadium in animals [180] and in humans following occupational and experimenal exposures [181].

Excessive cellular concentrations of vanadium result in toxic interactions which topple the cellular balance and the suggested regulatory role of vanadium causing increased cell death [182, 183, 184]. Later researches has shown vanadium to be capable of a beneficial behaviour under a thousand fold lower dose in comparison to the toxic higher doses.

This experience with vanadium led us to investigate another trace element selenium on the effect on experimental diabetes.

The objective of our study was to investigate the efficacy of low doses of selenium on the periods of progression of streptozotocin induced experimental diabetes in mice and which may be determined by some important markers that are responsible for biotransformation and detoxification.

A dose dependent study of different markers were done after feeding 3 different doses of selenium. It was found that Dose 2 i.e., 0.05 micro gm/0.1 ml was the most efficient dose in correction of different parameters as well as the blood sugar [113, 114].

Hepatic microsomal glucose-6-phosphatase (EC : 3.1.3.9) is potentially the most important enzyme involved in the homeostatic regulation of blood glucose concentrations. Substantial kinetic and genetic evidence indicates that glucose-6-phosphate hydrolysis in the glucogenic tissues is catalysed by a multi component system. It has been proposed that the active site of glucose-6-phosphatase in intact microsomes (microsomal fraction) is situated at the luminal

surface of the membrane and that a specific translocase (T_1) mediates entry of glucose-6-phosphate.

The level of glucose-6-phosphatase was elevated by 21.3% after production of experimental diabetes in mice and on selenium treatment the level fell to or below normal. Glucose-6-phosphatase catalyses the final step of glucose production by liver and kidney.

Miethke et al showed 150% increase of glucogenic enzyme glucose-6-phosphatase in diabetic mice [67].

Liu et al studied the effect of acute streptozotocin induced diabetes on hepatic microsomal glucose-6-phosphatase activity and m RNA expression in young, juvenile and adult rats. They found that acute streptozotocin diabetes increase expression of glucose-6-phosphatase m RNA and this contributes to the increased glucose-6-phosphatase activity seen with diabetes mellitus [87].

Burcelin et al showed 156% increase in glucose-6-phosphatase activity in streptozotocin diabetic rats. They proposed that in recent onset severly insulinopenic rats, an excessive glucose production via gluconeogenesis prevailed, mainly accounting for the concomitant hyperglycaemia. This excess glucose output cannot be attributed to liver insulin resistance : the gluconeogenic pathway is physiologically less sensitive than glycogenolysis to the inhibition by insulin [90].

Reddy et al studied the activity of glucose-6-phosphatase in liver under the influence of sepia shell extract in both normal and streptozotocin induced diabetic mice. Sepia shell possesses hypoglycemic effect. Activity of glucose-6-phosphatase was inhibited in both the normal and diabetic mice. The sepia shell extract enhances glycogenesis and reduces the formation of glucose from metabolic intermediates like pyruvate and glucose-1-phosphate and by suppressing gluconeogenesis [93].

Clore et al also found significantly increased hepatic glucose-6-phosphatase activity determined from freshly isolated microsomes in the type 2 diabetic patients. They concluded that increased endogenous glucose production which is a consistent feature of type 2 diabetes is mediated in part by increased glucose-6-phosphatase flux in type 2 diabetes [105].

Ghosh et al studied the effect of oral selenium on streptozotocin induced diabetic mice and found increase in glucose-6-phosphatase activity [115].

Dehydrogenation of glucose-6-phosphate to 6-phosphogluconate occurs via the formation of 6-phosphogluconolactone catalyzed by glucose-6-phosphate dehydrogenase, an NADP-dependent enzyme.

The level of glucose-6-phosphate dehydrogenase (EC : 1.1.1.49) was diminished by 20.8% in diabetic mice and on selenium treatment the level increased.

Shibib et al showed that Coccinia indica and Momordica charantia extracts lowered blood glucose by depressing its synthesis by enhancing glucose oxidation by the shunt pathway through activation of its principal enzyme glucose-6-phosphate dehydrogenase in streptozotocin induced hyperglycaemic rats [83].

Berg et al showed that administration of vanadate or Selenate in streptozotocin induced diabetic rats restore activity of glucose-6-phosphate dehydrogenase. Increase in cellular mRNA, responsible for increased synthesis of G6PD suggests the effect of insulin or mimetics (vanadate/ selenate in this case) occurs pretranslationally through gene regulation [116].

The level of pyruvic acid in liver was decreased to 78.9% in streptozotocin induced diabetic mice. On selenium treatment the level was almost normalised.

Reddy et al studied the status of pyruvate in liver under the influence of sepia shell extract in both normal and streptozotocin induced diabetic mice. The pyruvate concentration increased substantially in diabetic mice [93].

Glycogen is the major storage form of carbohydrate in animals and corresponds to starch in plants. It occurs mainly in liver (upto 6%) and muscle where it rarely exceeds 1%. However, because of its greater mass, muscle represents some three to four times as much glycogen store as liver.

The level of Glycogen in liver was decreased to 28.4% in experimentally induced diabetic mice. On selenium treatment the level of glycogen gradually recovered.

Miethke et al found that liver glycogen was reduced to 10% [67].

Khandelwal found marked decrease in liver glycogen and activities of glycogen-metabolizing enzymes in liver. Administration of oral sodium ortho vanadate restored the level [75].

Reddy et al studied the status of glycogen in liver under the influence of sepia shell extract in both normal and streptozotocin induced diabetic mice. The glycogen concentration was elevated steeply in both [93].

Ghosh et al studied the effect of oral selenium on streptozotocin induced diabetic mice. Reversal of the decrease in hepatic glycogen was observed in diabetic mice on oral selenium[115].

Lactate formed by the oxidation of glucose in skeletal muscle and by erythrocytes, is transported to the liver and kidney where it reforms glucose, which again becomes available via the circulation for oxidation in the tissues. This process is known as the Cori cycle or lactic acid cycle.

Diabetes and selenium feeding had no effect on the lactic acid level in heart. A 40.3% diminution in the level of lactic acid in muscle was noted in diabetic mice. Selenium feeding increased the level. The level of lactic acid in liver fell by 35.8% in diabetic mice. The level rose to almost normal in selenium Dose 2 fed diabetic group. The level of lactic acid in blood rose to 55.5% in diabetic mice and the level fell with selenium treatment and came to almost normal value.

Higher lactic acid concentration in blood promoted the gluconeogenesis and the decrease in tissue level revealed maximum utilisation of glucose that has been taken up by the muscle cells through the oxidative pathway.

Kondoh et al while investigating the methylglyoxal bypass in animals found diabetic and starved rats had significantly higher level of D-lactate in plasma, liver and skeletal muscle compared with the control group. In contrast, pyruvate levels in plasma, liver and skeletal muscle was markedly lower than normal in diabetic and starved rats. L-lactate level lowered markedly in plasma, liver and skeletal muscle of starved rats and elevated in liver of diabetic rats [81].

Tormo et al investigated the lactate production by the intestine of normal and diabetic rats and found the lactate produced was significantly higher in diabetic than in normal rats [92].

No simple or successful method for the separation of succinic dehydrogenase (EC : 1.3.99.1) from cellular inclusions has been devised to date; hence, any assay for this enzyme has to be carried out in the presence of some or all components of the succinic dehydrogenase-cytochrome system. Cytochrome oxidase may be inactivated by the use of cyanide or by carrying out the estimation anaerobically. Under either of these conditions and with the addition of a hydrogen acceptor, the activity of the succinic dehydrogenase system may be estimated readily. The expression succinic dehydrogenase system refers to the system which catalyses the anaerobic oxidation of succinate and which probably includes cytochrome b in addition to succinic dehydrogenase and the hydrogen acceptor [185].

The level of succinic dehydrogenase in liver was increased by 25.9% in diabetic mice. Following selenium treatment the level fell and with Dose 2 fed mice the level came almost to normal.

Armstrong et al studied succinate dehydrogenase activities of skeletal muscles in normal and streptozotocin induced diabetic rats. Enzyme activities in all muscles declined to a lower final level and exhibited a more rapid decay in animals receiving the larger dosage, both diabetic and karela juice fed rats [60].

Lenzen and Panten showed that succinate dehydrogenase activities in homogenates of rat and ob/ob mouse pancreatic islets were only 13% of the activities in homogenates of liver and were also several times lower than in homogenates of pancreatic acinar tissue. They also found that the activities of succinate dehydrogenase in tissue homogenates of pancreatic islets, pancreatic acinar tissue and the liver were significantly inhibited by malonate and diazoxide but not by glucose, mannoheptulose, streptozotocin or verapramil [64].

These findings are contrary to our finding which may be due to change of experimental animal.

The distribution of LDH-isoenzymes (EC : 1.1.1.27) from homogenate supernatant of muscle solens detected five LDH-isoenzymes in normal rats. Adipose lactate dehydrogenase isozyme distribution was altered in streptozotocin diabetic and fasting rats resulting from a relative reduction of subunit A.

The changes of lactate dehydrogenase in serum on selenium treatment showed that the value in diabetic group becomes almost double the normal value.

Wohlrab and Schmidt found that after 1-2 month duration of diabetes the LDH 1 was decreased and the LDH 4 was increased. The H-subunit value was decreased. In long term diabetes (11 months) the values of single fractions and the H-subunit value did not differ from those of controls [59].

Chang and Rothrock suggested that the redistribution of LDH-isozyme under these conditions is to prevent excessive accumulation of lactate in the tissue [61].

Increased LDH level may be due to cellular destruction that occurs in the diabetic pathology and posses an interference with the normal cardiovascular functions and is an associated risk.

Diabetes is more common now than it was before. Epidemiological studies have revealed diabetes to be a powerful factor for cardiovascular disease in general, particularly coronary disease. The most common lethal sequelae of diabetes was atherosclerotic large vessel disease. Diabetes predisposed subjects to all of the major atherosclerotic disease and coronary heart disease was the most common and most lethal. Incidence rate for cardiovascular disease is associated with increasing various risk factors including obesity, hypertension, cigeratte smoking etc. Although the incidence of diabetes on cardiovascular disease is greatly dependent on co-existent risk factors, there could be an independent effects of the diabetes in promoting the cardiovascular and coronary heart disease. This unique effect may derive from the disturbances in the metabolic effects of lipids and of the activities of enzymes like the lactate dehydrogenase and changes in the intrinsic catalytic behaviour of some of the enzymes that may be involved in the cardiovascular events. Fibrinogen status in diabetes may further contribute to this aspect. The study of the diabetic mice was undertaken to examine the level of fibrinogen, lactic acid, cholesterol, triglyceride, LDL, VLDL, HDL values and to measure the activity of lactate dehydrogenase and HMG CoA reductase (EC : 1.1.1.34) under the experimental diabetes condition; in order to assess the influence of diabetes on the cardiovascular system and to test the epidemiological observation in this regard.

The blood cholesterol level rose to 18.9% in diabetic mice in comparison to normal mice. With selenium treatment the level fell. In Dose 2 fed group the level came to normal level.

The level of serum LDL rose more than twice the normal value in diabetic mice. In Dose 2 fed animals the LDL level reached almost normal level.

The serum VLDL level rose 44.4% than the normal value, with production of diabetes. In selenium Dose 2 fed animals the level was almost normal.

The level of serum HDL was halved by induction of diabetes and in selenium Dose 2 fed animals the level approached near normal value.

The level of serum triglyceride was increased by 7 times in diabetic mice. Selenium feeding lowered the level.

The level of liver triglyceride was diminished by 7 times on induction of diabetes. In Dose 2 fed animals the level of liver triglyceride reached almost normal level.

In vanadium, selenium and vanadium & selenium fed normal mice the serum triglyceride level fell to half the normal level. But in diabetic groups vanadium alone failed to reduce the

triglyceride level. Selenium alone with Dose 2 is most effective in reducing the level. Also in the vanadium and selenium fed group the level is not satisfactorily reduced. So selenium Dose 2 is more effective than vanadium in respect to lowering of serum triglyceride level.

The activity of HMG-CoA reductase activity in liver was reduced by 22.8% in diabetic mice. In Dose 2 fed animals the activity neared the normal level.

Diabetes is associated with higher levels of triglyceride, cholesterol, lower HDL, increase in LDL and VLDL levels. These risk factors operate to enhance excess risk in diabetes. Our studies have confirmed the epidemiological observations (Framingham study) of William Kannel and others in respect of the variables involved in the cardiovascular derangements [186].

Diabetes is associated with lipolysis. Enhanced gluconeogenesis raise the serum triglyceride and mobilise fat from all tissues including liver which account for lowering of the liver triglyceride level in diabetic mice.

The unique effect of diabetes that is independent of the standard risk factors appears to be mostly explained by fibrinogen, mobilisation of lipid to blood and increase activity of LDH. Since the high risk of coronary heart disease in diabetes is concentrated in these with one or more associated cardiovascular risk factors, optimal management of cardiovascular sequelae require multivarious approach. Rational preventive measure must include raising HDL, lowering LDL, VLDL, decrease LDH activity instead of sole reliance on the correction of hyperglycaemia.

Verschoor et al have previously suggested that mechanisms other than reduced lipoprotein lipase activity might contribute to the defect in plasma removal of VLDL-triglyceride observed in insulin-deficient rats [65].

Goley et al found that plasma LDL-cholesterol was lower in alloxan induced diabetic rabbits [69].

Poucheret et al demonstrated that vanadyl sulfate can lower elevated blood glucose, cholesterol and triglycerides in a variety of diabetic models including the streptozotocin diabetic rat [100].

Sambandam et al found that diabetic rats had elevated triglyceride levels compared with control [106].

Douillet found that selenium or selenium or Vit E play a role in controlling oxidative status and altered lipid metabolism in liver, producing favourable fatty acid distribution in major tissues, affected by diabetic complication [123].

Xenobiotics are foreign toxic organic compounds, which must be detoxified by reactions making them less reactive or more water soluble and thus more amenable to excretion. Xenobiotic biotransformation end products are combined with endogenous molecules to form conjugates [187].

Glutathione (GSH) functions in the synthesis of important macromolecules and in the protection against reactive O_2 compound [188].

GSH was found to be effective in the management of various diseases of diverse aetiology. The level of glutathione in liver was raised by 39.1% in diabetic mice. With selenium Dose 2 feeding the level became almost normal.

The level of glutathione in blood in diabetic mice fell to almost half the normal level. With selenium Dose 2 feeding it was almost normal.

GSH is essential for the function of most if not all animal cells and maintains the functional and structural integrity of cells against the deleterious actions of metabolites and is maintained at a steady value to an autoregulatory mechanism [189].

The decrease in GSH level diorganises and disoriantes the mitochondria of the cell resulting in the reduced supply of ATP thereby limiting the optimum cellular and physiological functions in the diabetic plate. [190, 191, 192, 193].

GSH in blood reflects the GSH status in hepatocytes without considerable delay. But as GSH level is known to maintain the structural integrity of mitochondria, the decreased level of GSH may indicate the disorganisation of structure of mitochondria leading to the depletion of ATP synthesis. This finding is consistent with our previous result of inhibition in the activity of G-6-PDH limiting the supply of NADPH for maintaining the GSH in reduced state. Under such condition the cell might suffer from the lack of energy required for normal cellular and physiological processes. GSH is also an important cellular defense against oxidant injury and the significant role of GSH in the prevention of cellular lipid peroxidation has been well documented. The low level of GSH promotes an increase in lipid peroxidation resulting in a form of failure of adaptation on the part of GSH dependent defence mechanism against lipid peroxidation in rat liver microsomes to counteract the oxidative stress.

Mukherjee et al found a decline in GSH levels both in blood and liver compared with normal counterparts [84].

Kinalski et al found markedly diminished GSH level in the diabetic adult rats and their offspring in comparison to the control group [103].

Mukherjee et al showed marked decrease in glutathione. After 5 weeks of streptozotocin treatment, GSH level were reverted to normal by sodium selenite supplement [124].

Naziroglu et al indicated that intraperitoneally administered vitamin E and Se have significant protective effects on the blood, liver and muscle against oxidative damage of diabetes [130].

It is established that enhanced lipid peroxidation is followed by increased GSH oxidation to form oxidized glutathione (GSSG). In general, intracellular reduction of GSSG to GSH is mediated by glutathione reductase (EC : 1.6.4.2).

The level of glutathione reductase in liver fell by 20.9% in diabetic mice. With selenium feeding the level approached near normal level.

Godin et al showed in alloxan induced diabetic rats an increase in glutathione reductase activity [71].

Mukherjee et al showed a 20% decrease in GSH reductase activity in experimental diabetic condition [84].

Matkovics et al tested and compared antioxidant enzymes and glutathione reductase activity in streptozotocin diabetic animals. They concluded that streptozotocin treatment generally induces an oxidative predominance in tissues [97].

Glutathione-S-transferase (EC : 2.5.1.18) isoenzymes have a number of roles in the regulation of cellular metabolism. These include catalysis of the reduction of organic nitrates and hydroperoxides, steroid isomerization and the binding of non-substrate hydrophobic ligands such as steroid hormones, heme, bilirubin and numerous drugs. Multiple sub-units of GSH-S-T have been identified in a number of human and rat tissues and these can combine to form homo or heterodimeric isoenzymes. Upto 13 distinct isoenzyme subunits have been detected in human liver and lesser numbers in such tissues as kidney, testis, heart, lung, adrenals, brain, duodenum, ovary, placenta, spleen, eye lens and erythrocytes. This diversity and heterogenecity contributes to the inter tissue variation in several metabolic processes as well as the detoxification

of hydrophobic or electrophilic xenobiotics through catalytic conjugation with reduced glutathione (GSH) [194].

The GSH-S-T catalyze the conjugation of GSH with a variety of molecules that each have an electrophilic centre. These isoenzymes also exhibit selenium independent glutathione peroxidase activity and play an important role in the hepatic biotransformation and detoxification of xenobiotics. Consequently these enzymes may influence chemical carcinogenesis, mutagenesis and possible teratogenesis [195].

The level of glutathione-s-transferase in liver rose with production of diabetes and with selenium treatment the level continued to rise.

The glutathione-s-transferase is a multifunctional enzyme and it plays an important role in the detoxification of xenobiotic compounds with the help of GSH. The level of glutathione-s-transferase is found to be increased as compared to the control animals. The increase in this enzyme with the concomitant decrease in GSH level indicates accelerated detoxification pointing towards the possible accumulation of active metabolites and/or electrophilic molecules. Thus an increased utilization of GSH in the removal of toxic radical species through glutathione-s-transferase activity may eventually deplete the intracellular GSH pool.

Agius and Gidari demonstrated that streptozotocin increased the activity of mouse hepatic glutathione-s-transferases, but they suggested that this increase may be due to the direct action of streptozotocin and not as a result of the diabetic state the drug induces [66].

Thomas et al found reduced level of GSH-s-transferase in streptozotocin diabetic mice [72].

Mukherjee et al found an increase of GSH-s-transferase activity in experimental diabetic condition [84].

Raza et al found cytosolic glutathione concentration was decreased in diabetic rats and an increase of 20-30% in GSH-s-transferase activity in both diabetic and karela juice fed rats [94].

Giron et al fed diets with 5% olive, sunflower or fish oil for five weeks in streptozotocin diabetic rats and found in intestine GSH-S-T increased by diabetes [102].

Mukherjee et al found increase in glutathione-s-transferase activity after 5 weeks of streptozotocin treatment and reversion to normal by sodium selenite supplement [124].

Cytochrome P 450, Uridine diphospho glucoronyl transferase (UDPGT) and GSH-S-T enzyme systems are associated with the biotransformation for xenobiotics seen to be present in all vertebrates and has been best documented in mammals [196].

GSH-S-T catalyze the reaction between glutathione and a large variety of compounds bearing an electrophilic site [197].

UDPGT (EC : 2.4.1.17) on the other hand catalyzes the transfer of glucoronic acid to UDPGA to various phenolic, carboxylic acid and amine receptors.

The level of hepatic UDP-glucoronyl transferase rose on induction of diabetes and on selenium treatment went on rising.

Rouer et al measured the activities of UDP glucoronosyl transferase and cytosolic glutathione-s-transferase activities in the liver of streptozotocin induced diabetic mice. 2-3 fold increase was observed [63].

Numerous alterations in hepatic ultrastructure and metabolism occur during diabetes. These changes also seem to include the drug metabolizing enzymes as has been demonstrated for GSH-S-T and UDPGT and Cyt P 450 content [198].

Patterns in our observation show steady and progressive elevation of GSHT and UDPGT. Results indicate a simultaneous rythmicity of Cyt P 450 with UDPGT level followed by a decreased ATP synthesis. A failure of detoxification mechanism in the host under experimental diabetic conditions was evident that could induce a potentially increased risk of reactive metabolites under the condition of impaired detoxification capacities.

This study demonstrates the significantly altered levels of these drug metabolizing enzymes under the influence of pathophysiological condition like diabetes. Diabetes may act as proliferators of this altered state of xenobiotic degrading enzymes and could be expressive of intrinsic inductive potency.

The different rates of inductions of various drug metabolizing enzymes observed in the present study emphasise the need to carefully characterise the diabetic state for treatment with different drugs in different pathological conditions other than diabetes. Thus, considering the biological role of these different forms of enzyme, enough care has to be taken in balancing this status of enzyme activities, would be of interest in future.

Lipid peroxidation is primarily an outcome of the formation of free radicals, peroxides and superoxide anions. Active oxygen species interacts with unsaturated fatty acids present in phospholipid to initiate lipid peroxidation which is the major factor influencing the breakdown and turnover of biomembranes.

The level of lipid peroxidation in livers rose by six times in diabetic mice. With selenium Dose 2 feeding it approached the normal level.

The level of lipid peroxidation in brain rose by four fold in diabetic mice. With Dose 2 treatment the level fell almost to normal value.

The level of lipid peroxidation in kidney rose by five and half times in diabetic mice. With Dose 2 feeding it was almost normal.

The level of liver lipid peroxidation fell by one sixth part the normal value in both the vanadium and selenium fed normal mice groups. In both the vanadium and selenium fed diabetic groups the level fell by another one sixth part. Contrary to this result the level in vanadium plus selenium fed normal mice group only fell by a negligible amount and the level of liver lipid peroxidation came to normal in diabetic mice groups.

The present study documents a significant occurrence of an elevated lipid peroxidation in the tissues like brain, kidney and liver. The extent of increase in lipid peroxidation appear to be different in different tissues studied. This indicated that hyperglycaemia may affect different tissues in different fashions altering membrane lipid asymmetry that may have a role in the apparent reduced life span known to occur in diabetic patients.

Thomas et al depicted that high levels of free fatty acids or their metabolites which are known to accumulate in liver in both metabolic states may act as endogenous peroxisome proliferators [72].

Jain et al showed that lipid peroxidation levels were significantly higher in RBC of diabetic rats than in controls [78].

Mukherjee et al found that a single intraperitoneal injection of streptozotocin caused a four, eight and seven fold increase in lipid peroxidation in brain, lever and kidney respectively [84].

Kinalski et al evaluated lipid peroxidation and scavenging enzyme activity in streptozotocin-induced diabetes and suggested that diabetic pregnant rats and their neonates are exposed to

an increased oxidative stress and that vitamin E supplementation may reduce its detrimental effects [103].

Mukherjee et al showed increase in malondialdehyde levels in liver and blood after 5 weeks of streptozotocin treatment were reverted to normal by sodium selenite supplement [124].

Naziroglu et al determined the protective effects of intraperitoneally administered vitamin E and selenium on the lipid peroxidation as thiobarbituric acid reactive subsances (TBARS). They showed significant protective effects on the blood, liver and muscle against oxidative damage of diabetes [130].

Sato et al were the first to report increase level of lipid peroxide in plasma of diabetic patients [199]. Subsequent studies have confirmed this observation in diabetic patients and in animal and have suggested that hyperglycaemia may cause peroxidative injury to membrane.

Further, glutathione peroxidase has been demonstrated to participate in the conversion of toxic free radicals and organic hydroperoxides into hydroxy compounds in vivo and in vitro while oxidizing GSH into GSSG. In addition to glutathione peroxidase, catalase also acts as a scavenger of H_2O_2.

In our study diabetes and selenium feeding had no effect on catalase (EC : 1.11.1.6) level. This might ensure that the peroxidative damage due to H_2O_2 accumulation in the tissues is minimal in experimental diabetes [200].

Mukherjee et al found a marginal increase of catalase activity in streptozotocin induced diabetic rats [84].

Tatsuki et al studied the relationship between changes in lipid peroxides and those in catalase activity in pancreas, liver and heart of streptozotocin induced diabetic rats and suggested that the defense system in the pancreas to oxidative stress may be evoked in a early stage of streptozotocin induced diabetes [96].

Kakkar et al studied to identify whether oxidative stress occurs in the liver and pancreas in the initial stages of development of diabetes. They found increased catalase activity in liver and pancreas [98].

Dohi et al found selenium feeding in diabetic mice on liver and kidney catalase activity had no significant alteration [110].

The hepatic Cytochrome P 450 dependent drug metabolizing system catalyzes the metabolism of a wide variety of compounds such as xenobiotics, steroids, fatty acids and prostaglandins. Several forms of this family of enzymes exist in the untreated rat and several have been isolated and purified from chemically induced animals as well. Recently attention has been drawn to the constitutive forms of P 450 enzymes present in the untreated animal and presently 7 forms have been shown to exist in liver microsomes of the untreated male rat i.e. RLM 3 and RLM 5, UT-F, P 450f, RLM 2, RLM 5a and UT-H. Each of these forms is a separate and distinct Cytochrome P 450 based on such properties as absolute spectrum, molecular weight, isoelectric point, catalytic specificity and partial amino terminal amino acid sequence.

Although the levels of several of these constitutive forms have been shown to be unaltered, increased or decreased during treatment with chemical inducers or Cytochrome P 450, little is known regarding the regulation of these forms in altered physiological states not dependent on exogenous compounds. It is known, however, that certain hepatic microsomal P 450 dependent activities are altered as a result of streptozotocin induced diabetes and hypertension.

Studies have revealed that insulin dependent diabetes profoundly modulates the levels of inducible forms of Cyt P 450. The streptozotocin induced changes in P 450 related activity and apoproteins levels were successfully anatogonised by daily insulin therapy, demonstrating that the observed changes can be ascribed to the diabetic state rather than to the streptozotocin treatment [201].

The level of Cytochrome P 450 in liver fell by half in the diabetic group. With selenium feeding the level was more decreased.

Favreau and Schenkman measured the Cytchrome P 450 dependent hydroxylation of testosterone in hepatic microsomes of control, diabetic and insulin treated diabetic rats. Results provide evidence that specific constitutive Cytochrome P 450 enzymes are altered in the diabetic state and that these changes are not permanent since they can be overcome at least partially, by insulin replacement therapy [68].

Previous authors also made antibodies to monitor alterations in the content of the enzymes in livers of diabetic male rats. The results suggest there are atleast three types of responses by constitutents of the Cytochrome P 450 population to diabetes : no change in the microsomal content, a rapid increase when insulin level declines and restoration when insulin in supplied and a rapid decline when insulin level declines and a restoration by insulin treatment [70].

Barnet et al concluded that insulin dependent diabetes induces proteins of the P 450 III and P 450 IV families [74].

Donahue and Morgan showed vanadate is capable of separating the effects of diabetes on expression of individual P 450 isozymes [76].

It is now becoming increasingly evident that the induction and regulation of the Cytochrome P 450 dependent mixed function oxidase is not determined solely by exposure to xenobiotics, but may also be trigerred by pathophysiological conditions, presumably resulting from an increase in the levels of an endogenous chemical with inducing properties.

Two major inducible families of Cytochrome P 450 are P 450 III and P 450 IV. The former is inducible by glucocorticoids, macrolide antibiotics and some imidazole containing antifungal agents. The P 450 IV family is inducible by xenobiotics such as the drug clofibrate and its analogues and the phthalate ester plasticisers.

In diabetes the chances of atherosclerotic disease are high and coronary heart disease was most common and most lethal. Recent studies have implicated fibrinogen in the occurance of cardiovascular disease (Framingham Study).

Level of fibrinogen in blood rose to 3 times in diabetic group. With selenium treatment the level fell and in Dose 2 the level of fibrinogen fell most. Increase fibrinogen values may influence thrombogenic tendency affecting a series of vascular events including the rheology of blood flow viscosity of the blood and distortability of red cells squeezing through the capillary circulation, seemed to be a reasonable condition. In addition, fibrinogen appears to influence the aggregation of platelets [202].

Diabetes mellitus in experimental animals was reported to be accompained by different changes in brain neurochemistry. Concerning monoamines, a decrease in a turnover rate of brain serotonin, dopamine and norepinephrine was found.

The level of monoamine oxidase (EC : 1.4.3.4) in serum rose by 43.9% in diabetic mice. In Dose 2 group the level came almost to normal.

Lackovic and Salkovic found the concentration of serotonin, dopamine and norepinephrine to be increased in the brain of a diabetic rat. But after an intracerebro ventricular administration of non diabetogenic doses of streptozotocin or alloxan brain monoamine change were similar to those observed in experimentally induced diabetic animals [79].

Acetyl cholinesterase (EC : 3.1.1.7) catalyzes the hydrolysis of acetylcholine and other acetic acid esters, as well as certain esters and acyl halides of substituted phosphoric, carbamic and sulfonic acids. In view of the diversity of these molecules, it is generally believed that their binding patterns differ, while the hydrolytic part of the enzymic reaction remains the same. The widely used (pesticides) esters of phosphoric acid and carbamic acid are generally believed to exert their anticholinergic toxic effects essentially in a similar manner.

Although acetyl cholinesterase shares its nucleophilic activity of a serine hydroxyl group with many other hydrolases, notably chymotrypsin, its substrate specificity stems from the still ill defined binding phenomenon. The large variation in the chemical structure among the substrates and inhibitors that react with the enzyme precluded any attempt to oversimplify or unify the binding patterns [203].

The level of acetylcholinesterase in brain in diabetic group fell by 13 times. With selenium feeding the level rose.

Dash et al found hyperglycemia due to experimental diabetes induced in rats, causes a decrease in the activity of acetylcholinesterase in brain regions and heart [80].

Wahba et al estimated the activities of acetylcholinesterase in the seminal vesicle and in urinary bladder in streptozotocin induced diabetic rats. They found increase in the enzyme in detrusor muscles and insignificant result in seminal vesicles [82].

Urea biosynthesis is divided into four stages : (1) transamination, (2) oxidative deamination of glutamate, (3) ammonia transport, (4) reactions of the urea cycle.

The blood urea level rose by 20.4% in diabetic mice. With selenium treatment the levels rose and in Dose 2 fed group the level rose more than twice the normal.

With production of experimental diabetes the content of selenium in pancreas was increased by more than 10 times the normal value. The level of selenium in liver rose by one and half times in diabetic mice. In brain there is marginal lowering of selenium content and in blood also there is lowering of the selenium level.

Mice has 38 autosomes and 2 sex chromosomes. Mitotic chromosomes are observed at metaphase stage. Metaphase plates contained 40 chromosomal elements. An ideogram karyotype of mice somatic cells are made by arranging them according to their decreasing order in size. It has been shown that 40 chromosomes and the diploid chromosome set, though appeared to be

very similar to each other can be subdivided into 5 distinct groups, each including chromosomes of more or less similar sizes.

In our study no chromosomal abnormality was found on production of diabetes and on selenium treatment no change occured.

Homogenates of tissue from mature animals that do not show a rapid rate of mitosis readily undergo lipid peroxidation (e.g. brain, liver, kidney) whereas those tissues that are not susceptible to lipid peroxidation (e.g. testis and intestinal epithelium) undergo rapid cell division [204].

In histopathology of pancreas the islets of Langerhans showed a variety of changes, but in about one-third of pancreas no abnormality can be detected. In early diabetes a lymphocyte infiltrate (insulitis) may be present. In late stages the β cells show vacuolation due to an accumulations of glycogen and atrophy of the islets with replacement by amyloid or fibrous tissue.

Selenium treatment could not reverse the process of streptozotocin induced atrophy of the islets.

Low levels of dietary selenium do not cause cancer, but decreased levels of Se increase susceptibility to cancer given a carcinogenic exposure.

Slonim et al found that the mice fed on Vit E, selenium deficient diet, showed increased susceptibility to diabetes development by ordinarily non-diabetogenic doses of streptozotocin [109].

Douillet et al studied the effect of selenomethionine along with Vit E on platelet activity in diabetic rats in vitro and found a reduction in platelet thrombin, ADP induced aggregation, in adhesiveness to fibronectin and in sorbitol content [123].

Kowluru et al found dietary supplementation into antioxidants offers a means to inhibit multiple hyperglycaemia induced retinal metabolic abnormalities [125].

Naziroglu et al found Vit C to outweigh the effect of Vit E and selenium against oxidative damage to lens [126].

Kowluru et al suggested that antioxidants inhibit abnormal metabolic processes that may contribute to the development of cardiac disease in diabetes [127].

Goemen et al stated that a decrease in the sensitivity of the neurogenic impairment to antioxidant action may develop more rapidly than that of endothelial dysfunction in streptozotocin induced diabetic mice [128].

Kowluru et al stated that the alterations of retinal glutamate, oxidative stress and NO appear to be inter-related in diabetes and antioxidant therapy may be a suitable approach to determine the roles of these abnormalities in the development of diabetic retinopathy [131].

Reddi et al found that selenium supplementation to diabetic rats prevents not only oxidative stress but renal structural injury as well [132].

Kowluru et al found that long term administration of antioxidants can inhibit the development of the early stages of diabetic retinopathy [134].

In our study on the role of selenium in experimental diabetes we found that many parameters are normalised by selenium feeding in streptozotocin induced diabetic mice. But some parameters are not corrected, even some got worse.

So we may conclude thus that selenium is not a true replacement of insulin. A dose responsive action occurs on diabetes. The best dose has to be determined and an adjuvant has to be found to provide together the best result on diabetes.

This idea is leading towards the role of mixture of antioxidants for protection against the complication of diabetes.

Parameters	Statistical analysis
1. Blood Sugar	Dose-2 - deviation from normal is insignificant.
2. Glucose 6 phophatase in liver	Dose-2 - produces most significant reduction.
3. Glucose 6 po_4 dehydrogenase	Dose-2 - deviation from normal is insignificant.
4. Pyruvic acid in liver	Dose-2 - deviation from normal is insignificant.
5. Glycogen in liver	Dose-2 - deviation from normal is insignificant.
6. Lactic acid in blood	Dose-2 - produces most significant reduction.
7. Lactic acid in heart	No change.
8. Lactic acid in muscle	Dose-2 - produces insignificant deviation but at 3%.
9. Lactic acid in liver	Dose-2 - produces insignificant deviation but at 2%.
10. Succinic dehydrogenase in liver	Dose-2 - produces insignificant deviation from normal.
11. Blood cholesterol	Dose-2 - produces insignificant deviation from normal.
12. Serum LDL	Dose-2 - produces insignificant deviation from normal.
13. Serum VLDL	Dose-2 - produces insignificant deviation from normal.
14. Serum HDL	Dose-2 - produces insignificant deviation from normal.
15. Serum triglyceride	No dose shows significant improvement.
16. Liver triglyceride	No dose shows significant improvement.
17. HMG-CoA reductase in liver	Dose-2 - produces insignificant deviation from normal.
18. Glutathione in liver	D-2 & 3 - produces insignificant deviation from normal.
19. Glutathione in blood	D-2 - produces most insignificant deviation from normal.
20. Glutathione reductase in liver	Dose-2 - produces insignificant deviation from normal.
21. Glutathione S-transferase in liver	No significant variation from normal except Dose-3.
22. Hepatic UDP-glucoronyl transferase	No doses produce insignificant deviation from normal.
23. Lipid peroxidation in liver	Dose-2 - produces insignificant deviation but at 3%.
24. Lipid peroxidation in brain	No dose produces significant improvement to normal.
25. Lipid peroxidation in kidney	No dose produces significant deviation towards normal.
26. Catalase in liver	No change.
27. Cyt P-450 in liver	No dose show significant improvement.
28. Fibrinogen in blood	D-2 - produces insignificant deviation to normal at 6%.
29. Monoamine oxidase in serum	Dose-2 - produces insignificant deviation from normal.
30. Acetyl choline esterase in brain	No significant improvement.
31. Urea in blood	No dose show significant improvement.

SUMMARY

7.0 Summary :

Diabetes mellitus is a chronic disease characterised by hyperglycemia and glucosuria. The major forms of diabetes result from defects of insulin secretion, insulin action or both. It is associated with long-term damage, dysfunction or failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels.

Incidence rates of diabetes varies widely in different countries, with a high incidence in Scandinavia and Sardinia and a low incidence in Japan and China. However, in India data suggest the incidence rate 10.5/1,00,000/year.

The mainstay of treatment of diabetes is done by lowering the level of blood sugar through diet control, use of oral hypoglycaemic drugs and insulin injection. All have their limitations and long term complications and many fail to control the adverse effects of diabetes on different systems of the body.

Research on antioxidant leads to the identification of selenium as a potent oxygen free radical scavenger. Linkage of data showing relationship between selenium in soil and pancreatic cancer gave the idea of possible association of selenium in pancreas related disease like diabetes. Vanadium also showed an insulinomimetic action. Our study on selenium also showed the normoglycaemic action of this element on the experimentally induced diabetic mice by intraperitonial injection of streptozotocin. Selenium in the dose of 0.05 micro gm/0.1 ml had the best hypoglycaemic action.

The following are the major highlights of the effects of selenium on different biochemical pathways while producing hypoglycaemia in diabetic mice.

With respect to carbohydrate metabolism, the levels of glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, succinic dehydrogenase, pyruvic acid, glycogen, lactic acid were brought to or near normal value on treatment with selenium in streptozotocin induced diabetic mice. Only exception in case of lactate dehydrogenase in serum, the level went on rising on selenium treatment both in normal and diabetic groups.

With respect to lipid metabolism, the levels of cholesterol, LDL, VLDL, HDL, triglyceride, HMG-CoA reductase were almost normalised on selenium feeding in experimentally induced diabetic mice. In vanadium fed groups the serum triglyceride level was much higher than the normal value.

In the groups of experiments related to detoxicating microsomal enzymes the levels of glutathione and glutathione reductase were normalised in selenium treated experimentally induced

diabetic groups. But the levels of glutathione-s-transferase and UDP-glucoronyl transferase went on rising on selenium feeding in streptozotocin induced diabetic mice.

In the group of peroxidation reactions the levels of lipid peroxidation came to normal in selenium fed diabetic group. In the lipid peroxidation level in liver in both selenium and vanadium fed diabetic mice the levels were normalised. Catalase showed no effect on selenium feeding. Cyt-P-450 level was halved in selenium treated experimentally induced diabetic mice. In case of fibrinogen the level rose almost 3 times in diabetic group and with selenium treatment the level fell but remain more than twice the normal value. In monoamine oxidase the level was almost normalised in selenium fed diabetic group. The level of acetyl choline esterase in brain in diabetic group fell by 13 times. With selenium feeding the level rose more than 3 times the diabetic level. In blood urea the level rose more than twice the normal value in selenium treated expeirmentally induced diabetic group.

The selenium content in pancreas was increased by more than 10 times the normal value on production of experimental diabetes.

Chromosome preparation showed no change in normal, diabetic and Selenium Dose2 fed diabetic groups.

In histopathology of pancreas, streptozotocin treatment showed destruction of pancreatic islet cells. On selenium feeding there was no change of islet cells.

Thus it can be concluded that the activity of the enzymes lactate dehydrogenase, glutathione-s-transferase, UDP-glucoronyl transferase and the levels of fibrinogen, urea rose more in the selenium fed group than the normal and diabetic groups.

The activity of the enzyme acetyl choline esterase and the level of Cyt-P-450 monoxygenase fell in the selenium treated group. Lipid metabolism showed complete normalisation with selenium treatment.

It is seen that although selenium normalises the blood sugar level in the diabetic mice still it is not a complete substitute of insulin. To replace insulin some other compound may be added with selenium or the salt of selenium may be changed, like in the lipid peroxidation in liver addition of vanadium with selenium to diabetic animals normalised the blood sugar level. This hypothesis needs further investigations.

However, the mode of action of selenium on cellular level is a place of future research. The receptor locking activity and effect on the messengers for introduction of glucose inside cell is the field for further research.

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