REVIEW OF LITERATURE
Diabetes Mellitus (DM) is an ancient disease. The earliest description of its symptoms is found in the Ebers Papyrus of Egypt, dating back to 1500 B.C. One of the first clues to the pathology underlying diabetes came in 1889 when Minkawski and Von Merong noticed that urine of dogs whose pancreas were removed attracted an unusual number of flies and on analysis of the urine, they found high levels of glucose in it. These findings strongly suggested that the pancreas was secreting a substance which regulated the metabolism of glucose. Later in 1921, Banting and Best extracted insulin from the pancreas of a dog and injected it into a diabetic dog. This resulted in a fall in the blood sugar levels.

The discovery of insulin was hailed as a cure for diabetes because it lowered blood glucose levels, controlled the acute symptoms and prevented death from diabetic coma. But diabetics who had been on insulin for a long time were found to have an unusually high incidence of heart attack, stroke, kidney failure, gangrene and blindness. Insulin treatment controlled early symptoms but not the development of long term complications (Notkins 1979).
Definition, Classification and Aetiology of Diabetes Mellitus

Although DM may be regarded as a disease of ancient origin, it is also a "disease of civilization". The prevalence of diabetes clearly has been shown to increase as urbanization, working patterns and diet habits change from primitive to civilized modes (Skillman and Tzagournis 1973). The different and often conflicting findings about the nature of diabetes and its long term complications have led many to suggest that DM is heterogenous in aetiology, clinical presentation, susceptibility to complications and response to treatment. The spectrum is so wide that DM is presently regarded as a syndrome rather than a disease entity.

Definition: The syndrome of DM is characterized by the disorders of metabolism of carbohydrates, proteins and lipids due to insulin deficiency and/or insulin resistance (in other words absolute or relative deficiency of insulin) evolving from the interaction of a variety of genetic and environmental factors. Metabolic derangement is manifested by persistent hyperglycaemia with or without hyperlipidemia and a tendency to develop ketoacidosis. Also there may be progressive involvement of blood vessels and nerves resulting in accelerated atherosclerosis, microangiopathy and neuropathy (Tripathy 1983).
Classification: The staging and classification of DM has of late been subjected to basic alterations in view of newer informations on the evolution and clinical behaviour of this disorder. The classification adopted by WHO Expert Committee on DM (1985) is reproduced in Table 1.

Aetiology: DM arises from a complex interaction between the genetic constitution of the individual and specific environmental factors. Further, age, sex and race may exercise a good deal of influence in the genesis and development of DM.

A. Genetic Aspects of Diabetes. There is possibly no controversy on the question of importance of genetic factors in the development of diabetes. Positive family history can be obtained from 25 to 50% of diabetics. Compared to controls, the disease is 4 times more common among parents, 9.5 times among siblings, and at least twice as common among other relatives of known diabetics. Among offsprings it is 2.2 times more common in case one parent is diabetic and 3.9 times if both the parents are diabetic. Importance of hereditary factor has been further established from observations on twins. While diabetes affects both, 10-20% of fraternal twins, 50-80% of identical twins are concordant for the disease (Tripathy 1983). Further, in the largest study of NIDDM twin pairs, 48 out of 53 pairs were found
<table>
<thead>
<tr>
<th>A. Clinical Classes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Diabetes Mellitus</td>
<td></td>
</tr>
<tr>
<td>* Insulin dependent diabetes mellitus (IDDM)</td>
<td></td>
</tr>
<tr>
<td>* Non-Insulin dependent diabetes mellitus (NIDDM)</td>
<td></td>
</tr>
<tr>
<td>a) Non-obese</td>
<td></td>
</tr>
<tr>
<td>b) Obese</td>
<td></td>
</tr>
<tr>
<td>* Malnutrition related diabetes mellitus (MRDM)</td>
<td></td>
</tr>
<tr>
<td>* Other types of diabetes associated with certain conditions and syndromes such as pancreatic disease, disease of hormonal etiology, drug-induced or chemical induced conditions, abnormalities of insulin or its receptors, certain genetic syndromes, miscellaneous</td>
<td></td>
</tr>
<tr>
<td>(ii) Impaired glucose tolerance (IGT)</td>
<td></td>
</tr>
<tr>
<td>a) Non-obese</td>
<td></td>
</tr>
<tr>
<td>b) Obese</td>
<td></td>
</tr>
<tr>
<td>c) Associated with certain conditions and syndromes</td>
<td></td>
</tr>
<tr>
<td>(iii) Gestational diabetes mellitus</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Statistical risk classes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(subjects with normal glucose tolerance but substantially increased risk of developing diabetes)</td>
<td></td>
</tr>
<tr>
<td>* Previous abnormality of glucose tolerance (Prev AGT)</td>
<td></td>
</tr>
<tr>
<td>* Potential abnormality of glucose tolerance (Pot AGT)</td>
<td></td>
</tr>
</tbody>
</table>
to be concordant for the syndrome, a striking contrast to only 80 out of 147 IDDM twin pairs. Further 42% of the identical twin pairs with NIDDM have first degree relatives similarly affected. In contrast, 18% of the identical twins with insulin dependent diabetes have such a family history (Taylor 1989). These data suggest that the genetic basis of NIDDM is strong. But inspite of a stronger genetic basis, type II diabetes so far does not show any obvious association with HLA system. However epidemiological studies of HLA in IDDM have been found to vary among different diabetic populations. Among the Chinese, HLA-DR 3 was shown to be significantly high in those with IDDM in Beijing and Shangai. A similar association was observed in Chinese in Taiwan. In Chinese with IDDM in Singapore, HLA-AW33, B 17 and DR 3 were significantly high. In Thai, IDDM HLA-DR 3 was significantly high. Among three Indian studies, all reported from North India, one study showed significant association with HLA-B8 and BW 21, another failed to show any association with HLA-B8 but confirmed the association with BW 21, and the third confirmed both HLA-B8 and BW 21 along with DR 3. In Koreans with IDDM the incidence of HLA-BW 54 was reported to be significantly high (Tandhanand and Vannasaeng 1987).

B. Environmental Factors. Generally obesity, physical inactivity, urbanisation, stress and traumas have been
recognised as the factors that may predispose to or precipitate diabetes.

i) Nutrition: Overnutrition and obesity have been ascribed great significance in the aetiology of NIDDM by many writers. (WHO 1985, Rossini et al 1988) Incidence of diabetes is 4 times higher in persons with moderate obesity and may be 30 times higher in those with severe obesity.

In sharp contrast, diabetes also tends to occur in young subjects with chronic undernutrition. In several developing countries a sizeable proportion of older children, adolescents and young adults with diabetes behave differently than what is observed in western countries. Their total consumption of protein and calories falls far shorter than the required amounts, so that they are lean or underweight long before the development of diabetes. These ketosis resistant young diabetics hail from economically depressed families dwelling in remote rural areas and is now referred as Malnutrition Related Diabetes Mellitus (MRDM) (Tripathy 1983).

In certain areas patients of similar age and nearly similar economic status suffer from diabetes, associated with ductal lithiasis and fibrosis of pancreas. Large proportions of young diabetics seen in certain endemic areas have radiological evidence of pancreatic calculi. This form
of pancreatic diabetes is encountered in Southern States and Orissa in India. Majority of these patients are undernourished from childhood.

Thus, MRDM and pancreatic diabetes in the young are presently acknowledged to be related to nutritional deficiency (Tripathy 1983).

ii) Ageing and exercise: The prevalence of NIDDM increases markedly with age. Ageing per se is neither associated with decreased insulin secretion nor with decreased insulin sensitivity, provided that adequately nourished, physically active subjects are studied. If the decreasing levels of physical fitness in the elderly population as a whole are considered, then the effect of lack of exercise on insulin sensitivity may hasten the appearance of hyperglycaemia as it may at any age (Taylor 1989).

iii) Other factors: Numerous observations reveal higher prevalence of diabetes among the urban compared to the rural population. It is difficult to say if this is due to factors independent of nutrition, physical activity or stress and strain of life. While diabetes may often be diagnosed for the first time during pregnancy, this does not suggest that parity is a potent risk factor. The concept of trauma infection stress increased epinephrine hyperglycaemia
diabetes, is not acceptable without reservation for lack of sufficient data. Yet in subjects with latent diabetes each of the above factors does lead to development of overt disease or aggravate already existing florid diabetes (Tripathy 1983).

Viral infection and autoimmunity are not associated with development of type II diabetes.

**Prevalence of Diabetes Mellitus**

The incidence of diabetes is increasing all over the world and is now recognised as a common and universal disease. According to WHO (1985) about 30 million people are confirmed diabetics throughout the world with a continuous upward trend.

The prevalence of diabetes in Asia has been stated to range from \(\approx 1\%\) to almost 5%. The Chinese in the Peoples' Republic of China have the lowest prevalence rate (0.67%), whereas the Chinese who settled outside mainland China have prevalence rate of 1.6% in Singapore, 4.7% in Malaysia, 4.97% in Indonesia and 5.78% in Taiwan. Similarly Indian migrants to other parts of Asia have reported prevalence rates of 4.2% in Malaysia and 4% in Singapore (Tandhananand and Vannasaeng 1987). The prevalence within the country
based on a multicentric study of Indian Council of Medical Research (1972-75) showed a rate of 1.73% in those 15 years and older. Overall male/female ratio was 2.3% and 1.4% respectively. The results of age break-up were as follows:

Less than 30 years .. .. prevalence was 0.5%
30 - 39 years .. .. prevalence was 1.0%
More than 40 years .. .. prevalence was 4.0%

These data suggest that genetic predisposition to diabetes manifests itself in migrant population groups through better living conditions, marriage within close-knit migrant communities and/or psychological stress. In most of the reported studies, prevalence of diabetes tends to increase with age with peak prevalence in people 50-60 years old.

**Insulin in relation to diabetes mellitus**

1) Secretion: Insulin is initially synthesized in the beta cells of the pancreas as a larger single chain polypeptide precursor known as proinsulin (Steiner 1969). During its storage in the B-cell, the single chain pro-insulin is cleaved, resulting in the removal of a connecting strand (C-peptide) and the appearance of the smaller, double chain insulin molecule. Insulin and the C-peptide remnants are packaged in membrane-bound storage granules. Stimulation of insulin secretion results in the release of these granules
and the discharge of equimolar amounts of insulin and C-peptide (Rubinstein et al 1969). The rise in blood glucose associated with carbohydrate meal induces the B-cells in the islets of Langerhans to secrete insulin into the circulation. The insulin is then carried out in the blood stream to target cells throughout the body, where it binds to receptor molecules on the cell surface. This interaction triggers a series of events inside the cells that enhances the uptake of glucose from the blood and its subsequent breakdown for metabolic energy or storage as glycogen and fat. A defect anywhere along this pathway could result in DM (Notkins 1979). Possible causes include a) destruction of beta cells, b) abnormal synthesis of insulin, c) retarded release of insulin, d) inactivation of insulin in blood stream by antibodies or other blocking agents, e) altered insulin receptor or a decreased number of receptor on peripheral cell, f) defective processing of insulin message within the target cells— and g) abnormal metabolism of glucose.

Among the two major types, in IDDM, insulin secretion is either totally defective or severely impaired. Endogenous insulin production in these patients generally varies inversely with the duration of the disease. In the maturity onset diabetic, the secretory failure is less severe. Basal insulin concentration is generally normal or increased
whereas glucose stimulated insulin secretion is generally diminished.

**Action of Insulin**

A number of enzyme systems are known to be regulated by insulin and these enzymes have been demonstrated to be phosphoproteins. Such insulin-regulated enzymes controlled by phosphorylation-dephosphorylation reactions include glycogen synthetase, pyruvate-dehydrogenase, pyruvate kinase and acetyl CoA carboxylase (Czech 1981). Thus insulin with its anabolic properties conserves glucose as glycogen and amino acids as proteins, increases deposition of neutral fats and thus favors glycogenesis and lipogenesis. Further it promotes preferential oxidation of glucose to provide energy and spares fats and protein breakdown. These anabolic changes occur in three principal tissues i.e. liver, muscle and adipose tissue. The antioatabolic as well as anabolic effects of insulin in these tissues are summarized in Table 2. A reduction in circulating insulin leads to mobilization of endogenous fuels and reduced uptake of ingested nutrients.

In the case of mild insulin deficiency the fasting blood sugar level is usually normal, but hyperglycemia occurs after a load of carbohydrate. This is because of
Table 7  Action of insulin

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Adipose Tissue</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Anticatabolic effects</td>
<td>a) decreased protein catabolism</td>
<td>a) decreased lipolysis</td>
<td>a) increased fatty acid synthesis</td>
</tr>
<tr>
<td></td>
<td>b) decreased glucose-</td>
<td></td>
<td>b) increased amino acid output</td>
</tr>
<tr>
<td></td>
<td>neogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c) decreased ketogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Anabolic effects</td>
<td>a) increased glycogen</td>
<td>a) increased glycerol synthesis</td>
<td>a) increased amino acid uptake</td>
</tr>
<tr>
<td></td>
<td>synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) increased fatty acid</td>
<td>b) increased fatty acid synthesis</td>
<td>b) increased protein synthesis</td>
</tr>
<tr>
<td></td>
<td>synthesis</td>
<td></td>
<td>c) increased glycogen synthesis</td>
</tr>
</tbody>
</table>

Muscle Adipose tissue liver
failure of liver to trap glucose after a glucose load and this leads to hyperglycemia because of entry of larger amount of glucose into general circulation. Moderate insulin deficiency results in fasting and postprandial hyperglycemia. This is because of overproduction of glucose by gluconeogenesis and glycogenolysis and diminished peripheral utilization of glucose. In severe diabetic state, gross failure of insulin produces primarily hyperglycemia and secondary hyperlipidemia and hyper aminoacidemia. Due to lack of insulin, there is underutilization of glucose in insulin dependent tissues and an overproduction of free fatty acid (FFA) and amino acids from adipose tissue and muscle as alternate fuels. In a severe condition, the production of FFA exceeds that can be utilized by peripheral tissues (Stauffacher and Renold 1971).

In addition to increased fatty acid mobilization, the development of hyperketonemia requires an increase in the ketogenic capacity in the liver. The mechanism whereby insulin deficiency leads to augmented activity of the carnitine acyl transferase reaction is believed to involve augmented transfer of carnitine from extrahepatic sites to the liver as well as a fall in intrahepatic levels of malonyl Co A which is a potent inhibitor of the carnitine acyltransferase reaction. Also an elevation in glucagon
concentration increases the level of carnitine as well as the activity of carnitine acyl transferase and decreases the concentration of malonyl Co A in excess of that attributable to insulin deficiency alone. As a result of fatty acid oxidation, the increased availability of acetyl Co A exceeds the capacity for its oxidation to carbon dioxide via Kreb's cycle resulting in condensation of acetyl Co A molecules to form ketone bodies. Due to the deficiency of insulin, the ketones released by the liver are not metabolised at normal rates by muscle tissue and thus accumulate within the blood (Smith and Their 1981).

Hyperlipidemia and Diabetes

Hyperlipidemia is a relatively common problem in patients with poorly controlled DM. It has been estimated that the frequency of elevated plasma lipid levels in diabetic patients is between 20 and 90 per cent depending on the degree of diabetic control and the type of diabetes (Dunn, 1982). There are several associations for this: a) Insulin plays an important role in the regulation of intermediary lipid metabolism and fluctuations in the degree of diabetic control thus produce variable effects on plasma lipoprotein metabolism (Havel 1976, Nikkila 1974), b) many NIDDM patients are obese and obesity may lead to the development of
hyperlipidemia (Reaven and Bernstein 1978), although diabetes and hyperlipidemia represent different genetic disorders, each of these disorders is common in the population and the two disorders may co-exist by chance in the same individuals (Dunn 1982).

Atherosclerosis and Diabetes

One of the principal reasons of interest in the relationship between diabetes and hyperlipidemia is due to the unusually high prevalence of accelerated atherosclerosis in diabetic patients (Kannel and McGee 1979). Premature cardiovascular disease is one of the most common and serious chronic complications of diabetes. Diabetic patients tend to have higher lipid values and tend to have a higher incidence of hypertension and obesity than non-diabetics, thereby resulting in 2 to 3 fold increase in cardiovascular morbidity and mortality when compared with non-diabetics (Kannel and McGee 1979a, b).

Many factors appear to contribute to this enhanced atheromatous process in diabetes including alterations in platelet function, clotting factors, arterial smooth muscle cell metabolism and blood pressure regulation (Ganda 1980, Dunn 1982). Nevertheless, changes in plasma lipoprotein levels in diabetes remain one of the most important associated
risk factors in terms of accelerated atherosclerosis (Gorden et al. 1977, Santen et al. 1972). In addition, diabetes may alter lipoprotein structure and metabolism independent of increases in plasma lipid levels and these altered lipoproteins may be associated with accelerated atherosclerosis (Mahley 1981).

Lipid and Lipoprotein Metabolism

The lipoproteins transport lipids in the blood from sites of production to sites of removal or alteration. The task of transporting essentially water-insoluble lipids is achieved by virtue of the unique physicochemical features of the lipoproteins. This allows apolar triglyceride (TG) and cholesteryl esters to be sequestered within the core of the particle and free cholesterol and phospholipid to be arranged around this as a surface "membrane". Certain polypeptides called apoproteins (designated A through I*) specific for each type of lipoprotein, are thought to be arranged mainly on the surface extending in some cases into the lipid core. They participate in structure, serve as ligands for cell receptors, and modulate the activity of key enzymes.

The sites of origin of the lipoproteins are the gut and the liver - the former representing the site of entry of exogenous lipid destined largely for storage, while the
liver acts as a center for synthesis and collection of lipid from various sources and distributes these either into excretory (bile), secretory (lipoprotein), or storage pathways.

There are four major lipoproteins classified according to their density: Chylomicrons, Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL) and High Density Lipoprotein (HDL) (Table 3).

The chylomicrons consist almost entirely of dietary cholesterol and triglyceride. They are synthesized within luminal cells of the small intestine and are secreted into lymphatics. The regulation of this process is not fully understood. As part of the mixed meal, this process will be accompanied by elevated plasma insulin and glucagon levels. After gaining entry to the circulation, the chylomicron receives a specific apolipoprotein apo C II from HDL (Havel et al 1973). Apo C-II is an activator of the enzyme crucial to the metabolism of the chylomicron, namely lipoprotein lipase (LPL). This enzyme is located at the capillary endothelial junction in a number of tissues including adipose tissue and skeletal muscle. LPL hydrolyzes the chylomicron TG. As a result, the structure of chylomicron is altered. The excess surface material becomes detached from the chylomicron, to transfer to HDL, and to leave
<table>
<thead>
<tr>
<th></th>
<th>% content by weight</th>
<th>Origin</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Molecular weight</td>
<td>Triglyceride</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Chylomicrons</td>
<td>$10^9$</td>
<td>80-95</td>
<td>3-7</td>
</tr>
<tr>
<td>VLDL</td>
<td>$10^7$</td>
<td>50-70</td>
<td>15</td>
</tr>
<tr>
<td>LDL</td>
<td>$2.1-2.6 \times 10^6$</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>HDL</td>
<td>$2 \times 10^5$</td>
<td>3</td>
<td>16-20</td>
</tr>
</tbody>
</table>
behind a chylomicron remnant containing dietary cholesterol. The remnant lipoprotein is able to interact with a hepatic receptor specific for apolipoprotein E, which becomes exposed on the remnant. By this mechanism dietary cholesterol is transported to liver (Goldberg 1981).

While the chylomicrons bear exogenous fat, VLDL secreted by the liver and to a small extent by the intestine carries endogenous lipid. Hepatic VLDL TG is synthesized by the esterification of fatty acids (FA) (derived either from the circulation or from de novo hepatic synthesis) with glycerophosphate (derived from anaerobic glycolysis) but intestinal VLDL-TG is normally synthesized only from intraluminal FA. The lipids destined for transport are packaged together with specific apoproteins and secreted into the circulation. VLDL-TG formation by the liver is stimulated by the action of insulin, and a high CHO diet will increase VLDL production. Increasing the supply of FA substrate for TG synthesis to the liver will also stimulate VLDL production and secretion, although probably only suboptimally if insulin is deficient (Goldberg 1981).

In a similar fashion to the chylomicron, VLDL also is believed to receive the lipase activator, apo C II from HDL and undergo hydrolysis of its TG moiety (50-60% by weight of VLDL). An intermediate density lipoprotein (IDL) is
formed in small amounts during this process. The remnant produced at the end of this reaction is the cholesterol rich lipoprotein LDL. Part of the cholesterol in LDL is destined for peripheral tissues via a receptor mediated uptake process requiring as a ligand—the specific apoprotein in LDL—apo B (Goldberg 1981).

HDL is secreted by the liver but appears to receive important contributions from the intestines via chylomicrons and possibly from VLDL. HDL via its apoprotein A-I is an activator of a serum enzyme, lecithin cholesterol acyl transferase (LCAT) that is responsible for esterifying serum cholesterol. HDL is known to participate in the return of tissue cholesterol to the liver for excretion and may restrain the delivery of LDL cholesterol to the tissues (Miller and Miller 1975). Schematic model of plasma lipoprotein metabolism as proposed by Brown and Goldstein is given in Figure 1.

**Lipoprotein Lipase and Hormone Sensitive Lipase**

LPL is considered to be primarily responsible for the removal of major portion of triglyceride from TG rich lipoproteins (Chylomicrons and VLDL), although there may be differences in the modes of interaction of these lipoproteins with LPL. LPL is synthesized within tissue cells and
FIG. 1. MODEL OF PLASMA LIPOPROTEIN METABOLISM AS PROPOSED BY BROWN AND GOLDSTEIN

Dietary fat — Bile Acids + cholesterol

Intestine — Liver — Extra hepatic Parenchymal cells — Scavenger cells

Chylomicrons — VLDL — LDL

Lipoprotein lipase — HDL

Adipose and Muscle capillaries — Remnants — LCAT
transported to the endothelial surface. It is usually measured in plasma obtained following an intravenous bolus of heparin, which displaces it from its endothelial binding sites. More recently, it has been recognized that LPL in adipose tissue differs from that of skeletal or cardiac muscle in that adipose tissue LPL requires insulin for its activity and its capacity to increase in the fed state, whereas muscle LPL is less insulin dependent and is more active in the postabsorptive state (Goldberg 1981). Thus in the fed state circulating TG rich lipoproteins (chylomicrons and VLDL) will be hydrolyzed in adipose tissue and the released FA are partly stored. In the post-absorptive state VLDL-TG will be hydrolyzed in muscle where FA are required as fuel.

Following the hydrolysis of lipoprotein TG the released FA may circulate as part of the free FA (FFA) pool or may be esterified and deposited in the adjacent cells provided glycerophosphate is available. Both TG hydrolysis and glycerophosphate availability are activated by insulin. FFA may also be released from stored TG by the action of the intracellular enzyme hormone sensitive lipase. This enzyme is depressed by insulin but stimulated by the counter regulatory hormones. The amount of FA hydrolyzed from lipoproteins, deposited from tissue stores, or released from the latter is normally an integrated process, finely
tuned by the actions of insulin and the counter regulatory hormones, growth hormone, glucagon, cortisol, and the catecholamines. Insulin plays a major role in this regulation (Goldberg 1981).

Pathophysiology of Lipids and Lipoprotein Metabolism in Diabetes

Hyperlipidemia is the most common lipid abnormality observed in diabetic patients. This is usually caused by the accumulation of VLDL and rarely of chylomicrons in the plasma (Nikkila 1974). Hypercholesterolemia may also develop secondary to increased VLDL levels, though changes in LDL and HDL levels also occur with variable degrees of diabetic control (Dunn 1982). Various mechanisms have been reported to account for the effect of diabetes on lipoprotein metabolism and this appears to depend on both types of diabetes and the degree of insulin deficiency.

In type I diabetes, acute insulin deficiency results in a rapid increase in both free fatty acid mobilization from adipose tissue and secretion of VLDL and ketone bodies from the liver (Balasse et al 1972). This enhanced VLDL-TG synthesis from the liver appears to be shortlived since after 1 or more days of insulin deficiency, the ability of the liver to secrete VLDL-TG diminishes. At the same time,
clearance of TG from the plasma becomes impaired. This latter effect is thought to be the result of decreased activity of the enzyme lipoprotein lipase, which requires the presence of insulin for maintenance of adequate tissue levels (Havel 1976). Lipoprotein lipase is located at the endothelial cell surface of capillaries in adipose and muscle tissues throughout the body and is responsible for hydrolysis of TG from both chylomicrons and VLDL. After interaction with LPL, FFA and monoglycerides are liberated from lipoprotein to enter adjacent muscle or adipose cells, and the TG depleted chylomicrons and VLDL are then released from the capillary wall to re-enter the circulation as lipoprotein remnants. LPL requires insulin for maintenance of adequate activity and insulin deficiency results in a decrease in plasma and adipose tissue LDP levels. An indirect measure of plasma LPL activity which has been commonly used is the postheparin lipolytic activity (PHLA). Invivo studies in patients with Type 1 diabetes have confirmed decreases in PHLA that correlates with defects in plasma TG clearance (Bagdale et al 1968). On the other hand impaired catabolism of VLDL in diabetic patients may not totally be due to decreased LPL activity but may also reflect acquired abnormalities of VLDL structure which render this lipoprotein less amenable to interact with LPL (Bar-on et al 1981).
The situation in patients with Type II DM is somewhat different. Diminished PH1A has been observed in some patients in whom gross lipemia develops with chronically poor diabetic control. However, most patients in Type II diabetes are not insulin deficient, but rather have normal or elevated plasma insulin levels which are low only in terms of the relative plasma glucose level (National Diabetes Data Group 1979). These patients often develop mild to moderate degrees of hypertriglyceridemia but do not appear to have significant reductions in plasma LPL activity. Studies of invivo TG metabolism using radiolabeled VLDL-TG have suggested that the primary defect in poorly controlled diabetes is increased synthesis of VLDL-TG by the liver (Nikkila and Kekki 1973). In several groups of studies, overproduction of VLDL-TG was found in poorly controlled diabetic patients and the hepatic production of VLDL-TG decreased with improved diabetic control (Dunn et al. 1980). Thus these workers concluded that the primary defect leading to the development of hypertriglyceridemia in poorly controlled Type II diabetes is overproduction of VLDL-TG.

Diabetes also appears to have a significant effect on plasma cholesterol metabolism. Uncontrolled hyperglycemia has been reported to be associated with increased synthesis of total body cholesterol though this change may not be
solely due to insulin deficiency. Hypercholesterolemia can occur in diabetic patients for one of two reasons:

(a) Increases in plasma VLDL levels may result in secondary increases in the plasma cholesterol levels, since VLDL carries about 20 per cent of its total lipid content as cholesterol.

(b) Diabetes appears to have significant effects on plasma LDL metabolism. Increases in plasma LDL levels have been reported in both Type I and Type II diabetes which fall with improved diabetic control (Dunn 1982).

The reason for the effect of diabetes on LDL metabolism is not yet known, but several mechanisms have been suggested. VLDL is the major precursor for LDL in the plasma and increased synthesis of VLDL in poorly controlled diabetes might lead to increased formation of LDL. This relationship is supported by evidence suggesting an inverse correlation between plasma VLDL-TG levels and LDL-C levels in normolipidemic individuals (Philips et al 1981). Secondly, decreased LDL catabolism could be due to decreased ability of LDL to interact with its cell surface receptor (Gonen et al 1981, Witzman et al 1982). One reason for decreased LDL catabolism has been attributed to invivo glycosylation of plasma LDL which alters the configuration of LDL so that it is less able to interact with the specific LDL receptor.
responsible for the majority of LDL catabolism in normal individuals (Witzman et al 1982).

The effect of diabetes on HDL metabolism is variable. In Type I diabetes HDL-C levels have been reported to be normal (Kennedy et al 1978; Sosenko et al 1980; Mani and Mani 1987) or increased (Nikkila and Hormila 1978), when compared with age, weight and sex matched normals. It has been suggested that insulin treatment raises HDL-C levels in Type I diabetes and this may be partly responsible for these varied results.

In Type II diabetes, plasma HDL-C levels tend to be decreased (Kennedy et al 1978, Lopes-Varella et al 1977, Mani and Mani 1987). The data from the Framingham study showed that decreased HDL-C levels constituted a major risk factor for coronary atherosclerosis in diabetics. Also, Miller and Miller (1975) have concluded that HDL-C bears the same relationship to the risk of atherosclerotic vascular disease in diabetics as in non-diabetics. However, the correlation between HDL-C and vascular disease has not been observed in all the subgroups of diabetics. The association seems to be stronger in NIDDM patients, obese diabetics and in female patients (Kannel 1987, Gorden et al 1977, Mani and Mani 1987, Thelle et al 1982, Walden et al 1984, Haward et al 1984). These data suggest that the risk of arterio-
sclerosis is more in diabetic women than diabetic men.

The overall pathophysiology of diabetic hyperlipidemia is summarized in Table 4.

**Risk Factors for Macrovascular Disease in Diabetes**

The various putative risk factors for atherosclerotic vascular disease in diabetes is given in Table 5 and discussed below.

1) Genetic predisposition

The striking geographic differences in the frequency of the macrovascular disease among diabetics appear to reflect the frequency of macrovascular disease in the non-diabetic population, rather than there being a specific genetic basis for the complication. Where low rates of macrovascular disease are found in diabetic patients, the food intake pattern both in diabetic and non-diabetic groups is characterized by a greater contribution to energy intake from carbohydrate and a lesser contribution from fat (Rudnick and Anderson 1962, West and Kalbfleisch 1971).

ii) Sex difference

The impact of diabetic macrovascular disease in women is greater than it is in men. Coronary heart disease is 1.7 times as common in diabetic men as in non-diabetic,
Table 4  Summary of pathophysiology and clinical features of diabetic hyperlipidemia

<table>
<thead>
<tr>
<th></th>
<th>Type I DM</th>
<th>Type II DM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1 Nature of hyperlipidemia</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
  a) Hypertriglyceridemia  
  b) Usually mild to moderate  
  c) Occasionally severe |  
  a) Hypertriglyceridemia  
  b) Usually mild to moderate  
  c) Occasionally severe |
| **2 Abnormal lipoprotein** |  
  a) VLDL—occasionally chylomicron |  
  a) VLDL—occasionally chylomicron |
| **3 Pathophysiology** |  
  a) Decreased removal  
  b) LDL activity reduced  
  c) Related to insulin deficiency hyperglucagonemia |  
  a) Both decreased removal and increased production  
  b) Obesity, familial hypertriglyceridemia  
  c) Suggested role for insulin resistance, hyperinsulinism |
| **4 Status of other lipoproteins** |  
  a) LDL elevated in some |  
  a) LDL elevated in some  
  b) HDL normal or increase |  
  b) HDL tend to decrease |
Table 5  Risk factors for atherosclerotic vascular disease in diabetes

<table>
<thead>
<tr>
<th>Genetic predisposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex differences</td>
</tr>
<tr>
<td>Duration of the disease</td>
</tr>
<tr>
<td>Abnormal glucose tolerance</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
</tr>
<tr>
<td>Hypertriglyceridaemia</td>
</tr>
<tr>
<td>Low LDL concentrations</td>
</tr>
<tr>
<td>Elevated FFA concentrations</td>
</tr>
<tr>
<td>Altered FA patterns</td>
</tr>
<tr>
<td>Enhanced platelet aggregation</td>
</tr>
<tr>
<td>Cigarette smoking</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Obesity</td>
</tr>
<tr>
<td>Lack of exercise</td>
</tr>
</tbody>
</table>
but 2.7 times more common in diabetic women as in non-diabetic
(Garcia et al 1974, Kannel and McGee 1979a, b).

iii) Duration of the disease

Duration of diabetes is known to affect the prevalence
and severity of atherosclerotic vascular disease (Deckert,

iv) Abnormal glucose tolerance

Abnormal glucose tolerance without evidence of clinical
diabetes is associated with risk of macrovascular disease
intermediate between that of normal glucose tolerance and
frank diabetes (Wahlquist et al 1984). In the Whitehall
prospective study of cardiovascular mortality and the Paris
population study, the risk of hyperglycemia was associated
with the threshold effect which operates with the sharp
doubling of mortality at the 95th percentile of the 2 h
blood glucose concentrations (Fuller et al 1980, Eschege
et al 1980).

v) Hyperinsulinemia

Various experimental studies have supported the
hypothesis that elevated levels of circulating insulin
per se might predispose to atherosclerotic vascular disease
(Ganda 1980, Stout 1981). Population studies in Australia,
Scotland and Finland indicate that elevated serum insulin
levels are associated with CHD mortality as an independent risk factor (Wahlquist et al 1984). In a prospective study of 7000 non-diabetic men aged 43-54 years and followed for an average of 63 months, Ducimetiere and colleagues (1980) have found that the fasting serum insulin levels were predictive of subsequent coronary events as an independent variable together with systolic blood pressure and cigarette smoking.

vi) Hyperlipidaemia

Hypercholesterolemia has long been recognized as one of the cardinal risk factors for atherosclerotic vascular disease in population studies.

The metabolic aberrations of diabetes increase TG levels (Goldberg 1981). However hypertriglycercedemia is not shown to be an independent risk factor (Ducimetiere et al 1980, Steiner 1981).

Irrespective of actual lipid levels, lipoprotein metabolism is altered in diabetes and it has been suggested that diabetes might increase the sensitivity of arteries to risk factors such as LDL-C levels (Steiner 1981) without marked differences in the actual LDL-C levels. HDL-C concentrations can be low in some diabetic populations and the disproportionately low HDL-C concentrations in diabetic
women are cited as an explanation for the loss of protection of females from macrovascular disease (Gorden et al 1977, Reckless et al 1978).

FFA concentrations in plasma tend to be higher where there is lack of insulin or insulin action and this phenomenon is well recognized in diabetics. Experimental data indicate a relationship between plasma FFA concentrations and morphological changes in arteries in animal studies (Reinila 1981).

Fatty acid patterns may also be different in diabetics with possible implications for prostaglandin metabolism (Jones et al 1983).

vii) Enhanced thrombogenesis

Various aspects of haemostasis are altered in diabetes, including elevated fibrinogen levels and decreased fibrinolysis (Ganda 1980) and increased platelet aggregation.

viii) Cigarette smoking

In the Framingham study, diabetic men smoked fewer cigarettes each day than non-diabetic men. Smoking added to risk of CVD in diabetes without appearing to be synergistic with the diabetic state (Kannel and McGee 1979).

ix) Hypertension

Hypertension and particularly elevated systolic pressure
are risk factors by multi-variate analysis for diabetic macrovascular disease in population studies with the end point of CHD mortality or CVD mortality (Kannel and McGee 1979b, Fuller et al 1980, Jarett, Wahlquist et al 1984).

x) Obesity

Obesity not only increases the risk and severity of NIDDM patients but also the likelihood of associated risk factors such as hypertension, hyperlipidemia and hyperinsulinaemia. Obese women diabetics who sustain a myocardial infarction have a high mortality rate (Wahlquist et al 1984).

xi) Lack of exercise

Physical training in NIDDM patients improves glucose, insulin and lipid profiles. However its long term beneficial effect in terms of preventing the incidence of macrovascular disease, or favourably influencing mortality has yet to be studied prospectively.

Complications of diabetes mellitus

DM is one of the most important metabolic disorders which affects every cell in the body and its essential biochemical processes. The secondary complications that cause most of the morbidity and mortality are the result of biochemical abnormalities induced by an inability to control carbohydrate metabolism associated with the disease. The
secondary complications of DM are microangiopathy, retinopathy, nephropathy, neuropathy etc (Truel et al 1980). Although the acute and often lethal symptoms of diabetes can be controlled with insulin therapy, the long term complications of the disease reduces the life expectancy by as much as third. Compared with non-diabetics, diabetics show 25 times higher rate of blindness, 17 times higher rate of kidney disorders, 5 times higher rate of gangrene and of heart disease twice as high (Notkins 1979).

The secondary complications seen in diabetic patients is found to involve alterations in vascular basement membrane composition as well as accumulation of glucose derived reaction products due to over utilization of glucose in insulin independent tissues. Well known examples are the sorbitol pathway which is thought to result in hyperosmolar liaisons and increased biosynthesis of basement membrane thickening (Spiro 1976). Apart from these enzyme catalyzed pathways, more recently, another reaction has gained potential interest namely the non-enzymatic glycosylation (NEG) of proteins.

Non-enzymatic glycosylation of proteins

Glycoproteins are a class of conjugated proteins containing covalently linked CHO groups to the polypeptide
chain. They represent a large group of wide distribution and considerable biological significance. In animals glycoproteins occur as an integral part of cell membranes and structural tissues and also are free in the various body fluids and secretions. Thus virtually all the plasma proteins are glycoproteins except albumin, globulins, fibrinogen, various carrier proteins like lipoproteins etc. The CHO content of plasma glycoproteins is less than 50% and CHO units are usually N-glycosidically linked to proteins (Clamp 1975).

The formation of glycoproteins is generally under strict enzymatic control. However, sugars can also bind nonenzymatically to proteins under physiologic conditions. Thus NEG of proteins may contribute to an understanding of two important problems in diabetes. They are:

(a) assessment of metabolic control
(b) pathogenesis of the long term complications.

Biochemical basis of NEG

NEG of proteins is the covalent attachment of glucose to free amino groups of the proteins (lysine residues). The reaction proceeds through an initial Schiff-base formation and then a subsequent isomerization (Amadori rearrangement) to form a stable ketoamine linkage (Fig 2).
Figure 2 Chemistry of non-enzymatic glycosylation

Glucose Aldimine Ketoamine
(Schiff base)

1. Proteins with T½ of days to weeks
Glucose + NH₂-protein → Schiff base → Amadori product

2. Long lived structural proteins
Glucose + NH₂-protein → Schiff base → Amadori product
Advanced Glycosylation End Products
(Brown fluorescent pigments which crosslink proteins)
There are distinctly two different types of NEG products, depending on the half life of the protein involved (Fig 1).

i) Short lived proteins i.e. enzymes, serum albumin and apoproteins will react with sugar and form Schiff-base at a rate reflecting the ambient concentration of glucose (Vlassara et al 1986). Within a few weeks a slow chemical rearrangement will form a more stable but still reversible adduct, the Amadori product. The chemistry of this product has been extensively studied in glycosylated haemoglobin. Within weeks the concentrations of Amadori products reach equilibrium and thus will not increase any further and process becomes irreversible. Proteins from diabetic tissue consistently contain two- to three-fold greater concentrations of Amadori products than do proteins from normal tissue regardless of the length of exposure to hyperglycemia (from weeks to years).

ii) In contrast, proteins that turn over at a much slower rate, such as crystallins, collagen, elastin, and myelin proteins will accumulate different NEG products derived very slowly from Amadori products. Unlike the Amadori products, the formation of these slowly glycosylated products is chemically irreversible. At this stage of advanced glycosylation, the end products will continue to accumulate for the life of the protein, leading to significant
structural and functional alterations, primarily increased cross-linking between protein molecules and their derivatives (Gerami et al 1988).

**Main physiological determinants of NEG**

Several independently acting variables determine the extent of NEG, pH, temperature, protein, glucose concentrations and time of exposure to glucose. In vivo, the most important factors are glucose concentration and incubation time. The duration of hyperglycemia is critical, not only because the concentration of Amadori products increases as a function of time, for these soon reach equilibrium but rather mainly because the advanced glycosylation and products (AGEP) continue to accumulate over the entire lifetime of slowly turning over proteins such as collagen (the single most widespread protein in the body, present in every vessel, small or large, and all basement membranes). The net increase of those end products, which occurs at a much faster rate in patients with diabetes than in normal individuals, appears to be the link between chronic hyperglycemia and a number of altered physiological processes (Brownlee et al. 1984, Vlassara et al 1986, Cerami et al 1988, Kennedy and Lyons 1989).
Pathophysiological Consequences of NEG

The major biological effects of excessive NEG include:

i) inactivation of enzymes
ii) inhibition of binding of regulatory molecules
iii) crosslinking to glycosylated proteins and trapping of soluble proteins by glycosylated extracellular proteins
iv) decreased proteolysis
v) recognition and endocytosis of glucose modified protein by macrophages
vi) abnormalities of nucleic acid function, and
vii) possibly increased immunogenecity and tissue cytotoxicity.

Changes in Serum

The interest in NEG gained momentum by studies of haemoglobin $A_1c$ which was found to be present at more than 3 times the normal concentration in the poorly controlled diabetic patients (Koenig et al 1976). Since the rate of NEG depends on the ambient blood glucose concentrations and also on the life span of the protein involved (e.g. RBC 120 days) the GHb levels reflect integrated glucose concentration over the previous few weeks and hence represent an index of long term blood glucose control (Bunn 1981).
i) Plasma Proteins - Albumin

Plasma proteins are subject to glycosylation more severely than any other kind of proteins. This phenomenon is best characterized with albumin. The serum glycosylated albumin levels reflect fluctuations of glycemia within shorter period of time, perhaps 1-2 weeks in keeping with its half life of approximately 17 days (Schultze and Heremans 1966). Although the mean values for per cent GHb and per cent G-Alb were not greatly different in the control population, the range of increase in the diabetic population was greater for G-Alb (upto 30%) than for HbA (9-20%) suggesting that G-Alb may be a more sensitive indicator of integrated blood glucose, albeit over a shorter time span (Gutthrow et al 1979). Dolhofer and Wieland (1979, 1980), Nayak and Pattabiraman (1982) and Mani et al (1987) have also reported that NEG of serum albumin is increased in diabetics and levels correlated with the mean fasting blood glucose. Various investigators have mentioned that the estimation of glycosylated protein level could serve as a sensitive indicator for glycemic control in diabetic patients (McFarland et al 1979, 1984, Yue et al 1980, Krishnaswamy et al 1981, Kennedy et al 1981, Mani et al 1987).

ii) Lipoproteins

NEG of serum lipoproteins has been demonstrated by many investigators (Schleicher et al 1981, Gonen et al 1981,
Witzum et al 1982, Mani et al 1987). There was a two-to three-fold increase in the amount of lysine-bound glucose of apoprotein B of LDL isolated from the serum of diabetic patients. This finding is of special interest since it is known that chemical modification of lysine amino groups of apoprotein B e.g. acetylation interferes with specific receptor mediated cellular LDL uptake and favours cholesterol ester accumulation via the atherogenic scavenger pathway (Goldstein and Brown 1977). NEG of apoprotein B is the first example of an in vivo covalent modification of lysine residues of LDL. This may change properties of LDL as suggested by studies on human fibroblast indicating a decrease in cellular binding, uptake and degradation of G-LDL (Gonen et al 1981, Witzum et al 1982).

Thus the increased level of G-LDL in diabetic patients may contribute to a better understanding of the high incidence of atherosclerosis in diabetes.

All these studies have clearly indicated that NEG of serum proteins steadily increases in DM because of which the origin and significance of increased serum glycoproteins are of considerable interest in DM. Serum glycoproteins may also reflect metabolic changes occurring in tissues.
Changes in tissues

The ability of glucose in hyperglycemia to aggregate and cross-link lens proteins, leading to opacification under otherwise physiological conditions is long known. Two types of cross-links are formed from NεG of lysine amino groups:

(a) The disulfide bond type from oxidation of previously unexposed sulphydryl groups (Monnier et al 1979).

(b) The AGE type which forms over the long half life of the crystalline lens protein.

The latter type lends to the lens characteristic spectroscopic properties similar to those of brunescent or senile human cataracts (Monnier and Cerami 1981).

Collagen from normal subjects shows an age-related linear increase in the accumulation of AGE pigment as well as an increase in diabetic like morphological changes and mechanical properties consistent with high cross-linking of proteins (Cerami et al 1979, Monnier et al 1984, Andreassen et al 1981, Yue et al 1983). The single most important consequence of advanced glycosylation of structural proteins is that the generated reactive groups can trap "innocent bystander" soluble proteins, some of which can be rendered damaging (Fig 3). Albumin and IgG, for example, entrapped on glycosylated collagen, retain their ability to form
FIG. 3 COVALENT TRAPPING OF POTENTIALLY DAMAGING NON GLYCOSYLATED PLASMA PROTEINS BY ADVANCED GLYCOSYLATION END PRODUCT OF COLLAGEN

Hyperpermeable vessel wall

Albumin

IgG

Extra vascular Matrix

NH₂

Protein deposition
Basement Membrane thickening

Advanced Glycosylation En & products

Complement activation
Immune Complex formation
Tissue damage

Another important aspect of the advanced glycosylation of connective tissue components is their potential role in atherogenesis by trapping lipoproteins on the arterial wall. Recent studies have indicated that LDL at an LDL-C concentration of 1030 mg/l is covalently bound by glycosylated collagen more than 3 fold as much as by normal collagen (Brownlee et al 1985). Although LDL has a rather short circulation half life, its subsequent immobilization on long-lived glycosylated vessel wall proteins can promote lipid accumulation in fibrous plaque. Further, by preventing the diffusion of LDL out of the intima, this immobilization enhances formation of AGEF on the LDL particle itself. Subsequent uptake of G-LDL by scavanging macrophages may further contribute to the atherogenic process by endocytosis.
of the modified LDL leading to foam cell formation (Cerami et al 1985, Cerami et al 1987).

Recently it has been proposed that the structural alterations observed due to NEG process are largely dependent upon hydroxyl radicals produced by glucose autoxidation indicating a therapeutic role for antioxidants (Hunt et al 1988).

In summary, AGEP can covalently trap extravasated serum proteins such as IgG, IgM, albumin and LDL by glucose derived cross-linking to extravascular matrix. This trapping may contribute to capillary narrowing in the retina and the glomerulus, and to the arterial narrowing in the coronary, cerebral, and peripheral circulation. Similarly entrapped immunoglobulins may contribute to tissue damage. Thus the structural alterations of basement membrane collagen due to overglycosylation are found to be involved in diabetic micro-angiopathy which is unique to diabetes.

A spectrum of proteins that are glycosylated and are found to be the underlying causes for the life threatening secondary complications which the diabetics are more prone to is given in Table 6.
<table>
<thead>
<tr>
<th>Protein</th>
<th>Physiologic function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Hemoglobin</td>
<td>Oxygen exchange</td>
</tr>
<tr>
<td>2 Red Cell Membrane</td>
<td>Deformity in Microvasculature</td>
</tr>
<tr>
<td>3 Antithrombin III</td>
<td>Inhibition of excessive coagulation</td>
</tr>
<tr>
<td>4 Fibrinogen</td>
<td>Plasma viscosity and clot formation</td>
</tr>
<tr>
<td>5 Fibrin</td>
<td>Clot maintenance</td>
</tr>
<tr>
<td>6 Endothelial cell membrane</td>
<td>Maintenance of vascular integrity</td>
</tr>
<tr>
<td>7 Lens crystallins</td>
<td>Transmission of light to retina</td>
</tr>
<tr>
<td>8 Lens capsule</td>
<td>Focussing of light on retina</td>
</tr>
<tr>
<td>9 Myellin</td>
<td>Nerve impulse conduction</td>
</tr>
<tr>
<td>10 Tubulin</td>
<td>Axonal transport</td>
</tr>
<tr>
<td>11 Glomerular basement membrane</td>
<td>Renal filtration barrier</td>
</tr>
<tr>
<td>12 Collagen</td>
<td>Tissue structural property, scar and plaque formation</td>
</tr>
<tr>
<td>13 Coronary artery proteins</td>
<td>Vessel integrity for myocardial perfusion</td>
</tr>
<tr>
<td>14 Low density lipoprotein</td>
<td>Lipid transport and metabolism</td>
</tr>
<tr>
<td>15 High density lipoprotein</td>
<td>Lipid transport and metabolism</td>
</tr>
<tr>
<td>16 Albumin</td>
<td>Osmotic regulation: transport of metabolites</td>
</tr>
<tr>
<td>17 Cathepsin B</td>
<td>Intracellular protein degradation</td>
</tr>
<tr>
<td>18 Pancreatic RNA se</td>
<td>Hydrolysis of RNA</td>
</tr>
<tr>
<td>19 Ferritin</td>
<td>Iron storage</td>
</tr>
</tbody>
</table>
Collagen in relation to Diabetes Mellitus

Collagen is the main fibrous protein of connective tissue and the most abundant protein in the human body. The collagen molecules after being secreted by the cells, assemble into the characteristic fibres responsible for the functional integrity of tissues such as bone, cartilage, skin and tendon.

Collagen is a glycoprotein which is unique in its amino-acid composition as it contains unusual amino-acids such as 4 and 3 hydroxyproline, 5 hydroxylysine, extracellular location, tertiary structure and mode of biosynthesis (Nimni 1983). Each collagen molecule consists of three strands of polypeptide chains that have a unique helical structure. Each of the polypeptide chains contains a glycine residue at every third position. Proline and hydroxyproline follow each other relatively frequently and the gly-pro-hyp sequence makes up about 10% of the molecule. Tryptophan and sulphur containing amino-acids are not usually present and very small amounts of phenyl alanine and tyrosine are present. This triple helical structure generates a symmetrical pattern of three left handed helical chains, that are in turn slightly displaced to the right, superimposing an additional "Super coil" (Ramachandran 1967). The structure of collagen molecule is shown in Figure 4.
FIG. 4. SCHEMATIC DRAWING SHOWING THE COLLAGEN TRIPLE HELIX.

- Glycine
- Predominantly Imino Acids
Detailed investigations in this area have resulted in the characterization of five structurally and genetically distinct types of collagen present in different parts of the body as shown in Table 7 (Prockop et al 1979). In mammalian collagen the amount of hydroxyproline (an imino acid rarely found in proteins other than collagens) varies from 11 to 14% and therefore, the biochemistry of hydroxyproline is intimately connected with that of collagen. Extensive studies with radioactive tracer using $^{14}C$ proline have shown that both biosynthesis and degradation of collagen follow unique steps. The biosynthesis of collagen is shown in Table 8 (Kivirikko and Risteli 1976) and degradation of collagen in Figure 5 (Adams and Frank 1980).

There are two general types of collagen namely interstitial (Type I to III) and basement membrane (Type IV and V). Interstitial collagens are predominant and are responsible for the great tensile strength of the connective tissue. The basement membrane is a delicate non-cellular layer of variable thickness interposed between the epithelium and the adjacent connective tissue of the intestinal tract, blood vessels, renal glomeruli, and tubules and most other tubular and granular structures.

The collagen of the basement membranes have several distinctive features. They are:
Table 7. Structurally and genetically distinct collagens

<table>
<thead>
<tr>
<th>Tissue Distribution</th>
<th>Molecular Form</th>
<th>Chemical Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone, tendon, skin dentin, ligament, fascia, arteries and uterus</td>
<td>1(I)₂ 2</td>
<td>Hybrid composed of 2 kinds of chains. Low in hydroxylysine and glycosylated hydroxylysine</td>
</tr>
<tr>
<td>Hyaline cartilage</td>
<td>1(II) 3</td>
<td>Relatively high in hydroxylsine and glycosylated hydroxylsine</td>
</tr>
<tr>
<td>Skin, arteries and uterus</td>
<td>1(III) 3</td>
<td>High in hydroxyproline and low in hydroxylysine; contains interchain disulfide bonds</td>
</tr>
<tr>
<td>Basement membranes</td>
<td>1(IV) 3</td>
<td>High in hydroxylysine and glycosylated hydroxylysine; high in hydroxyproline (3 &amp; 4); may contain large globular regions</td>
</tr>
<tr>
<td>Basement membranes and perhaps other tissues</td>
<td>A &amp; B</td>
<td>Similar to Type IV</td>
</tr>
</tbody>
</table>
### Table 9: General steps in the biosynthesis of collagen

<table>
<thead>
<tr>
<th>Biosynthetic step</th>
<th>Biological significance of the biosynthetic step</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Transportation and translation</td>
<td>Primary structure</td>
</tr>
<tr>
<td>2 Hydroxylation of prolyl residues</td>
<td>Essential for triple helix formation at 37°C</td>
</tr>
<tr>
<td>3 Hydroxylation of lysyl residues</td>
<td>Essential for glycosylation reactions</td>
</tr>
<tr>
<td>4 Glycosylation of hydroxylsyl residues</td>
<td>Essential for stability of cross-links</td>
</tr>
<tr>
<td>5 Chain association and disulphide bonding</td>
<td>Possibly affect fibre formation</td>
</tr>
<tr>
<td>6 Triple helix formation</td>
<td>Essential for normal rate of procollagen secretion into the extracellular matrix</td>
</tr>
<tr>
<td>7 Secretion of procollagen into the extracellular matrix</td>
<td>Essential for extracellular modifications</td>
</tr>
<tr>
<td>8 Conversion of procollagen into collagen</td>
<td>Essential for normal fibre formation</td>
</tr>
<tr>
<td>9 Aggregation of collagen molecules</td>
<td>Fibre formation</td>
</tr>
<tr>
<td>10 Cross-link formation</td>
<td>Essential for stability of fibres</td>
</tr>
</tbody>
</table>

* These steps are not entirely sequential. For instance, steps 2-4 are initiated while the polypeptide chains are growing on the ribosomes and they are continued after the release of completed polypeptide chains from the ribosomes. Also, in certain instances, step 5 can occur before steps 2-4.
Fig 5  Degradation of Collagen Molecule

Collagen

Collagenase

{ Rat uterus
{ Human skin
{ Synobial fluid
{ Granulocytes
{ Crab hepatopancreas
{ Bone

Polypeptides and Oligopeptides

Dipeptidase
Prolidase
Imino peptidase

Free Hyp and Hyp-containing Peptides

(Reabsorbed by kidney and rapidly metabolised to pyruvate and glyoxalate)

(80 Heterogeneous
Pro - 4 Hyp,
4 Hyp - Glu,
Gly-5 Hyp-4 Hyp)

excreted in urine
1) it is rich in hydroxylysine which is almost fully glycosylated

2) is relatively abundant in 3-hydroxyproline besides 4-hydroxyproline and

3) is low in alanine and arginine content.

Studies by Kafalides (1979, 1980) have shown that the biosynthetic pattern of basement membrane collagen is similar to that of interstitial collagens. These observations have demonstrated that the structural and functional integrity of both the connective tissues and basement membranes are dependent on the net collagen biosynthesis and degradation. Changes in collagen structure occur with age and alterations in the rate of synthesis and degradation of collagen may be critical in a number of diseases such as diabetes, atherosclerosis and kidney disorders.

Urinary Excretion of Hydroxyproline

As 4-hydroxyproline is an imino acid synthesized during post-translational hydroxylation of appropriate proline residues, formation of the bulk of 4-hydroxyproline in animals depends on the synthesis and maturation of collagen. This relationship influences every aspect of 4-hydroxyproline metabolism and since 4-hydroxyproline cannot
be directly reutilized for protein synthesis it is committed to excretion or catabolism (Adams and Frank 1980).

The 4-hydroxyproline released as a free compound from protease susceptible peptides is efficiently reabsorbed by the kidney and rapidly catabolised. The peptides resistant to proteolysis are largely excreted in urine in the form of mainly small peptides. Thus a very small fraction of total excreted 4-hydroxyproline, less than 5% is free, an additional fraction 5 to 10% of the total represents non-dialysable peptides up to at least 10,000 molecular weight and rest is in the form of small peptides approximately di and tripeptides. Almost 80, 4-hydroxyproline containing peptides have been described and identified in the urine (Adams and Frank 1980). The excretion of hydroxyproline in urine has provided a convenient tool for studies on the collagen metabolism since hydroxyproline is almost exclusively present in collagen and therefore forms an in vivo label for this protein. Numerous studies of urinary hydroxyproline have shown that young animals excrete more hydroxyproline than older animals presumably because there is higher turnover of collagen in the growing stage (Adams and Frank 1980). An increased excretion of hydroxyproline is observed in diseases affecting the bone-like Paget's disease, bone tumors, rickets, osteomalacia, osteoporosis, acute osteomyelitis etc. due to higher
turnover of collagen. However, very few investigators have studied the urinary hydroxyproline levels in diabetics. Studies by Recchia et al (1967) indicated slightly increased level of urinary hydroxyproline in a few patients with juvenile diabetes mellitus. Recent study by Mani and Mani (1986) indicated a higher excretion of hydroxyproline in the case of diabetics in general and insulin dependent diabetics in particular. The most striking observation was the difference in the hydroxyproline content of the two major fractions "HP fraction 1" and "HP fraction 2" from normals and diabetics. While HP fraction 2, an acidic fraction predominantly containing large molecular weight polypeptides with hydroxyproline present in only small amounts (5-10% of the total urinary hydroxyproline pool) in the case of normals, it turned out to be the major fraction in IDDM subjects possibly due to a higher turnover of collagen or abnormalities in the degradation of collagen.

Pathobiochemistry of Basement Membrane Thickening

Patients with diabetes develop premature and extensive disease of their blood vessels compared to the non-diabetics of comparable age. The vascular disease appears as arteriosclerosis and as microangiopathy. The latter is a disorder of capillaries and many small vessels essentially unique to diabetes (Siperstein 1972). This vascular lesion which is
unique to diabetes is a disorder characterized by thickening of basement membrane that surrounds capillaries. Studies have revealed that the basement membrane contains both collagen (Type IV) and non-collagenous components such as laminin, fibronectin, glycosaminoglycans etc. whose metabolic fate is influenced by the ambient glucose concentration (Spiro 1969).

Several groups of investigators have performed compositional studies on human GBM from normal and diabetic subjects (Beisswenger and Spiro 1970, Parthasarthy and Spiro 1982, Shimamura and Spiro 1987). Beisswenger and Spiro were the first to demonstrate an increase in basement membrane protein in glomeruli from patients with chronic diabetes who showed histologic evidence of nephropathy. In addition the composition of this material was found to be distinctly different from that of normal basement membrane (Beisswenger and Spiro 1973, Spiro 1973). When compared with normal GBM, membrane from diabetics showed an increase in hydroxylysine content and a proportional decrease in lysine content such that the sum of the two was constant. Diabetic membrane also exhibited elevations in glucose and galactose reflecting a rise in hydroxylysine linked disaccharide residues. In addition there were smaller, yet significant increases in hydroxyproline and glycine as well
as decreases in valine and tyrosine. These results suggest that there is an increase in the hydroxylysine rich subunits composed of collagen-like amino acids in the diabetic BM.

Many workers (Kilo et al 1972, Vracko 1970, Seiss et al 1979) have confirmed the thickening of basement membrane in skeletal muscle capillaries in diabetics. The relation between diabetic control and width of skeletal muscle capillary BM was also studied by Raskin et al (1985). Their studies on Type 1 diabetics revealed a significant reduction in the BM thickness as the blood glucose control was improved. Although the pathogenesis of diabetic nephropathy is unknown, morphological, immunological and biochemical studies by Micheal et al (1978) demonstrated certain unique features of this disease. A consistent finding in glomerular and tubular basement membrane was the demonstration of a striking immunofluorescence for IgG and albumin, a finding which is not present in other diseases.

Effects of ageing and diabetes on basement membrane thickening of six cell types was studied by Vracko (1978). He found that BM lamina accumulate primarily in tissues in which basal lamina layering is a normal byproduct of all cell replenishment and same tissues are involved in diabetes.

GBM synthesis was studied by Cohen (1978). The earliest changes detectable in the diabetic renal glomerulus
consists of an increase in the width of peripheral BM. The findings of the study suggested that excess GBM in diabetes results from the net effect of hyperglycemia and insulin deficiency and that metabolic control is an important factor in delaying the progression of the diabetic nephropathy. As stated earlier various investigators have shown an increase in hydroxylysine, hydroxyproline, glycine levels in the human diabetic GBM which represents a relative enrichment of BM collagen components. Klein et al (1975) showed that diabetic glomeruli from human kidneys were larger and heavier than non-diabetic glomeruli. Diabetic glomeruli had greater hydroxyproline content than non-diabetic glomeruli and glomeruli from diabetics of largest duration showed greatest increase in mass and hydroxyproline values.

Thus the results of the various studies showed that BM collagen synthesis reacted very sensitively to the metabolic control of the disease.

Kivirikko and Ristelli (1976) reviewed the biosynthesis of collagen and its alterations in pathological states. Collagen comprises of 40-60% of protein in human fibrous atherosclerotic plaques and its deposition in the arterial intima has been suggested as being largely responsible for the occlusive and irreversible nature of coronary and cerebral arterial disease. In diabetes there is an increased
synthesis of collagen and thereby increasing the risk of atherosclerosis.

Parthasarthy and Spiro (1982) demonstrated the effect of diabetes on the glycosaminoglycans (GAG) component of the human GBM. A significant decrease in the GAG content of diabetic membrane accompanied by a significant elevation of hexoses, which are primarily associated with collagen components was observed, thereby suggesting that a redistribution of BM molecules occurred in the diabetic state.

Interaction of certain serum lipoproteins with arterial tissue macromolecules is considered as one of the mechanisms of cholesterol accumulation in human atherosclerotic lesions. The interaction of several macromolecular components such as collagen, proteoglycans, glycoproteins and lipoproteins in the normal morphological characteristics of the arterial wall integrity and the possible changes during the pathogenesis of the disease leading to atherosclerosis was studied by Berenson et al (1984). DM is thus found to play a key role in accelerating the development of atherosclerosis, due to excessive LDL trapping by hyperglycemia induced advanced glycosylation end products on collagen.

Recently Spanheimer et al (1988) have demonstrated that in experimental rats the collagen production was significantly
reduced in bone and cartilage of diabetic rats within two weeks after induction of diabetes. Factors that were found to correlate with altered collagen production were the duration of the disease and the amount of weight loss. They concluded that marked changes in collagen production may contribute to the chronic connective tissue complications in diabetes.

Shimomura and Spiro (1987) in their study on macromolecular components of human glomerular basement membrane have shown that there is an increase in the GBM collagen. This data indicates that in diabetes there is an alteration in the macromolecular architecture of BM and this may be responsible for the filtration defect and the ultimate glomerular occlusion.

Thus from the various studies it has been established that collagen plays a potential role in the acceleration of secondary complications due to

(a) its glycoprotein nature, and
(b) excessive cross-linking ability.

Further, urinary hydroxyproline levels would serve as an important index of collagen metabolism. Thus based on the above literature it can be concluded that DM is a chronic disease which precipitates various secondary complications which are life threatening. Also it has been observed that
glycemic control plays an important role in reducing/reversing the risk for various complications. Hence fundamentally the primary purpose of nutrition therapy is to achieve normal blood sugar level by optimizing the glucose utilization, normalising glucose production and enhancing insulin sensitivity. Therefore the primary aim should be good management that would reduce the risk for micro and macrovascular complications.

Management of Diabetes Mellitus

The management of DM is one of the important aspects in the control of the disease. The aims of nutritional advice for diabetics should include the following:

1) Maintain ideal body weight
2) Maintain or improve glucose tolerance
3) Maintain as near as practicable euglycaemia
4) Maintain normolipidemia including cholesterol, triglycerides and high density lipoprotein
5) Ensure an adequate intake of all nutrients
6) Allow ample physical activity
7) Consider the social function of the food.

Management of DM includes:

a) diet alone
b) diet and hypoglycemic drugs
c) diet and insulin.

Luft (1979) have reported that among the diabetics 20 to 30% are maintained on diet alone, 40 to 50% on insulin, and oral hypoglycemic drugs are used where diet alone does not give satisfactory control of diabetes.

Dietary control is the most preferred treatment for DM as it is free from all objectionable side effects (Antia 1975).

Prior to the discovery of insulin in 20th Century, the treatment of DM included intermittent fasting, undernutrition and carbohydrate restriction. With the advent of insulin a more liberal diet was made possible and a variety of diets were advocated with the variations in total calories as well as in proportion of CHO, protein and fat contents.

Till recently the fundamental principle of the diabetic diet was to restrict CHO intake and the diet consisted of about 33% CHO, 17% protein and 50% fat. In 1968 Vishwanathan observed better results with diet containing 60% CHO, 10% protein and 30% fat. Brunzell et al (1971) noted improvement in glucose tolerance by feeding 85% CHO. Similar results were also reported by Vishwanathan et al (1974), Tripathy et al (1975), Kiehm et al (1976), Anderson and Ward (1978),
Anderson et al (1979), Jenkins (1979). Since then the evolution of high CHO diet started.

Subsequently it was noticed that protein content of the diet was insufficient and in order to make the diet more balanced, the protein contents were raised to 19%, keeping the CHO content almost constant (67%). This resulted in reduction of fat content e.g. 14% (Vishwanathan et al 1974) and showed rapid and effective control of diabetes (Vishwanathan and Vaishnava 1977, Vishwanathan et al 1978).

The hypoglycemic effect of CHO in DM is attributed to their fibre contents. Among various foods which provide fibre such as cereals, pulses, fruits and vegetables have shown hypoglycemic or hypolipidemic properties. Because of a number of beneficial properties, DF has achieved a new status in the field of Nutrition and Medicine.

**Dietary Fibre**

Ingestion of large amounts of plant fibre has become one of the latest food fads and has been strongly advocated for diabetic patients. Since the identity of the dietary fibre source plays an important role in exhibiting the known beneficiary effects, an understanding of the nature and chemical composition of the macromolecules which go into the formation of "fibre network" is essential.
Dietary fibres (DF) are the macro-molecular polysaccharides and lignins present in the cell walls of plants, which resist the digestion in the human gut (Trowell 1972). Thus the DF differs from the crude fibre (CF) which is the residue obtained after chemical treatment of the plant material with weak acid followed by weak alkali (AOAC 1970). Crude fibre normally amounts to 20-50% of the total dietary fibre in most diets. All foods of vegetable origin contribute to the fibre content of the diet, while whole grain cereals and cereal by-products such as bran are rich sources of dietary fibre.

Physico-chemical nature of the dietary fibre

The indigestibility of DF is because of the structural complexity of the different macromolecules due to the cross-linking of several polysaccharides.

The polysaccharides are:

1) Cellulose

It is the most abundant macromolecule in nature. It is a long (upto 10000 sugar residues) linear polymer of 1,4 Beta-linked glucose unit. Hydrogen bonding between sugar residues in adjacent chains imparts a crystalline microfibril structure. Cellulose is insoluble in strong alkali (Aspinall 1973).
ii) Hemicelluloses

They are those cell wall polysaccharides solubilized by aqueous alkali after removal of water soluble and pectic polysaccharides. They contain backbones of Beta-1,4 linked pyranoside sugars, but differ from cellulose in that they are smaller in size (often less than 200 sugar residues), contain variety of sugars, and are usually branched. The hemicelluloses are subclassified on the basis of the principal monomeric sugar residue present in the molecule. Acidic and neutral forms differ in the content of glucuronic and galacturonic acids (Theander and Amen 1979). Uronic acid formation involves the oxidation of the terminal - CH₂OH to COOH and is of biological importance since the sugar residues become available for methylation, amidation, and the formation of cation complexes. Hemicelluloses, especially the hexose and uronic acid components are somewhat more accessible to bacterial enzymes than is cellulose (Southgate et al 1976).

iii) Pectins

Pectins are a complex group of polysaccharides in which D-galacturonic acid is the principal constituent. They are structural components of plant cell walls and also act as intercellular cementing substances. This includes a water insoluble parent compound (protopectin, pectinic acids and
pectic acids) and pectin. The backbone structure of pectin is an unbranched chain of axial-axial L (1-4)-linked D-galacturonic acid units. Long chains of galacturonan are interrupted by blocks of L-rhamnose rich units that result in bends in the molecule. Many pectins have neutral sugars covalently linked to them as side chains, mainly arabinose and galactose, and to a lesser extent xylose, rhamnose and glucose. It has also been shown that small quantities of glucuronic acid may be linked to pectin in a side chain (Pinnilc and Voragen 1973, Worth 1967, Knuss 1974). The carboxyl groups of the galacturonic acids are partially methylated and the secondary hydroxyls may be acetylated. Pectin is highly water soluble and is almost completely metabolised by colonic bacteria. Other non-cellulose polysaccharides include gums, mucilages and algal polysaccharides (Aspinall 1973, McNeely and Kovacs 1976).

iv) Mucilages

These polysaccharides consist of mainly uronic acid polymers with a high water holding capacity. They tend to form viscous gel when foods containing them are homogenised with water.

v) Gums

Gums are secretary cells of plants which they produce
at the site of injury. These gums contain complex polysaccharides.

vi) Algal polysaccharides

The polysaccharides of algae vary in uronic acid and sulfate content derived from algae and sea weeds.

vii) Lignin

Lignin is not a carbohydrate. It is usually considered as a structural material acting as a cementing and anchoring agent in the cell wall. Lignin is a polymer of aromatic alcohols of a group of complex aromatic polymers consisting of phenylpropane units. It is virtually insoluble in strong acid and alkali and is not digested by the colon.

The major chemical features of the polysaccharides are illustrated in Figure 6.

Thus, all these polysaccharide macromolecules have a great affinity for water molecules and get hydrated. They can also bind bile salts and other organic materials and may delay or impair the small intestinal absorption of associated nutrients.
FIG. 6 MAJOR POLYSACCHARIDE COMPONENTS OF DIETARY FIBER

CELLULOSE

PECTIN

HEMICELLULOSE (MAJOR COMPONENT SUGARS)

a) BACKBONE CHAIN

D-XYLOSE  D-MANNOSE  D-GALACTOSE

b) SIDE CHAINS
Fibre related materials

In addition to polysaccharides and lignin, the human diet contains a number of plant-derived materials that are similar to fibre in that they resist digestion in the upper intestine. These are cutin, suberin and polymeric esters of fatty acids. The former is a water impermeable substance secreted into the plant surface, whereas suberin is deposited in the later stages of cell wall development. These are enzyme and acid resistant materials recoverable in the lignin fraction. Other dietary components which associate with the lignin residue are the products of Maillard reaction.

Certain fibre rich foodstuffs contain significant amount of plant sterols. Also in close physical and chemical association with fibre in the plant cell wall are proteins, viositol, hexaphosphate, silica, saponins and other glycosides and polyhydroxyphenolic materials such as tannins. It is often difficult to dissociate the physiological effects of these materials from those of true DF components (Southgate 1976).

Table 9 depicts the different polysaccharides and non-polysaccharides fibres from plant cell wall (adopted from Yahouny 1982).
<table>
<thead>
<tr>
<th>Polysaccharides:</th>
<th>Main Chain</th>
<th>Side Chain</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>Glucose</td>
<td>None</td>
<td>Main structural component of plant cell wall. Insoluble in concentrated alkali; soluble in concentrated acid.</td>
</tr>
<tr>
<td>Non-cellulose:</td>
<td>Xylose</td>
<td>Arabinose</td>
<td>Cell wall polysaccharides which contain backbone of 1-4 linked pyranoside sugars. Vary in degree of branching and uronic acid content. Soluble in dilute alkali.</td>
</tr>
<tr>
<td>Hemi-cellulose</td>
<td>Mannose</td>
<td>Glucuronic acid</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pactic substances</td>
<td>Galacturonic acid</td>
<td>Rhamnose</td>
<td>Components of primary cell wall and middle lamella vary in methylester content. Generally water soluble and gel-forming.</td>
</tr>
<tr>
<td>Mucilages</td>
<td>Galactose-mannose</td>
<td>Galactose</td>
<td>Synthesized by plant secretory cells prevent dessication of seed endosperm. Food industry use, hydrophilic, stabilizer e.g. guar</td>
</tr>
<tr>
<td>Gums</td>
<td>Galactose</td>
<td>Xylose</td>
<td>Secreted at site of plant injury by specialized secretory cells. Food and pharmaceutical use e.g. Karaya gum</td>
</tr>
<tr>
<td>Galactose</td>
<td>Glucuronic acid-mannose</td>
<td>Galactose</td>
<td></td>
</tr>
<tr>
<td>Glucose-mannose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algal polysaccharides</td>
<td>Mannose</td>
<td>Galactose</td>
<td>Derived from algae and seaweed. Vary in uronic acid content and presence of sulfate groups. Food and pharmaceutical use e.g. carrageenan, agar</td>
</tr>
<tr>
<td>Xylose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coniferyl alcohol</td>
<td>Sinapyl alcohol</td>
<td>3-dimensional structure</td>
<td>Non-carbohydrate cell wall component. Complex cross-linked phenyl propane polymer, insoluble in 72% $\text{H}_2\text{SO}_4$. Resists bacterial degradation.</td>
</tr>
<tr>
<td>P-coumaryl alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Associated substances</td>
<td>Saponins, phytates, silica, proteins, lipids, ions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source: Vahauny (1982)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Physico-chemical Properties of Fibre**

The functional role of the fibres are due to the physico-chemical properties which are exhibited by these macromolecules in an aqueous environment (Eastwood 1987). These properties are:

(a) susceptibility to bacterial fermentation
(b) the ability to imbibe water molecules forming gels
(c) as an adsorbent
(d) as cation exchanger.

(a) **Bacterial fermentation**

Human upper intestinal enzymes are unable to hydrolyze the fibres, but the passage through the ileocecal valve exposes these macromolecules to bacterial enzymes that selectively degrade many of these macromolecules. Bacterial degradation of dietary fibres involves extra-cellular hydrolysis of polysaccharides into component mono- and disaccharides, followed by intracellular aerobic glycolysis. The products of fermentation of DF are short chain fatty acids, acetate, propionate, butyrate, lactic acid, formic acid, ethanol, methane, CO₂ and H₂. These products are excreted by different routes. Most of the H₂ and methane are absorbed into the circulation and excreted via the pulmonary route. Lignin is excreted unaltered in the stool. Short chain fatty acids are to a smaller extent absorbed
or excreted. The production of these gaseous compounds causes flatulence.

(b) Gel formation and water holding capacity

The water holding capacity of DF has important physiological effects in both the upper and lower intestine. Hydration of fibre occurs by adsorption to the surface of the macromolecule and by entrapment within the interstices of fibrous or gel matrix. The fibre saturation capacity or upper limit of water held is determined by the chemistry and morphology of the macromolecules and by the pH and the electrolyte concentration of the surrounding medium.

The initial event upon exposure of fibre to an aqueous medium is surface adsorption of water molecules. The presence of sugar residues with free polar groups confers a significant hydrophilic capacity to polysaccharides whereas intermolecular bonding such as the other cross linkages between chains of cellulose molecules has the opposite effect. Aqueous swelling of cellulose fibres does not alter the X-ray diffraction pattern, suggesting that water adsorption is limited to monocristalline regions occupied by other sugars or uronic acids. Lignin is relatively a polar and much less hygroscopic than other fibre components.
(c) **Adsorption**

The gel formation due to the water holding capacity of the fibre produces a larger surface area, thus acting as an adsorbent. A number of organic materials such as bile acids, steroids, various toxic substances may be reversibly bound to the fibre as it passes through the gastro-intestinal tract. The adsorption properties of DF and their components have been attributed to their ability to sequester conjugated or unconjugated bile acids. This property is influenced by a variety of factors including the physical and chemical forms of the fibre material, the type of bile acids and its micellar form, pH and osmolarity of the surrounding medium (Story and Kritchevsky 1976, Eastwood and Mowbray 1976). In vitro binding studies with bile acids on different cell-wall components indicated that cellulose and hemicellulose are not sequestrants of the bile acids, whereas the gel forming fibres such as guar gum and pectin and the hydrophobic lignins are reasonably good sequestrants. This property is responsible for the hypolipidemic action of the fibres.

(d) **Cation exchanger**

The action of the fibre as cation exchanger is attributed to the presence of free carboxyl groups on the sugar residues (Grant et al 1973, McConnell et al 1974). This property of the fibre favours the binding of cations.
and thus calcium binding is due to the formation of co-ordinated complexes with acidic polysaccharides. The property is reflected in their effects on mineral balances, electrolyte adsorption and heavy metal toxicity.

The overall physico-chemical properties of the fibre are given in Table 10.

Besides the above important properties, fibres also exhibit certain other characteristics (Van Itallie 1978, Heaton 1973). They are:

(a) They require chewing which slows down the intake of foods,
(b) Promote secretion of saliva and gastric juices, which distend the stomach and promote satiety,
(c) Displace available nutrients from the diet,
(d) Reduce the absorptive efficiency of the small intestine.

Effect of dietary fibre on glycemic responses

Early studies using viscous fibre such as guar gum demonstrated that such fibres added to glucose tolerance tests resulted in flattened glycemic and insulin responses (Mahalko et al 1984, Jenkins et al 1976, 1977, Miranda and Horwitz 1978, Anderson and Chen 1979). Although DF is one factor which may influence the glycemic response, only
Table 10. Physicochemical properties of dietary fibres and fibre components

<table>
<thead>
<tr>
<th>Property</th>
<th>Type of dietary fibre or components</th>
<th>Predicted physiological response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-holding capacity</td>
<td>Polysaccharides enriched with polar groups, particle size of material</td>
<td>Increased stool bulk, laxation, decreased intraluminal pressure</td>
</tr>
<tr>
<td>Gel formation</td>
<td>Pectin, mucilaginous polysaccharides</td>
<td>Delayed gastric emptying, delayed nutrient absorption, increased transit time</td>
</tr>
<tr>
<td>Cation exchange</td>
<td>Free ionic (carboxy) groups (hemicelluloses)</td>
<td>Trace element imbalance</td>
</tr>
<tr>
<td>Adsorption</td>
<td>Lignin, pectin, mucilaginous fibres</td>
<td>Bile acid imbalance, laxation</td>
</tr>
<tr>
<td>Bacterial digestibility</td>
<td>Polysaccharides</td>
<td>Altered microbial growth, altered chemical environment</td>
</tr>
</tbody>
</table>

Source: Yahouny (1982)
certain types of DF are found to be effective. Soluble fibre in the form of viscous gums have been extensively investigated and numerous commercial preparations are now available and recommended for use in diabetes treatment. To be effective guar products need to be of high viscosity and preferably mixed with food. However, as guar is a highly unpalatable product, efforts are under way to discover more acceptable sources of DF that will be capable of improving glucose tolerance.

Studies with soyabean dietary fibre (SDF) in rats have resulted in decreased fasting blood glucose levels improved glucose tolerance curve (Madar 1983, 1985, Madar et al 1985, Shorey et al 1985). SDF is acceptable to human subjects mainly due to its ease in usage either when mixed with water or in milk products and in cooking. The short term effect of SDF in NIDDM has been shown to reduce the postprandial glycemic response while the insulineemic response remained unchanged (Shorey et al 1985, Tsai et al 1983, Madar et al 1988). The beneficial effect of SDF could be attributed to the nature of the fibre, as it contains pectin, galactomannans and arabinogalactans with high viscosity.

Physical properties of the fibre and the structural arrangement of the molecules may be altered during heat processing rendering the food less available to enzymatic
digestion. SDF when administered in bread was more effective in reducing the glucose levels than when administered as powder (Madar et al. 1988). Jenkins et al. (1983) demonstrated that spaghetti made of wheat flour raised the blood glucose level to a lesser amount than the same amount of flour consumed as bread. Thus, methods of processing and cooking CHO food may also influence glucose and insulin responses probably reflecting differences in structural degradation and gelatinization dependent on these methods (Lindsay et al. 1984, Tappy et al. 1986, O'Dea et al. 1980).

Rice fibre has shown only minor effect as the rice fibre is composed mainly of cellulose and hemicelluloses of low viscosity (Madar and Thorne 1987). Similarly, other particulate fibre - wheat bran have shown only a marginal fall (significant but a non-physiological reduction) in blood sugar levels in diabetic patients (Hall et al. 1979, Nygran and Hallman 1982, Parson 1984, Mani et al. 1987).

Studies with Fenugreek (Trigonella foenum graecum) have also been made extensively. It is commonly used for seasoning purposes and as an ingredient of curry powder and sauces. Various investigators have confirmed in diabetic rats and dogs that fenugreek has the potential to reduce the post-prandial glucose levels (Ribes et al. 1984, Madar 1984). Shani et al. (1974) have shown that the antidiabetic property
of fenugreek seeds is associated with defatted seed material which is rich in fibre. Fibre analysis revealed that fenugreek contains 60% DF mainly pectin. Fenugreek has also been shown to exhibit a hypocholesterolemic effect (Sharma 1986).

Legume seeds are one food group that apparently elicits low postprandial glucose and insulin responses. This was demonstrated in experiments that compared responses after consumption of legume seeds and after consumption of other CHO containing foods (Jenkins et al 1980a, b, Dilawari et al 1981, Kamaih et al 1982, Fleming and Shaheen 1988). Legume seeds contain several constituents that could impart comparatively low glucose and insulin responses. For example, legume seeds contain substantial quantities of both water-soluble and water-insoluble fibre (Kamath and Belavady 1980, Fleming 1981, Southgate et al 1976).

There are alternative hypothesis regarding the mechanism whereby legume seeds may elicit low postprandial glucose and insulin responses. In particular there is speculation that the starch is not readily available for hydrolysis (Jenkins et al 1982, Wong et al 1985) and that the presence of certain substances may reduce digestive enzyme activity so that the rate of starch digestion is reduced. Constituents such as trypsin inhibitors, tannins and phytic acid were implicated as being responsible for reducing starch digestibility.

Pea-fibre has shown excellent results. Postprandial blood glucose response was markedly reduced by pea-fibre. The fibre content in the peas are of water-soluble nature and consists of mainly arabinose, galactose and uronic acid. The main mechanism in lowering the blood glucose levels is thought to be at the intestinal level by decreasing the adsorption rate of glucose (Hamberg et al 1989).

Changes in the small intestine following DF administration

DF may change the motility of the gut which, in turn, may affect the mixing, digestion rate and availability of CHO to enzymes especially pancreatic amylase. These effects could manifest themselves even after a short period of fibre administration. The presence of fibre in intestine plays the role of a barrier between the intra aluminal transport and diffusion areas. Hence, there will be differences in the rate of diffusion and less glucose will enter the circulation. Long term administration of DF will probably change the intestine morphology and the unstirred layer thickness (Vahouny and Kritchevsky 1982). These changes may result in slower glucose transfer. It is apparent that both gastric emptying and small intestine changes following supplementation of DF play a part in the reduced rate of absorption
observed mainly with the viscous fibres.

Another factor which may contribute to the lower rate of CHO digestion is the effect of DF on digestive enzymes (Schneeman and Gallaher 1985). The effect of DF sources on the rate of digestion in in vitro systems has been evaluated and wheat bran and cellulose have been found to inhibit amylase activity. Thus sources of fibre could interfere with digestive enzyme activity and could contribute to the reduction in the amount of glucose diffused into the circulation.

**DIETARY FIBRE AND LIPID METABOLISM**

A picture is beginning to emerge on the effect of DF and fibre components on lipid metabolism. Various types of DF have been shown repeatedly to lower cholesterol, others have had no positive effect while some have yet to be fully tested. In many experiments larger amounts of DF have been consumed than may not appear practical on a daily basis and one can question whether lowering plasma cholesterol levels by such means is beneficial.

Much of the initial interest in DF was centered around wheat bran as a source of fibre and many of the early studies investigating lipid lowering effects used this source of
fibres. Truswell (1984) in his excellent review article mentioned about the conflicting observations with wheat bran. Studies from our laboratory by Goelwamy et al (1985) and Mani et al (1987) with wheat bran supplementation have indicated no beneficial effect with respect to both serum lipids and tissue lipids in diabetic rats and in serum lipid profile in NIDDM patients.

Rice fibre has been found to have no effect on lipid and cholesterol levels in the diabetic rat (Madar 1983). Similarly maize bran (Munoz et al 1979) and cellulose have not lowered plasma cholesterol in human experiments (Eastwood et al 1973, Keys et al 1961, Prather 1964, Stanley et al 1973).

By contrast pectin has produced mean falls of plasma TC in various studies (Kay and Truswell 1977, Durrington et al 1976, Meittinen and Tarpila 1977, Jenkins et al 1975, Kay 1976, Chen et al 1981). The reduction in TC values was due to the fall in the LDL-C fraction. Pectin produces a small or even no increase of faecal bulk probably because it is all or nearly all digested by bacterial action in the human colon. It delays gastric emptying but does not appreciably shorten or prolong the total mouth-anus transit time. The major mechanism by which pectin lowers plasma TC is from increased faecal loss of bile acids.
 Guar has also been more frequently shown to lower plasma TC in man than other types of DF. The effect has been seen in subjects with normal plasma lipids (Fahrenbach 1965 and Jenkins et al 1975) and in hyperlipidemia (Jenkins et al 1979, 1980).

Sharma (1984) studied the hypocholesterolemic activity of some Indian gums in hypercholesterolemic rats. Guar gum, gum acacia and pectin showed hypolipidemic activity. The reduction in serum TC was primarily due to decrease in LDL + VLDL fraction. HDL-C and TG levels were unaffected. Increased excretion of fecal steroids was noted with each source of fibre.

Oat fibre has been shown to have hypocholesterolemic effect (Fisher and Griminger 1961, Chen et al 1981, Anderson et al 1984, Shinnick et al 1988). The hypocholesterolemic effect of oats is attributed to soluble B-glucans and neutral sugar components of its DF.

Different legumes have lowered plasma cholesterol in several controlled trials (Jayakumari et al 1979, Anderson and Chen 1979, Hockaday 1981, Soni et al 1982, Mathur et al 1968). Sharma (1987) has shown that when legumes such as Bengalgram, soyabean, winged bean, Rajmah and Khesri dal were included in the hypercholesterolemia inducing diet of
rats, they prevented the elevation of serum lipids.

Freeze dried peas (30 g/day) have been reported to lower plasma cholesterol by 15 mg/dl (Truswell 1984).

Recently SDF has received greater consideration regarding the regulation of lipid and cholesterol metabolism. SDF offered for 12 weeks to NIDDM with normal blood lipids and cholesterol had no significant effect on either TG or TC (Madar et al 1986). Similar results have been observed in mice (Madar 1985) and rats (Madar 1985) with a normal range of blood TG and TC levels. Soyabean polysaccharide taken by young men also resulted in unchanged lipid levels (Shorey et al 1985). Thus SDF does not seem to contribute to the reduction of circulating lipid and cholesterol levels in healthy men or NIDDM with initial low levels.

Studies with Colocasia leaves (Colocasia antiquorum) on serum and tissue lipids in hypercholesterolemic rats showed that colocasia leaves are lipogenic in nature (Mani et al 1989). Feeding colocasia to hypercholesterolemic rats led to accumulation of lipids in liver.

Truswell (1984) in his review article has summarized about fibre-rich foods on plasma cholesterol as follows:
Mechanism by which dietary fibre regulates lipids and cholesterol metabolism

It has been shown that the hypocholesterolemic effect of DF is related to its ability to adsorb bile salts (Story 1985). The adsorption depends on the hydrophobic groups of DF. The variety of DF sources adsorb bile salts differently. Cellulose was found to bind only a small amount of bile acids while lignin binds more than any other DF sources. These observations show that the effect of DF to adsorb or to bind bile acids resulted in reduced adsorption and therefore reduced recycling of bile acids. This effect is believed to reduce serum cholesterol levels in a two-part relationship. The lack of recycling of bile acids leads to a need of cholesterol for replenishment of the pool, and the decreased recycling means that an incomplete adsorption of dietary cholesterol takes place.
Soluble fibre is postulated to modulate lipid metabolism in the following manner:

(a) altering cholesterol and/or acid absorption
(b) altering hepatic production of lipoproteins
(c) altering peripheral disposal of lipoproteins.

Thus in a nutshell the above studies indicate that DF contributes significantly to the prevention of metabolic diseases—DM and hyperlipidemia. Physiological effects of DF appear to depend heavily on the source and composition of the fibre. A variety of fibre from different sources may prove to be ideal in optimizing the benefits. Finally, palatability and availability are the two major factors limiting the widespread use of fibre products. Palatability is particularly important as it has proven to be the limiting factor in patient compliance (e.g. guar gum) in addition to excessive and unacceptable flatus, abdominal bloating and diarrhoea. Keeping these points in mind the present study was planned to study the effect of curry leaves and dry Sundakai powder supplementation, on Diabetes mellitus.