Diabetes mellitus characterised by hyperglycaemia is believed to affect 30 million people throughout the world. Individuals with long standing diabetes are prone to various secondary complications which include: retinopathy, cataracts, atherosclerosis, nephropathy, neuropathy and limited joint mobility. As a consequence of these complications, the life expectancy of diabetics is only two third of that of the general population. Hyperglycaemia is a major causative factor in the development of these complications and diabetics with poor blood glucose control are particularly at risk. A number of hypotheses have been proposed to explain how hyperglycaemia may be involved in the pathogenesis of diabetic complications. One of these is the high activity of the sorbitol pathway causing accumulation of sorbitol within cells. As a result of this accumulation, a high intracellular osmotic pressure develops and could account for the cell damage. Furthermore, the high activity of the sorbitol pathway is accompanied by depletion of myoinositol levels and decreased activity of Na-K ATPase. Although there is good evidence that the sorbitol pathway may be involved in the development of diabetic cataract, its role in other complications is highly debated. A more recent and increasingly attractive explanation is that of glycation which is the focus of this review. In addition, there is evidence for interplay between the sorbitol pathway and glycation of other tissue proteins in diabetes. Glycation is a spontaneous reaction between reducing sugars in the acyclic form and proteins, and until recently it was referred to as non-enzymic glycosylation. In contrast, enzymic glycosylation is highly regulated, controlled by enzymes and required for the synthesis of glycoproteins which have a specific function. The term glycation refers to a reaction between any reducing sugar and protein whereas glucation, fructation and ribation are often used for specific sugars like glucose, fructose or ribose respectively. This reaction between sugars and proteins has been known since 1912 when Louis Maillard noted that solutions of amino-acids heated in the presence of reducing sugars developed a yellow brown colour. As early as 1912, Maillard proposed that this reaction could occur under physiological conditions and may be of significance in diabetes. However, this finding was ignored by medical scientists for almost 60 years. Instead it became of considerable interest in food science where the reaction is known as the Maillard or "browning" reaction. It was not until 1968 that the significance of in vivo glycation was recognised, prompted by the discovery of glycated haemoglobin (Hb A1c). Further work showed that glycated haemoglobin measured in diabetes reflected the average glycaemia over the preceding 120 days i.e., the life span of the protein. Following the discovery of glycated collagen, it was quickly realised that glycation may have a role in diabetic complications. In vitro work has shown that the process of glycation can be divided into two stages as outlined in Figure 1.
First, a nucleophilic addition reaction occurs between a free amino group (usually on a lysine residue) from the protein and a carbonyl group from the sugar to form a labile Schiff base (SB). This first step is freely reversible but some of the SB will rearrange to a more stable ketoamine or Amadori product (AP). In vivo, the extent of glycation will depend on the degree and duration of hyperglycaemia, half life of protein and permeability of the tissue to free glucose. Extracellular proteins are therefore particularly susceptible to glycation and in diabetes there is a two third fold increase in their extent of glycation. Once formed AP’s can undergo several fates: firstly, they can be catabolized; secondly, they can be oxidized to form inert compounds such as carboxymethyllysine (CML) which has been detected in urine, lens protein and collagen\(^7\) and thirdly they can undergo dehydration regenerating the free amino groups to yield reactive dicarbonyl compounds called deoxyglucosones\(^8\). This latter route is of considerable interest since deoxyglucosones can react with free amino groups on other unmodified proteins or AP’s to form advanced glycation endproducts (AGE) or Maillard products. As a consequence of AGE formation, proteins become irreversibly cross-linked, exhibit browning and generate fluorescence. The limited information on AGE suggests that they may be, (a) fluorescent cross-links, (b) non fluorescent cross-links or (c) fluorophores. Studies on the chemistry of AGE suggest that they may contain pyrroles, imidazoles or pyrazines\(^9\). In vitro studies have shown that cross-linking and fluorophore formation may occur independently since cross-linking of proteins cannot be accurately predicted from their fluorescence changes alone\(^10\). AGE form very slowly and since proteins are continually turned over, only long lived proteins will accumulate them. In contrast virtually any protein can be glycated, at least in vitro. Both glycation and AGE formation can alter protein conformation and may impair function in several ways: decreased enzymatic activity, decreased ligand affinity, modified protein half life, blocking of proteolytic sites, and altered immunogenicity. Although it is generally accepted that it is the AGE which contribute to the development of diabetic complications, there is some evidence that glycation alone can impair function. In vitro studies have shown that certain enzymes and hormones have reduced biological activity after glycation. For example cathepsin B shows reduced activity when glycated and as a consequence the conversion of proinsulin to insulin becomes impaired\(^11\). Glycation of insulin results in a loss of its biological activity. This reduced activity of insulin can be demonstrated on fat cells through reduced glucose oxidation increased fatty acid synthesis and reduced\(^12\) antilipolytic properties. In Vivo glycation of insulin would not be expected due to its very short halflife, so the above findings are only of theoretical interest.
Increased glycation of albumin, the major serum protein occurs during hyperglycaemia. Although glyca. Lion of albumin does not affect its half life, it has been shown to induce a conformational change in vitro. As a result, binding of bilirubin and fatty acids decreases by two and twenty fold respectively. Glycation of serum albumin can change its interaction with endothelial cell plasma membranes. Unmodified albumin is excluded whereas glycated albumin is ingested avidly. The increased passage of albumin through endothelial cells could account for the leakage of albumin from the capillaries of diabetic retina. There is increased glycation of LDL in diabetes compared to normal individuals. In vitro, glycated LDL shows reduced recognition, binding and degradation by cultured human fibroblasts. This could explain the elevated plasma levels of LDL and cholesterol in diabetes which could contribute towards the development of atherosclerosis. In diabetics, there is increased glycation of fibrinogen and fibrin. In vitro studies have demonstrated that glycated fibrinogen has a reduced half life, whereas glycation of fibrin reduces its degradation by plasmin. Antithrombin III is a coagulation-regulatory factor and prevents excessive coagulation. However, this ability is impaired by in vitro glycation. Furthermore, antithrombin III activity has been reported to be decreased in patients with insulin-dependent and insulin-independent diabetes. Excessive glycation of clotting factors could contribute to the development of thrombophilic states and vascular dysfunction in diabetes. The best studies of in vivo formation of AGE concern the lens crystallins since they have virtually no turnover. In vitro and in vivo studies with animals have shown that glycation of crystallins can cause opacification of the lens very similar to that seen in diabetic cataract. Conformational changes of lens protein induced by glycation can expose SH groups to oxidation and disulphide formation. For this reason, increased disulphide in addition to AGE cross-links are believed to produce the opacification. Normally SH groups are protected against oxidation by reduced glutathione. In diabetes, increased activity of the sorbitol pathway results in the depletion of NADPH and therefore reduced glutathione. The sorbitol pathway and glycation may act together to produce diabetic cataract. Collagen, the major structural protein in the body can be glycated in vitro and shows increased cross-linking, rigidity, fluorescence and reduced susceptibility to proteolysis. The fluorescence is often used as an assay for AGE and is higher in collagen samples from diabetics. It has been proposed that increased AGE cross-links of collagen in the joints may be responsible for the limited joint mobility (LJM) syndrome seen in 40% of insulin-dependent diabetics. This possibility has been supported by the finding that collagen from patients with LJM has higher fluorescence compared to those without. In vitro, the AGE on collagen can trap other proteins covalently such as albumin, immunoglobulins and LDL. Albumin and immunoglobulins trapped by collagen in basement membranes might also be part of AGE. Hence glycation could contribute to basement membrane thickening in diabetic cataract. Similar trapping of LDL could by glycation can contribute to the accelerated development of atherosclerotic plaques in diabetics. AGE formed by glycated myelins in diabetes increases their susceptibility to phagocytosis by macrophages in vitro. Furthermore, glycated myelin can stimulate the secretion of proteases from macrophages. Both of these processes might contribute to the demyelination of nerve fibres seen in diabetic neuropathy. Glycation of nucleic acids has been shown to occur in vitro but at a slower rate than proteins. DNA glycated in vitro exhibits fluorescence spectra characteristic of AGE. In vivo, glycation and the subsequent AGE on nucleic acids could cause mutations and defects in replication and transcription. Whether this could account for the increased frequency of congenital abnormalities seen in infants of diabetic mothers is not yet known, because there is no evidence for in vivo DNA glycation. Since glucose is the major metabolic sugar, glycation has received the most attention. In vitro studies have shown that other sugars including their phosphorylated derivatives are more reactive than glucose. Many of these sugars are present in minute concentrations and it has therefore been assumed that they do not contribute significantly towards protein glycation in vivo. However, there is growing interest in fructose since it can induce AGE formation up to 10 times faster than glucose in
Fructose is an important constituent of fruits, honey and sucrose and is often recommended as an alternative to glucose for diabetics since it does not require insulin for its metabolism and has a plasma concentration of less than 1mM. However, diabetics have higher serum fructose concentration after ingestion of a fructose rich diet compared to normal individuals and taking the above considerations into account, in vivo fructation cannot be ignored. Furthermore, increased activity of the sorbitol pathway in diabetes can result in accumulation of fructose in certain regions of the body such as the eye lens, nerves, kidneys and arteries i.e., regions associated with diabetic complications. The sorbitol pathway is a metabolic shunt where excess glucose is converted to fructose under the influence of the enzyme aldose reductase. The fructose levels here can reach and exceed those of glucose and so significant fructation could occur. In contrast to other enzymes, in vitro glycation of aldose reductase has been reported to increase its activity. Furthermore, evidence has been presented for in vivo fructation of lens protein. The possible role of glycation in diabetic complications can be outlined as in Figure 2.

In recent years it has been suggested that the body may possess defence mechanisms against AGE. An enzyme a-ketoglutaraldehyde found in liver can inactivate deoxyglucosones in vitro. Such inactivation in vivo could prevent AGE formation. Macrophages possess receptors which can recognise AGE proteins enabling them to be endocytosed and degraded. However, the efficiency of these
mechanisms in vivo is not yet known. At present there is considerable interest in inhibitors of glycation and AGE formation because of their therapeutic potential in preventing diabetic complications. A number of compounds have been shown to inhibit in vitro glycation and include acetylsalicylic acid (aspirin), pyridoxal phosphate, vitamin E, aminoguarridine, lysine and penicillamine. Aminoguanidine, a nucleophilic hydrazine with three amino groups has received the most attention. The amino groups from this compound can react with the carbonyl groups of free sugar, AP or deoxyglucosones to block glycation and AGE formation. In diabetic rats administration of aminoguanidine reduced cross-linking of aortic collagen and prevented basement membrane thickening in the kidneys. The possibility that long term administration of aminoguanidine or its analogues may prevent diabetic complications in humans is under investigation. Acetylation at amino groups by aspirin has been shown to block glycation in vitro and in diabetic animals. A study by Cotlier in 1981 has suggested that frequent use of aspirin may protect against diabetic cataract. It was shown that the prevalence of cataract was significantly lower in diabetics with rheumatoid arthritis receiving high doses of aspirin compared to a matched population on no aspirin. This led to the suggestion that aspirin may protect against cataract by preventing glycation. However, other analgesics such as paracetamol and ibuprofen also have a protective effect against cataract but cannot acetylate proteins. Therefore the protective effect of aspirin may be due to other mechanisms since aspirin can also reduce blood glucose levels, inhibit sorbitol pathway activity and stimulate the release of insulin. For any antiglycation drug to be successful, it will need to have a long half life and virtually no toxicity. Obviously, these requirements are not easy to fulfill. An alternative and more realistic suggestion proposed by Furth has been to change feeding habits so that hyperglycaemic episodes are avoided. Hyperglycaemic episodes can occur after ingestion of a high sugar meal on an empty stomach and will be more pronounced in diabetics and individuals with impaired glucose tolerance. The above suggestion has been supported by the finding that glycated haemoglobin and plasma protein levels are reduced after 18-20 days of fasting during the month of Ramadhan. Since evidence for the role of glycation in diabetic complications is accumulating, the importance of maintaining normoglycaemia in diabetes cannot be stressed too strongly.

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