CHAPTER III

REVIEW OF LITERATURE - II

LITERATURE REVIEW ON CORONARY HEART DISEASE AND ANTIDIABETIC DRUGS

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The term coronary heart disease (CHD) includes three distinct but overlapping syndromes in middle age - myocardial infarction, angina pectoris and sudden death. Wherever coronary heart disease is prevalent there are certain risk factors present in the population which include hypertension, cigarette smoking and diabetes mellitus, obesity, lack of physical activity, raised uric acid levels, emotional stress and soft drinking water. Virtually coronary heart disease occurs on the background of severe atherosclerosis. Hence cardiologists and biochemists are much concerned about the aetiology and pathogenesis of coronary heart disease which can be regarded as a complication of severe atherosclerosis.

Atherosclerosis :

Atherosclerosis is primarily a disease of large and medium sized arteries such as aorta and its principle branches and in particular the coronary arteries. Cerebral vessels are also involved. It is very rarely

found in veins. It is found in a variety of animals such as birds, pigs and nonhuman primates. In man, the first appearance is in the form of a fatty streak in the proximal aorta and the thoracic segment. Later on fibrous plaques and lesions laden with lipid appear but in the distal aorta in the abdomen. Atherosclerosis is the main underlying cause of coronary heart disease, stroke and gangrene of the extremities and dilatation of the abdominal aorta. Atherosclerosis produces clinically significant disease by various mechanisms. By narrowing the lumen of arteries it produces ischaemia to some degree. Atherosclerosis sets the stage for sudden complete occlusion with more ischaemia and resultant death of tissue in an organ such as the heart or the brain or the leg. Atherosclerotic lesions in the aorta can be a source of emboli to the extremities. Atherosclerosis can produce clinical sequelae by weakening the wall of the aorta.

The types of grossly detectable lesion are defined as follows :

Fatty streak :

This is a fatty intimal lesion that is stained distinctly by sudan IV and shows no other underlying

change. Fatty streaks are flat or only slightly elevated and do not significantly narrow the lumen of blood vessels.

Fibrous plaques :

This is a firm, elevated intimal lesion which, in the fresh state, is pale grey glistening and translucent. A fibrous plaque has fat in its base with a thick fibrous connective tissue cap covering the fat. If a lesion shows hemorrhage, thrombosis, ulceration or calcification, the portion so involved is classified under that category.

Complicated lesion :

This is a lesion in which there is hemorrhage, ulceration or thrombosis with or without calcium deposit.

Calcified lesion :

This is a lesion in which insoluble mineral salts of calcium are visible or palpable without overlying hemorrhage, ulceration or thrombosis.

Experimental work in animals is concerned mainly with the induction of changes in blood and arterial wall lipids and with the formation of fatty

streaks. The feeding of cholesterol in the amounts of 0.5 to 1 gm/kg body weight daily for 50 days given to the rabbit is sufficient to produce moderate atheroma. Atherosclerosis can also be produced by damaging the aorta with a slight scratch. Feeding of saturated fat can also produce atherosclerosis. Feeding of sufflower oil showed partial regression of atheromatous lesions in rhesus monkeys (Chakrabarti et al 1977). Long term feeding of sucrose is known to cause atherosclerosis.

Pathogenesis of atherosclerosis :

Much research in the area of atherosclerosis has incriminated risk factors like hyperlipidaemia and hypertension. Other research has concentrated on the morphologic and chemical characterization of lesions observed at autopsy or on the investigation of animal models of the disease. Recently our attention has been directed towards the pathobiology of the arterial wall and to the key role of smooth muscle cell proliferation in lesion formation (Ross and Glomset 1973; Ross and Glomset 1976). The fundamental facts relative to the development of atherosclerosis have also been reviewed by Prof. J. L. Beaumont (1975).

Normal artery is composed of three distinct morphological layers. The intima or the innermost layer consists of narrow region bounded on one side by continuous layer of endothelial cells and on the other by a sheet of elastic tissue called the internal elastic lamina. The media or the middle layer of arterial wall contains large number of smooth muscle cells which spiral around the artery. The outer most layer, the adventitia consists of a loose mixture of collagen, proteoglycan or glycosaminoglycans and some elastic fibres which surround the arterial wall along with fibroblasts and smooth muscle cells. There are certain areas like aorta where the artery is more elastic, the media is lamellated by additional interspersed layers of elastic lamina separating the smooth muscle cells into lameller layers. This difference in architecture is responsible for differences in morphologic pattern of lesions of atherosclerosis of the aorta, the coronaries and the cerebral arteries.

The artery has a normal endothelial barrier. The lumen has intact endothelial cells in single layer. The vesicles on cell surface lead to transport of materials from lumen to arterial wall across the endothelial barrier by pinocytosis. Ordinarily lipoproteins

of high density can be carried across but larger low density chylomicron molecules can not cross the barrier. If the endothelium of the artery gets damaged, the macromolecules and lipoproteins pour massively into the arterial wall. Injury can be caused by experimental trauma, injection of homocystein, stress of stretch in hypertension, presence of induced hypercholesterolaemic state etc.

The loss of endothelial surface exposes the subendothelial connective tissue to platelets and circulating substances. The platelets adhere to subendothelial collagens and release the contents of their granules. The entry of platelet factors leads to reaction between platelet factors and the plasma constituents on one hand and the arterial wall on the other. Due to massive infiltration of platelet factors, plasma lipoproteins and possibly other factors such as hormones and smooth muscle cells start migrating into intima and their active multiplication takes place within the intima. The focal proliferation of smooth muscle cells at the site of injury leads to deposition of large amount of connective tissue. The intimal cells thereafter regenerate and cover the thickened area. This is the genesis of fibromusculoelastic lesion (Fig.1).

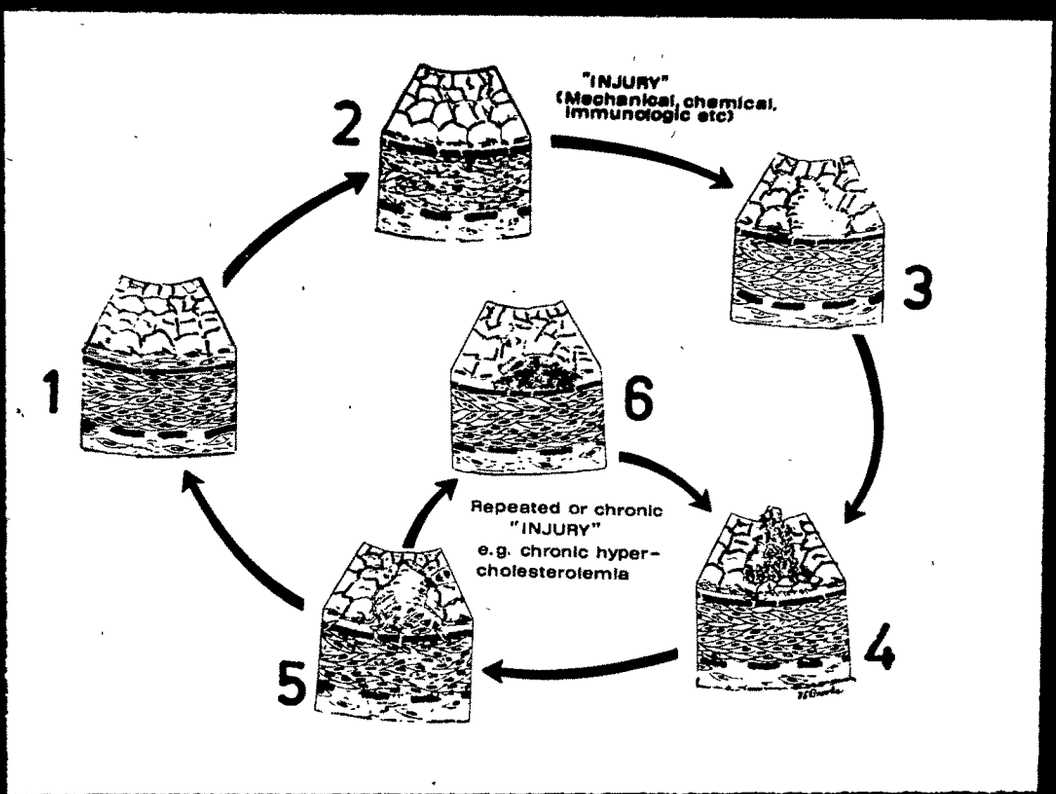

Fig.1 : 1. Normal muscular artery or artery with fully regressed lesion.
2. Injury to the surface endothelium (at this stage lipids can pass across the injured endothelium.
3. Denudation of surface endothelium with breach in internal elastic lamina.
4. Proliferation of smooth muscle cells of media through the breaches of the elastic lamina creating raw surface in denuded area.
5. Adherance of platelets and disintegration of platelets on the surface area.
6. Adherance of thrombi deposition of lipid, embibation of calcium and ulceration.

Note : The above diagrams are schematic and in three dimensions. (The New England Journal of Medicine, Vol.295, No.8 p.423, 1976).

In absence of risk factors, there is regression of the lesion leaving a slightly thickened intimal layer containing only a few layers of smooth muscle cells.

With further progression, characteristic lipid deposition occurs within and outside the cells. The fibrous connective matrix gets laden with the lipid deposit. The smooth muscle cells undergo necrosis. The proliferated cells envelope round the lipid and the debris. Such a change occurs in case of chronic injury or hypercholesterolaemia.

Repeated injury and stress can tip off the balance between reendothelialization, cell proliferation and cell destruction. The risk factors interfere with repair. The increased plasma concentration of low density lipoprotein leads to endothelial injury and a tissue response with proliferation and lipid deposit. If onto this is added repeated proliferation and regression, a complicated lesion containing newly formed connective tissue and lipids is formed which may eventually calcify. This sequence of events could lead to lesions that produce clinical sequence of thrombosis and infarction. This response to injury hypothesis dates back to the pioneering work of Virchow which has

been modified by many workers including French (1966) and Mustard and Packham (1975).

A second hypothesis concerning the cause and pathogenesis of atherosclerosis has been proposed by Benditt and Benditt (1973). This hypothesis suggests that each lesion of atherosclerosis is derived from a single smooth muscle cell that serves as a progenitor for the remaining proliferative cells. According to them each lesion is a benign neoplasm derived from a cell that has been transformed by agents such as viruses or chemicals. This is monoclonal hypothesis.

A third hypothesis concerning the atherosclerosis and smooth muscle proliferation is clonal senescence hypothesis suggested by Martin and Sprague (1973). The normal proliferation of smooth muscle cell is an activity of precursor stem cells. Their proliferation leads to liberation of chalones which inhibit replication. The stem cells multiply in media and chalones infiltrate from media to intima to inhibit smooth cell proliferation. With ageing the number of available stem cells in media to replace smooth muscle cell is too less. Hence there is loss of inhibiting chalones.

This leads to replication of stem cells of intima. This clonal hypothesis is of interest because it specifically relates to ageing which is well recognized to be a risk factor in atherosclerosis though it does not explain the associated lipemia.

A number of studies have been made to show that constituents and platelet factors released in the content of their granules have a vital mitogenic role in proliferating the smooth muscle cells.

Serum macromolecules such as low density lipoproteins have supporting role. Since low density lipoproteins appear to be taken up and degraded by smooth muscle cell in culture (Bierman and Albers 1975), as they are the fibroblasts in cell culture (Goldstein and Brown 1974; Brown et al 1975) their role may be to provide lipids for cell membrane formation. One of these lipids is probably cholesterol, although sphingomyelin and polyunsaturated fatty acids are suspected to be involved. Another serum factor that supports proliferation of arterial smooth muscle cells is insulin (Stout et al 1975). It specific role remains unknown but its actions on other cell types suggest that it also may be increasing the supply of substrates for cell growth. The extra cellular matrix of atheromatous lesion differs from normal in the type of collagen present.

Most important constituent of arterial extra cellular matrix is glycoseaminoglycans. According to Wight and Ross (1975) glycoseaminoglycans in the arterial

smooth muscle cells consist of approximately 60 to 80 percent of dermatan sulfate, 10 to 20 percent chondroitin sulfates A and C, and less than 5 percent hyaluronic acid. Large quantities of dermatan sulfate by these cells in culture are important from the point of view of marked propensity of these glycosaminoglycans to bind low density lipoproteins (Iverius 1972). A large number of studies have been conducted by Kurup and others on synthesis of glycosaminoglycans by liver and aorta after administration of insulin and other antidiabetic drugs. Decrease in the sulfated glycosaminoglycans was observed in the aorta of rats on hypercholesterolaemic diet (Seetanathan and Kurup 1971; Vijay Kumar and Kurup 1973). Biosynthesis of many acid mucopolysacchrides is decreased in atherosclerosis (Ishida and Kalent 1968; Nakamura et al 1968). Other findings do not agree with this (Braunstein 1960; Bertelesen 1961). Saxena and Nagchaudhari (1970) studied the effect of high fat diets on acid mucopolysaccharide fraction of aorta in rabbits. On feeding coconut oil, chondroitin sulfates were increased and hyaluronic acid and heparin sulfate were decreased significantly. Initially, similar changes occurred on feeding 'Dalda' and later on hyaluronic acid

was decreased as the treatment was prolonged. These changes are associated with elastic fiber breakdown in media. On feeding coconut oil, chondroitin sulfates were increased and hyaluronic acid and heparin sulfate were decreased significantly. Initially, similar changes, occurred as treatment was prolonged. These changes are associated with elastic fiber breakdown in media. On feeding 'Suffola', heparin sulfate, low sulfated chondroitin sulfate, dermatan sulfate and chondroitin sulfate were increased and hyaluronic acid was decreased significantly. Saraswathy Devi and Kurup (1970) studied the effect of administration of mucopolysacchride fraction from bovine aorta on lipids of serum, liver and aorta in rats given atherogenic diet for 3 months. Mucopolysacchrides were found to lower lipid levels. Malathy et al found decrease in the L-glutamic:D-fructose-6-PO_4 transferase activity in liver of rats fed atherogenic diet (1970).

Lipids in cardiovascular disease :

The association between the abnormalities of lipid metabolism and increased prevalence of atherosclerosis in man are well known particularly over the last twenty years. The relevance of hypertriglyceridaemia to the

development of atherosclerosis has been shown in past (Albrink and Man 1959; Brown et al 1965; Carlson and Bottinger 1972). It has been proposed that the most frequent underlying biological risk factor is the excessive flux of fat to the liver (Bortz and Walter 1974). Paranjape et al (1977) reported 52 percent of hyperlipoproteinemic patients in a series of 300 patients who suffered from myocardial infarction. When typed according to Fredrickson's classification, 15 percent had type IIa, 6 percent had type IIb, 13.5 percent had type III, 16.6 percent had type IV and 0.6 percent had type V.

Serum enzymes :

Several studies have been conducted on serum enzymes in heart diseases. The change in the nature of enzyme systems in response to environmental changes is an adaptation mechanism in the body. Atherosclerosis appears to be accompanied by disturbances in the concentration of enzyme systems within the tissues including the arterial tissue. The value of serum enzymes such as creatine phosphokinase (CPK), glutamic oxaloacetic transaminase (GOT), alpha hydroxybutyric dehydrogenase (HBD), lactate dehydrogenase (LDH) and its isoenzymes

as diagnostic as well as prognostic tool in cases of acute myocardial infarction have been studied by many workers (Singh 1977; Kothari 1977).

Insulin :

Over 100 years ago, Langerhans saw the islets in the pancreatic tissue for the first time. For a long time, these cells have been intensively studied. Since the discovery of Banting and Best, rapid advances have been made in the past decade concerning the synthesis, storage and secretion of insulin. Insulin is a polypeptide containing 51 aminoacids. 'A' chain of insulin comprises of 21 and 'B' chain consists of 30 amino acids. The two chains are linked by two disulfide, bridges between position 7 of both the chains and positions 20 and 19 of the 'A' and 'B' chain respectively. Human insulin differs from that of pig, dog, sperm whale-in whale and rabbit only with respect to the c-terminal amino acid of the 'β' chain (B_{30})-from ox insulin with respect to the three amino acids, two of which are within the internal ring of the 'A' chain. Most vertebrates including mammals, birds, amphibia, reptiles and fish synthesize insulin, though recently insulin has been found even in starfish and snail (Wilson and Falkmer 1965).

Proinsulin-a precursor of insulin was first isolated by Steiner (1969). It is speculated that in diabetes an inherited defect of the proteolytic enzyme responsible for conversion of proinsulin into insulin or mutation in a structural gene for proinsulin may result in the production of a defective hormone.

Insulin secretion or release is a complex phenomenon and is regulated by variety of factors, the knowledge of which is still expanding and undergoing rapid changes. Electric stimulation of the vagus nerve and stimulation of βadrenergic receptors is found to cause release of insulin (Frohman et al 1966). It is well established that glucose stimulates the release of insulin from the pancreas both invivo and invitro. The administration of ketone bodies to experimental animals is also found to either cause hypoglycaemia or interfere with peripheral glucose utilization (Mebbane and Madison 1964; Felt et al 1964). Successful attempts to elicit insulin release by the addition of leucine to the pancreatic tissue incubated invitro have been reported (Milner and Hales 1967). The perfusion of dog pancreas in situ with leucine, histidine or glycine was shown to cause an increase of insulin like activity into pancreatic vein blood (Ninomya et al 1966). Recently,

short chain fatty acids have been shown to stimulate the release of insulin (Manus et al 1967; Horino et al 1968). In man, prolonged administration of growth hormone brings about increased levels of insulin like activity in normal as well as hypopituitary patient. (Zahnd et al 1960; Stein et al 1962; Luft and Cerasi 1964; Kipnis 1965). ACTH has been shown to stimulate insulin release in normal and in adrenalectomized mice (Genuth and Labovitz 1965). Serum immunoreactive insulin increases after glucagon injection (Samols et al 1965). Recent observations suggest that glucagon involved in the regulation of insulin release may be of intestinal origin (Unger et al 1968). The administration of insulin to dogs whose blood sugar level is kept constant, decreases pancreatic insulin release (Kosaka et al 1964). Together with metabolic substrates 3'-5' cyclic adenosine monophosphate may be the most important mediator of the insulin releasing stimulation (Sutherland et al 1965; Senft 1966). Calcium ions are essential for insulin release in response to any stimulation (Sussmen et al 1966). It is suggested that glucose acts through adenyl cyclase system (Cerasi et al 1972). The findings of Gabby and Tze (1972) indicate that conversion of glucose to sorbitol

may be an essential preliminary step in insulin release. Whether sorbitol activates adenal cyclase system is not known. Prolactin, cortisol, prednisolone, progesterone, testosterone, oestrogens and thyroxine also regulate the secretion of insulin.

Serum insulin response in diabetes :

A direct relationship between insulin secretory rates or levels of serum insulin and blood glucose concentration has been established (Metz 1960; Cahill et al 1966). According to Cerasi (Cerasi 1975), the initial defect in the diabetic β-cell consists of a specific step in the recognition of hyperglycaemia as the acute trigger of the processes that lead to release of insulin.

Inspite of its limited character, its strategic location may cause important delays in the feed back loops that regulate the glucose homeostasis of the organism and therefore be sufficient to impair glucose tolerance. When acute and late phases of insulin secretion were studied in mongrel dogs before and after the induction of mild alloxan diabetes, it was found that alloxan diabetic dogs had significantly decreased early phase insulin responses to glucose and slower

plasma glucose disappearance rates. In contrast, these mildly diabetic dogs achieved comparable insulin levels and higher glucose levels during a four hour 40 mg/min glucose infusion than prealloxan control values. It is suggested that reduced early phase secretion and intact later phase of insulin secretion are not dependent on genetic determinants and may be induced in a model of acquired diabetes (Pupo et al 1976). Although normal, nonobese subjects develop glucose intolerance and a blunted insulin response, obese persons either normal or mildly diabetic, show no change in glucose tolerance and maintain essentially unchanged serum insulin responses. Carbohydrate restriction imposed upon mild, nonobese diabetics for period of four months appears not only to improve glucose tolerance but also to reduce a greater insulin response to glucose (Rudnick and Taylor 1965). Raheja et al (1971) studied immunoreactive insulin in 54 Indian subjects with or without diabetes after giving oral glucose load of 75 gms at 30', 60' and 90'. They found maximal and prompt response with rapid fall in normal weight nondiabetic controls with sustained high response in nondiabetic obese subjects. Higher immunoreactive insulin was also seen in maturity onset diabetics and

in young controls given sulfonylureas. Maturity onset diabetics with severe carbohydrate intolerance and mild ketosis or children with juvenile onset diabetes and ketosis show a pattern of insulin response that is characterized by abnormally low insulin values throughout the duration of test (Ehrlich and Bamers 1964). Thus, the insulin secretory pattern in the ketosis prone young diabetic appears to resemble a state of absolute insulin deficiency.

There is ample evidence to show that sulfonylureas enhance insulin secretion by mechanisms different from those of glucose. The patient with maturity onset diabetes appears to show a significantly low mean serum insulin five minutes after the intravenous administration of tolbutamide, but a total insulin response which is normal (Perley and Kipnis 1966). In prediabetic subjects in whom both glucose and tolbutamide were given intravenously for acute stimulation of insulin secretion, there was a significantly blunted serum insulin response to glucose but the tolbutamide stimulated insulin response was normal (Rojas et al 1969). An improvement in glucose tolerance and a decrease in serum insulin like activity (ILA) have been seen after the oral administration of glucose to

diabetic subjects treated with chlorpropamide (Feldman and Lebovitz 1967).

In obese diabetics, Abramson and Arky (1967) compared the results of the chronic administration of phenformin with those of chlorpropamide. Although glucose tolerance tests showed decreases in blood glucose levels following treatment with either drug, the serum insulin response decreased following phenformin therapy. It is not quite clear whether the long term antidiabetogenic effects of the sulfonylureas are due to the effects of the drug upon the beta cell and subsequent normalization of serum insulin blood glucose relationships or whether the extra pancreatic effects play a predominant role.

Biguanides do not appear to stimulate insulin secretion and may infact suppress insulin release.

Effect of amino acids on serum insulin levels is well established. Action of hormones and catecholamines have been discussed elsewhere in the thesis.

Serum insulin antagonists :

Stadie and coworkers (1949) showed that a buffer containing 2.5 percent albumin could inhibit insulin induced glycogen synthesis in the rat diaphragm. Plasma

from untreated or uncontrolled insulin dependent diabetic patients markedly inhibited the effect of insulin added invitro. The antagonistic activity was subsequently demonstrated to be in the albumin fraction of the plasma proteins (Vallance Owen et al 1958; Lowy et al 1961). This antagonism to insulin could be demonstrated in both normal and diabetic subjects in albumin fractions at high concentrations. The term "Synalbumin antagonist" was proposed for the responsible factor. Mahler and associates (Mahler et al 1968) have shown insulin antagonism on the perfused limb of the rat by a reduced insulin B-chain albumin complex. An increase in the insulin antagonist activity has been seen following the intravenous administration of sodium tolbutamide or insulin in normal subjects (Devrim et al 1968).

It is of interest that Vallance Owen and Ashton (1963) found increased insulin antagonism in patients with myocardial infarction, and in such patients Peters and Hales (1965) found raised plasma insulin concentrations in fasting and during oral glucose tolerance tests.

Metabolic effects of insulin :

Most of the studies on insulin have been performed by using rat epididymal adipose tissue. In particular, it is easily accessible and also extremely sensitive to the metabolic effects of insulin although adipose tissue from other sites in these animals is equally sensitive. Rat adipocytes have also been used. Human adipose tissue is less responsive to insulin than that of the rat.

Insulin in physiological amounts increases the uptake of glucose by rat adipose tissue invitro (Krahl 1951; Winegrad and Renold 1958). Insulin does not act only on glucose but fructose, mannose and galactose are also taken up. Crofford and Renold (1965) showed that insulin was more effective on adipose tissue by a mechanism similar to its action on muscles. They further showed that facilitation of glucose transport was the principle site of insulin action on fat. Insulin accelerates the rate of formation of glycogen in adipose tissue (Leonards and Landau 1960). Insulin increases the oxidation of carbon-1 of glucose much more than it does the oxidation of carbon-6 in adipose tissue. Insulin also stimulates lipogenesis in adipose tissue, and to an

equal extent from both glucose-1-C^{14} and glucose-6-C^{14}. The most important general effect of insulin is that it stimulates the incorporation of carbon from acetate, acetaldehyde, malonate and pyruvate into fatty acid but only in the presence of glucose. The effects of insulin on fatty acid synthesis from these four substrates thus appear to be secondary to its effect on carbohydrate metabolism in adipose tissue. (Winegrad et al 1960). Insulin increases the incorporation of C^{14} from glucose into glyceride glycerol. This effect represents increased rate of triglyceride synthesis. Glucose plays a role as the major precursor of glycerol phosphate by providing a supply of energy for the esterification processes by the metabolism of acetyl CoA class formed during glycolysis in the Kreb's cycle. It stimulates the biosynthesis of fatty acid. It is thus of fundamental importance in the regulation of triglyceride synthesis.

With ageing, the rate of lipogenesis from acetate decreases. In mice rendered obese by intra-peritoneal injection of gold thioglucose, the pancreas increases both its store of insulin and its capacity to secrete insulin invitro in response to glucose (Malaises et al 1968). In alloxan diabetic rats there

is marked decrease in the invitro synthesis of monoenoic acids from acetate by adipose tissue. The addition of insulin invitro restores lipogenesis in normal (Benjamin and Gellhorn 1964).

Major mechanism of increased lipogenesis by insulin in adipose tissue seems to be because of increase in glucose uptake. The role played by NADPH produced in the pentose shunt in the process of lipogenesis is already well known. In short, increased metabolism of glucose in presence of insulin produces more amount of factors and cofactors required for fatty acid biosynthesis and esterification - pyruvate and acetyl CoA in the Embeddon Meyerhof pathway, NADPH in the pentose shunt, energy in the Kreb's cycle and L-glycerol phosphate in glycolysis.

Halperin and Robinson (1971) demonstrated that insulin stimulates glucose conversion to fatty acid both by increasing glucose transport and by augmenting pyruvate incorporation into fatty acids by a mechanism distinct from the known stimulation of glucose transport.

Insulin in presence of glucose decreases the rate of release of fatty acids from adipose tissue by converting glucose to more L-glycerol phosphate, which

can be used to reesterify fatty acids. In absence of glucose, insulin inhibits loss of glycerol from adipose tissue. Mahler et al (1964) showed that insulin appeared to exert a restraining effect on a hormone sensitive lipase activating mechanism. Insulin inhibits lipolysis by inhibiting the adenyl cyclase system in adipose tissue (Brown et al 1969).

Goldman and Cahill (1964) analysed adipose tissue after intravenous injection of glucose-C^{14} with or without insulin in fed, fasted and refed rats. In fed rats, insulin reduced the specific activity of blood glucose, liver glycogen and liver triglyceride glycerol and increased incorporation into adipose tissue glycogen, fatty acids and glyceride glycerol. Fasting for 48 hours eliminated all insulin effects except those on blood glucose, liver glycogen and adipose glyceride glycerol. Refeeding over corrected this unresponsiveness and there was increased incorporation of the glucose label with or without insulin into liver glycogen, phospholipids and triglyceride fatty acids and triglyceride glycerol, (Goldman and Cahill 1964). Mirsky (1963) has demonstrated an antilipolytic effect of insulin invivo in dogs. Both insulin and tolbutamide have been reported to inhibit

fatty acid and glycerol release from adipose tissue (Rudman and Shank 1966; Fain et al 1966). It is suggested that this may be due to increased synthesis and reesterification of fatty acids (Winegard and Renold 1958; Jungas and Ball 1963; Renold et al 1965). Hollenberg (1970) in an extensive series of studies concluded that the increase in adipose mass produced by refeeding or insulin administration was due to deposition of lipid in existing cells and not due to accelerated formation of new fat cells.

Jungas (1971) has shown that in rat fat insulin accelerates the conversion of lactate or pyruvate or of endogenous glycogen to fatty acid, the effects seeming to result from increased conversion of pyruvate to CO_2 and acetyl CoA in presence of insulin, due to an increase in pyruvate dehydrogenase activity. The activity of this enzyme was greater in homogenates from tissue exposed to insulin prior to homogenisation.

Insulin increases the activity of several enzymes such as glucokinase, glycogen synthetase, glucose-6-phosphodehydrogenase and 6-phospho gluconate dehydrogenase which results in increased peripheral

glucose uptake and conversion to glycogen in the liver. Evidence has also accumulated indicating that insulin acts as a suppresor of the biosynthesis of pyruvate carboxylase and phosphoenol pyruvate carboxylase - the key enzymes of gluconeogenesis. Insulin also suppresses adenal cyclase activity. It favours glycolysis and increases the amounts of reduced NADP available for the synthesis of fatty acids. Mehlman et al (1971) replaced the carbohydrate in the diet of the normal, diabetic and diabetic insulin treated rats with 1-3, butanediol. They found that in adipose tissue malic enzyme activity was greatly decreased in diabetic and increased in diabetic insulin treated animals.

Own et al (1967) showed both qualitative as well as quantitative differences between human and rat fat. In particular, these workers showed that the addition of NADPH invitro increased oxidation of glucose by human fat and depressed oxidation by rat epididymal tissue. However, it has been suggested by several workers that human adipose tissue is the major store of neutral fat whereas liver is the major site of lipogenesis in man. In the diabetic, insulin has an antiketotic effect. Jones and Arky (1965) have studied

the response of triglyceride and free fatty acid production in man after acute and after prolonged mild hypoglycaemia induced by insulin in normal and confirmed that insulin caused acute falls in plasma glucose and free fatty acid concentrations with secondary rises in each moeity. They observed no consistent alteration in triglycerides during the period of hypoglycaemia.

Insulin, obesity and diabetes :

The relationship between diabetes and obesity is well known. An increased insulin response to a carbohydrate load is a well recognised finding in non-diabetic obese patients (Karam et al 1963; Perley et al 1966; Solomon et al 1968; Chiles and Tzagournis 1970). Marked decrease in insulin values following physical training of the obese was interpreted as being due to increased insulin sensitivity of tissues and indicating that muscle is an important determinant for insulin sensitivity in obesity. Hyperinsulinism has also been noted during glucose tolerance testing in latent diabetics and in patients with mild maturity onset diabetes though fasting hyperglycaemia is associated with an absolute as well as a relative deficiency of

endogenous insulin (Yalow and Berson 1960; Chiles and Tzagournis 1970). Studies of Vallance Owen suggest that in subclinical and mild clinical diabetes of the maturity onset type, an early manifestation may be compensatory over secretion of insulin, either because of the presence of a circulating antagonist (Vallance Owen 1962, 1964 and 1966) or of peripheral resistance to insulin (Galton and Wilson 1970). While studying the effects of prolonged starvation on glucose tolerance and plasma insulin levels in obese patients, Jackson and his colleagues (Jackson et al 1969) observed improved glucose tolerance in diabetic group and impaired tolerance in nondiabetic group after prolonged fast and concluded that obesity is a heterogenous condition and the differences observed indicate separate aetiological factors in the genesis of obesity.

Obese insulin dependent diabetic patients commonly require larger than usual doses of insulin to establish a proper control of diabetes. The adipose tissue of these patients shows a decreased sensitivity to exogenous insulin (Boshell et al 1964; Daweke et al 1963). High plasma insulin concentration is the indicator of the loss of peripheral insulin effectiveness

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in the pathogenesis of diabetes (Reaven et al 1972). Since weight reduction diminishes the insulinogenic response to glucose, it is suggested that insulin action upon glucose uptake in enlarged fat cells is slow (Salans et al 1968; Robinowitz and Zierler 1962). Thus, positive relationship between hypersecretion of insulin and weight gain is indicated.

Synthetic oral hypoglycaemic agents :

Sulfonylurea compounds :

Oral antidiabetic drugs belong to either sulfonylurea group or biguanide class. The sulfonylurea compounds such as tolbutamide, chlorpropamide, carbutamide, tolazamide etc. are the modification of the same general structure having a benzenering + a sulfonyl group + urea group. Major effects of sulfonylurea oral hypoglycaemic compounds is the lowering of blood sugar level by increasing output or triggering the release of endogenous insulin. Effectiveness of the sulfonylurea is suggested to be due to damage to the alphacells of the pancreas, inhibition of the production of glucagon, increased utilization of glucose by peripheral tissues and inhibition of insulinase or insulin antagonism. Some investigators

find decrease in the rate of release of glucose from the liver by means of the inhibition of enzyme systems (Renold et al 1956).

Tolbutamide is carboxylated in the liver, rapidly metabolized and entirely excreted as carboxy-tolbutamide. Its duration of action is about 6 to 10 hours. Very little amount of chlorpropamide is metabolized in the body. All sulfonylureas get bound to serum protein. However chlorpropamide gets very firmly bound to protein. Duration of action of chlorpropamide may be as long as 60 hours.

Sulfonylurea compounds are goitrogenic in some animals. Unduly high incidence of hypothyroidism has been found in patients treated with these drugs (Brown and Solomon 1958). Side effects of sulfonylureas include flushing of skin in some alcoholics, sudden hypoglycaemia and nausea. Of the new sulfonylureas undergoing clinical trial, glibenclamide (Glyburide, HB 419) or 'Daonil' appears to be more promising with respect to the effectiveness and low toxicity effects.

The biguanide compounds :

The biguanide oral hypoglycaemic agent N'-betaphenethyl formamidinyliminourea hydrochloride

(phenformin DBI, N'-phenthyl biguanide HCl) was discovered in 1956. Presently available biguanides are phenformin, butaformin and metformin.

There appears to be no single decisive mechanism of action. The hypoglycaemic activity of the biguanides is independent of the pancreas. Williams and colleagues (1958) suggested that phenformin might act by increasing the glucose utilization by anaebolic pathway. Thus, it may directly interfere with the formation of high energy phosphate bonds and inhibit the regeneration of NAD. Later, several workers have supported this view (Moorhouse and coworkers 1958; Forbath and Clarke 1959; Sadow 1963). Though some reports do not agree with this view (Butterfield and Whichelow 1963). Other mechanisms suggested by which phenformin may act is by decreasing the rate of intestinal glucose absorption (Hollobaugh et al 1970; Kruger et al 1970) or it may exert an action on human insulin inhibitors present in diabetic individuals (Sterne 1964). Biguanides or phenformin in particular are often preferred for obese diabetics as these drugs are found to decrease fat synthesis and reduce body weight (Patel and Stowers 1964).

Phenformin concentrates in the liver and nearly 60 percent is recoverable in the urine with the elimination rate reaching its maximum value about 4 hours after oral ingestion. The biological half life of phenformin and its metabolite: is just over three hours.

The side effects of phenformin are usually limited to gastrointestinal tract and include metallic taste, anorexia, nausea, vomiting, flatulence and diarrhoea.

Various combinations of biguanides and sulfonylureas are also found effective in the treatment of diabetes. Beaser (1958) was the first one to document its use in the therapy of diabetes. Sulfonylureas increase the endogenous supply of insulin while phenformin causes greater utilization of available insulin.

Vascular disease in diabetes :

Diabetic vasculopathy involves two major processes. (1) Increased severity/earlier occurrence and increased prevalence of atheromatous disorders of coronary and cerebral arteries, premature damage to larger arteries and increased incidence of arterio-

sclerosis. (2) Microangiopathy associated with thickening of the capillary basement membranes in many tissues with specially disturbing consequences in the eyes and kidneys.

The increased incidence and severity of significant occlusive vascular diabetics in the heart and extremities among diabetics are evident from experience of many specialists and have been reported by several workers. In 1966, in United States, cardiovascular disease was responsible for 54 percent of all deaths (Bradley 1971) and in diabetic population death rate due to cardiovascular disease was 77 percent. Butterfield (1968) noted increased incidence of atheromatous lesions in those with even marginally impaired carbohydrate tolerance. At all age levels, diabetics have increased incidence of ischaemic heart disease (Epstein 1967). Raheja (1971) also observed higher incidence of ischaemic heart disease in Indian diabetics than in nondiabetics though Ginde and Talwalkar (1975) found that vascular complications are less common and renal disease, gangrene and diabetic coma are more common than in the western literature.

Recently, there has been some interest in glycoseaminoglycans in association with antidiabetic drugs. While studying the long term effect of tolbutamide and phenformin in rats, Prasanna and Kurup (1973) found that total cholesterol level of aorta was not appreciably affected whereas triglycerides and phospholipids showed considerable increase in the animals receiving tolbutamide and phenformin. The total sulfated glycoseaminoglycans to hyaluronic acid shows considerable decrease in both the tolbutamide and phenformin treated animals. Levels of different glycosaminoglycans in diabetic sera was studied by Malathy and Kurup (1973). They found an increase in the hyaluronic acid and a decrease in the heparin sulfate, chondroitin sulfate A and C. Glycosaminoglycans forms insoluble complex with lipoproteins. This happens more with sulfated glycosaminoglycans and less with hyaluronic acid. It has been proposed that high concentration of sulfated glycosaminoglycans normally present in aortic wall may limit the entry of lipoproteins by complex formation at the arterial wall surface. These workers have further stated that the concentration of sulfated glycosaminoglycans decrease in diabetic aortas while concentration of lipoproteins increases. Hence,

lipoproteins which are in free state enter the arterial wall. This partially explains the changes in atherosclerotic aorta. Decrease in the activity of enzyme L-glutamine, D-fructose-6-PO_4 amino transferase was found after long term administration of tolbutamide and phenformin which would lead to decrease in glycosaminoglycans synthesis (Prasanna and Kurup 1973). Reported increase in the incidence of atherosclerotic condition in tolbutamide administered diabetics has been explained by the observation that decreased concentration of glycosaminoglycans can result into increased lipid accumulation leading to vascular complications.

Saraswati Devi and Kurup have done pioneering studies on the hypolipidaemic effect of certain Indian pulses. They have studied the effect of dietary proteins on metabolism of glycoproteins (Menon, Leelamma and Kurup 1976). They also observed decreased glycosaminoglycans levels in the aorta of rats on hypercholesterolaemic diet (Seetanathan and Kurup, 1971). Concentrations of hyaluronic acid decreased in the rats receiving blackgram proteins whereas heparin sulfate and chondroitin sulfate C increased. (Menon and Kurup 1975).

There is no doubt that wise and intelligent use of oral hypoglycaemic drugs helps in achieving good control of blood sugar. It has often been said that those individuals who have maintained good control from the time diabetes was diagnosed are the ones in whom vascular disease occurs later and with less severity (Forsham et al 1958; Mathews 1954; Roots and Bradley 1959). However, some studies have raised doubts on the prevailing opinion thatn oral substitutes could prevent or delay the vascular lesion in diabetics nearly by promoting proper control of blood sugar level (Danowski 1957, Vaishnava et al 1968; Raheja et al 1970). Knowles (1970) showed the beneficial effect of good control. In 1964, Shipp et al reported for the first time hyperlipemia following long term therapy with sulfonylurea compounds in young diabetics and warned against their long term therapy in treating young diabetics which may lead to the development of lipemia retinalis and eruptive xanthomatosis. Cardiovascular mortality in diabetes and its relation with the long term use of antidiabetic drugs has been reported by Boyle et al (1972), Hadden et al (1972) and Garcia et al (1972). Boyle et al (1972) in a six year prospective study found greater frequency of myocardial infarction in the

patients treated by oral drugs than in patients treated by diet alone. According to recent morbidity and mortality statistics on diabetes in U.S.A., there has been no increase in the longgevity of the elderly diabetic during the last decade or two i.e. very much about the time the oral agents were discovered (Reid and Evans 1970). Hadden et al (1972) in a retrospective survey of the incidence of myocardial infarction and causes of death in maturity onset female diabetic patients found 25 percent deaths due to myocardial infarction in diet controlled diabetics as compared to 31 percent in oral drug treated and 35 percent in insulin treated. In a 16 year follow up Framingham study on morbidity and mortality in diabetics in a general population the larger number of deaths due to cardiovascular diseases were found to occur in the insulin treated group whose mortality was two and half times that of nondiabetics. The excess cardiovascular mortality in this group, was especially high in the insulin treated women where it was triple that of comparable nondiabetics (Garcia et al 1970).

UGDP studies :

The University Group Diabetes Programme (UGDP) is a long term prospective clinical trial designated to evaluate the effect of hypoglycaemic agents on vascular complications in patients with noninsulin dependent, adult onset diabetes. The principal design features of this clinical trial included the establishment of a common study protocol to provide for the collection of comparable data, inclusion of a placebo treated group with double blind evaluation of the oral hypoglycaemic drugs under study and central collection and monitoring of all study data. Study was conducted in 12 diabetic centres in U.S.A. Only patients with recently diagnosed diabetes in whom the diagnosis had been confirmed by a glucose tolerance test were selected for study. All patients were instructed regarding diet intended to achieve or to maintain normal body weight. Five groups of 200 patients each with maturity onset diabetes were randomly allocated to one of the following treatments; insulin variable (insulin dosage in the amount required to maintain "normal" blood glucose levels), insulin standard (ISTD) (insulin dosage of 10 to 16 units per day depending on the patients estimated body surface),

tolbutamide (1.5 gm daily given in split dosage), phenformin (100 mg of long acting phenformin per day in split dosage), or placebo (PLBO) (dosage schedule similar to those used for the two oral drugs under study).

The excessive cardiovascular mortality in patients treated with tolbutamide was an unexpected finding (Prout and Goldner 1970 - UGDP Report Series). The observed mortality from all causes and from cardiovascular causes was higher than that observed in any other treatment groups. In addition, there was no evidence that phenformin was more effective than any of the other treatments in preventing the occurrence of nonfatal vascular complications associated with diabetes (Knatterud et al 1971). Latest publication of UGDP does not show higher frequency of cardiovascular events in patients treated with tolbutamide (Miller et al 1976). Insulin therapy has also been found to fail in preventing vascular complications of diabetes. Despite of the fact that poor blood glucose control was present in only 11.6 percent of patients treated with variable doses of insulin in the UGDP, there is no evidence so far that this treatment group was spared degenerative vascular complications to any greater degree than the patients in the PLBO and the ISTD treatment group.

In 1971, Cornfield scrutinized the results of UGDP. In his opinion the management through sulfonylurea drugs in maturity onset type of diabetes gave inferior results in the matter of prolonging life than the management based on diet alone. Evidence of myocarditis and microgranulomata in the viscera of post mortem cases with prolonged administration of oral antidiabetic drugs before death was reported much earlier by Bloodworth and Hamwi (1961). An inotropic effect of sulfonylurea compounds upon the heart muscle has also been shown by some workers (Palmer et al 1971; Wolff and Grant 1971). The Joslin clinic has shown lowest number of cardiovascular deaths in diabetics treated on diet, highest on sulfonylureas and not quite as high on insulin. However, in this study, subjects had varying severity of the disease and results were statistically insignificant.

Studies not in favour of UGDP :

On the otherhand, there are also a few studies which present the evidence contrary to the results of UGDP. Balodimos and coworkers (1968) evaluated the post mortem findings of patients divided into three

groups of treatment with diet, oral agents or insulin. The frequency of myocardial infarction, cerebral vascular accidents and peripheral vascular disease occurred with equal frequency in both the groups. Similar findings were observed by Keen et al (1970) in London. Passikivi in Stockholm (1970) reported beneficial effect of tolbutamide treatment on high risk group comprising of those with arrhythmia or heart failure. In a study of 456 patients in Jerusalem assessed for vascular complications, it was found that in all age groups, there was no greater incidence of complications in the tolbutamide treated group than in the diet group whereas slightly higher incidence was found in insulin treated group (Fidel et al 1971). Data were analysed by Moss and Dewitt (1973) of 1840 patients in whom diabetes started after the age of 35 and before the age of 70, and who were under the constant care from one to fifteen years. According to the predictions of life insurance tables, these patients lived longer than nondiabetic patients. There was a significant decrease in the longevity of those who had persistent hyperglycaemia. The greatest increase in longevity occurred in those whose disease could be controlled by diet alone or

with sulfonylurea drugs. There was less apparent benefit from phenformin or insulin in this respect. It is indicated that in the treatment of diabetes sulfonylurea drugs have a beneficial effect upon longevity when they are used properly.

Hyperlipemia occurs commonly in untreated patients with diabetes mellitus. An increase in plasma concentration of fatty acids, triglycerides phospholipids and cholesterol is not infrequent even in well controlled diabetes. Because of the possible interrelation between glucose intolerance and elevated plasma lipids, interest in the effects on plasma lipids of hypoglycaemic agents designed to correct glucose intolerance continues. There are conflicting reports on the effects of plasma lipids of the marketed oral hypoglycaemic agents.

Lipid studies with oral drugs and insulin :

It is an accepted fact that the lipid abnormalities are closely associated with the development of atherosclerosis and cardiovascular disease so commonly found in diabetes mellitus. In maturity onset diabetics sulfonylureas have been found guilty of altering the lipid picture.

Butterfield and coworkers (1967) have shown that insulin and sulfonylureas induce lipogenesis and that the antilipolytic effects of insulin differ from those of the hypoglycaemic agents. Insulin acts on the adenyl cyclase system while the hypoglycaemic agents inhibit lipolysis by enhancing the phosphodiesterase activity and by inhibiting the activity of triglyceride lipase. Effect of diabetic diets on Serum cholesterol, triglycerides and free fatty acids, glucose and body weight was studied in 50 maturity onset diabetics by Hulse and Gershberg (1969). Absolute decrease in cholesterol and triglyceride after 9 months was more striking than tolbutamide and less than phenformin. Augusti and Kurup showed that tolbutamide inhibits lipoprotein lipase activity invitro and the hyperlipemia observed on tolbutamide therapy may probably be due to this inhibition. Insulin has only negligible effect on lipoprotein lipase activity (Augusti and Kurup 1967).

Bowers et al (1964) and Schwartz et al (1966) reported no effect of oral hypoglycaemic agents on triglyceride and cholesterol levels in diabetic patients. Later studies by Schwartz et al (1977) revealed that subjects receiving phenformin alone or

in combination had significantly lower serum cholesterol and triglyceride levels than matched diabetics treated with insulin and tolbutamide alone. Belknap et al (1967) performed a doubleblind cross over study with chemical diabetes and showed that tolbutamide did not to any noteworthy degree modify triglyceride levels that were experimentally elevated by high carbohydrate feedings. Shipp and Munroe (1962) and Morris and Bolinger (1964) reported definite reduction in the blood concentrations of total lipids and cholesterol after the use of oral hypoglycaemic agents. Bressler and Katz (1965) showed that tolazamide appreciably reduced serum cholesterol in maturity onset diabetes and that this decrease was achieved even in the face of unsatisfactory blood sugar control (Stone and Brown 1966). Stout et al (1974) proposed that the predominant effect was on levels of very low density lipoproteins with no change in low density lipoproteins. The sulfonylurea compounds were found to induce lipogenesis and rise in serum cholesterol (Meenakshi et al 1969). Experimental evidence also supports this finding (Stamler et al 1955; Hartal et al 1968). Prasannan and Augusti (1973) also found increase in the total as well as

free cholesterol in serum of the phenformin and tolbutamide treated rats. The total lipids were found to be lowered with phenformin therapy in diabetic hyperlipemia (Schwartz et al 1966; Alterman and Lopez Gomez 1968; Gershberg et al 1968). The effect of tolbutamide on the lipolytic response of rat adipose tissue and of a partially purified lipoprotein lipase fraction to adrenaline and ACTH has been studied and compared with action of insulin by Augusti and Kurup (1968). Tolbutamide and insulin reversed the stimulatory effect of adrenaline and ACTH both in case of adipose tissue and partially purified lipoprotein lipase preparation, the inhibition in the adrenaline being much more pronounced than in case of ACTH. Both insulin and tolbutamide have been reported to inhibit fatty acids and glycerol release from adipose tissue (Rudman and Shank 1966; Fain et al 1966). It is suggested that this inhibition may be due to increased synthesis and reesterification of fatty acids (Jungas and Ball 1963; Renold et al 1965). Prasannan and Kurup (1973) found no change in total cholesterol levels of aorta on long term administration of tolbutamide and phenformin whereas aortic triglycerides and phospho-

lipids showed considerable increase in the same animals receiving tolbutamide and phenformin. Insulin and the oral hypoglycaemic agents - chlorpropamide, acetohexamide and phenformin inhibited both the basal lipolysis and adrenaline stimulated lipolysis in rat adipose tissue and cyclic AMP reverses the inhibitory effect of these agents (Augusti 1975). Brown et al (1965) suggest that endogenous production of very low density lipoproteins is presumably due to phenformin's reduction of basal insulin levels (mean change of 23 percent) and free fatty acid levels (mean change of 27 percent). However, phenformin may also impair triglyceride clearance from plasma.

Murthy and Steiner (1972) have reported insulin stimulated lipogenesis in brown adipose tissue slices in absence of glucose. They have further suggested that insulin might have promoted lipogenesis in brown adipocytes by lowering cyclic AMP levels as it reduces the cyclic AMP levels in brown and white adipose tissues. Baldimos et al (1967) found rise in mean serum cholesterol level in their female patients treated with insulin or tolbutamide. Cahill (1971) has shown that insulin serves to

coordinate fuel mobilization into and out of the various body depots with the needs of the organism and with the availability or lack of availability of fuel in the environments. High insulin levels herald the "fed" state, and this initiates tissue uptake and storage of fuels. Low insulin levels herald the "fasted" state and this initiates mobilization of stored fuels from the tissue stores, the rate being proportional to the lowness of insulin. As the insulin levels increase, lipid synthesis occurs (when fuel is carbohydrate or fat). Even excess protein calories end up as fats. Diabetics well controlled with long acting insulin preparations will maintain higher insulin levels during the period of action of the preparation concerned. During that period, insulin levels are not likely to fall to low fasting levels even after the exogenous fuel has been dealt with. As compared to pretreatment levels and inspite of lower blood sugar levels, significantly higher insulin levels particularly in the fasting state were found in 14 well controlled diabetics who had sulfonylurea for 3-5 years (Boshell et al 1967). Thus, treatment with insulin or sulfonylurea tend to maintain the "fed" state. These observations thus

explain some of the reported findings in patients treated with insulin or sulfonylurea i.e. lipogenesis and weight gain (Butterfield and Whichelow 1967) rise in serum cholesterol (Baldimos et al 1967; Meenakshi et al 1969) or serum triglycerides (Camerini Davelos et al 1967).

Atherogenesis, oral hypoglycaemia agents and insulin :

One of the major complications of diabetes is atheroma of the blood vessels, particularly of the coronary and cerebral arteries and of the arteries of the lower limb. Its incidence has not diminished and indeed, appears to have become greater since insulin became freely available. Albrink and her associates have shown that such incidence was less when insulin was expensive. It is therefore, pertinent to examine in greater detail, what effect diabetes and its therapy have on the metabolism of the arterial wall.

i) Lipid formation in arterial tissue :

Metabolism of heart and arteries has been extensively reviewed by Opie (1973). The lipid found in atheroma may be derived partially from the circulation and partially from increased local synthesis or decreased local breakdown of lipid.

In arterial tissue, formation of glycerides from exogenous or endogenous fatty acids can occur because α-glycerophosphate for esterification can be provided by glycolysis. The activity of the pentose-phosphate pathway which is required for truelipid synthesis, is high in arterial tissue as judged by the activity of G-6-P-D. Exogenous long chain free fatty acids in man are mainly used for lecithin than for triglycerides in aorta. There is little evidence to show that significant synthesis of cholesterol can occur in arterial tissue. The major part of cholesterol in the atheromatous lesion in cholesterol fed rabbits is derived from plasma. Esterification of cholesterol can occur in the intima of atherosclerotic rabbit aortas and the rates of esterification may be increased by endogenous or exogenous free fatty acids. Such esterified cholesterol becomes fixed in the arterial wall. Once arterial lipid is formed by the above processes, further arterial degeneration could be promoted. As the lipid concentration in arterial tissue increases, diffusion of nutrients and O_2 becomes more difficult. Hypoxia may stimulate lipid synthesis in the arterial wall, as in other tissues, by increased reduction of cofactors (including reduced NADP). As

mentioned earlier, hypoxia, hypertension and mechanical injury could increase the permeability of the endothelium of the arterial wall to exogenous lipid in the form of cholesterol and low density lipoproteins.

ii) Arterial wall metabolism in diabetic state :

Overt diabetes mellitus is linked with increased incidence of arterial disease and coronary heart disease. In this link, possible explanations have been given by Renold (1973). According to him, initially the insulin independent conversion of glucose to glycoproteins and other metabolites might be taking place in the vascular membranes and it may result in the material found in atheromatous and microangiopathic lesions. Accumulation of sorbitol formed by polyol pathway (in which glucose could be converted without phosphorylation to sorbitol) in the arterial wall could osmotically damage the arterial wall and encourage lipid synthesis by nonoxidative pathway. An increased insulin concentration thought by some to be an important early lesion in diabetes, might have direct effect in promoting atherosclerosis. Enhanced lipid synthesis found in the rat aortic wall can be related to increased levels of circulating glucose and insulin.

iii) Role of insulin and oral hypoglycaemic agents :

Abnormalities in insulin secretion have often been described in patients with ischaemic heart disease. These abnormalities in insulin secretion consist of raised insulin responses to oral carbohydrates with or without high fasting plasma insulin levels (Mirsky et al 1966) and are found in atherosclerotic subjects who are neither obese nor hyperglycaemic (Nikkila et al 1965; Peterson and Hales 1965; Christiansen et al 1968; Tzagournis et al 1967). It has been suggested that hyperinsulinism may be the common factor linking atherosclerosis with diabetes, obesity and hyperlipidaemia (Stout and Vallance Owen 1969). Stout and Vallance Owen have shown that intravenous injection of rats with insulin and C^{14} - labelled substrate containing either glucose or acetate led to much greater incorporation of these substrates into the aortic lipids than when the substrate alone was injected. They have further suggested that the C^{14} labelled lipid in the aortic wall is probably triglycerides synthesized from fatty acid and α-glycerophosphate - both of them having been metabolized from the injected substrates. Mahler (1966)

has shown inhibition of tissue lipase in arterial wall by insulin and suggests that this results in the accumulation of lipids and hence the formation of atherosclerosis. There is an indirect evidence that insulin has an effect on arterial lipid metabolism. Administration of insulin inhibits regression of arterial lesions induced by a high cholesterol diet which occurs when the animal is transferred to a normal diet and abolishes the protective effect of oesterogens on experimental atherosclerosis (Stamler et al 1960). In pancreatectomized rats fed on atherogenic diet, treatment with insulin reduces the serum cholesterol to that of nondiabetic controls but does not decrease the incidence or severity of their vascular lesions, as happens when a comparable reduction of hypercholesterolaemia is affected by dietary methods (Wilson et al 1960). Obese spiny hyperglycaemic rat which has both hyperglycaemia and hyperinsulinism develops lipid infiltration of its coronary arteries at a much earlier age than its normal littermates (Renold et al 1968). Stout (1970) further showed that treatment with long acting insulin resulted in vascular lesions in chicks similar to the earlier lesions in human atherosclerosis.

To further test the role of insulin in the pathogenesis of atherosclerosis, rats were injected intravenously with sodium ($1\text{-}C^{14}$) acetate with and without insulin. It was found that insulin stimulated the incorporation of sodium acetate into total lipids, cholesterol and phospholipids to a significant extent whereas incorporation into triglycerides just failed to reach statistical significance (Stout 1971). Increased arterial cholesterol synthesis after insulin treatment was unexpected finding. The exact site of insulin on cholesterol metabolism is not very clear. In this connection, studies by Nepokroeff et al (1974) have brought out interesting findings. They found that in streptozotocin induced diabetes, rat liver B-hydroxy-B-methyl-glutaryl CoA reductase activity and the amplitude of the diurnal variation of this enzyme were progressively reduced to very low levels whereas daily insulin therapy to 7-day diabetic rats restored the activity and the amplitude of this diurnal variation.

Vallance Owen has suggested that late onset diabetics are obese because the insulin synalbumin antagonist in their plasma does not interfere with the effect of insulin on adipose tissue and therefore,

would permit accumulation of fat in the artery. Insulin dependent diabetic is not really insulin deficient. There must be periods during the day when he has an excess of exogenous insulin in the plasma covered by the dietary carbohydrates.

All these studies lead to a vague conclusion that insulin could be responsible for the accumulation of fat in the arteries. Long term effect of insulin on lipid metabolism and atherosclerosis has not been explored much even after the hypothesis put forward by Stout. Hence, present study aimed at exploring the effect of insulin and oral hypoglycaemic agents which potentiate the insulin release. Since it was extremely rare to get completely well controlled insulin dependent Indian diabetic patients, the present study was extended to rabbits - a species sensitive to insulin as well as atherosclerosis.

There is considerable epidemiological evidence presented during recent years warning public against the use of tolbutamide. This aspect has been reviewed earlier. However, comparatively there are very few reports on experimental work invivo or invitro. Khachadurian and Badeer (1961) have shown that physiological concentration of tolbutamide increases glucose

uptake by the isolated heart lung preparation and decreases the coronary blood flow. Higher doses caused severe cardiac failure. Hyperlipemic effect of long term use of tolbutamide is well recognised. However, there are hardly any strong evidences showing tolbutamide induced atherosclerosis. Therefore it seemed pertinent to us to study the effect of long term administration of insulin and tolbutamide on lipid metabolism and atherogenesis in rabbits.

Enzyme studies :

Inspite of the existing controversies regarding the long term effect of insulin and oral hypoglycaemic drugs, the mechanism by which this hormone acts in various tissues causing lipid accumulation is not explored to a satisfactory degree. While studying the effect of insulin deficit and excess on macromolecular changes in connective tissues of rats, Beramon et al (1972) commented that insulin is the driving force in the synthesis of macromolecules and as such the long term administration of insulin could contribute to certain complications seen with long term insulin therapy. Thus, a disturbance in the level of insulin would account for far more than the changes in the arterial wall.

Insulin changes the activity of several enzymes involved in the metabolism of fats and carbohydrates. Hansen et al (1970) demonstrated that insulin increased the hexokinase content of epididymal fat pads invitro using glucose, pyruvate or alanine as energy substrates. These workers concluded that insulin stimulation of hexokinase activity is accompanied by the de novo synthesis of hexokinase protein. Mehlman et al (1971) replaced the carbohydrate in the diet of normal, diabetic and diabetic insulin treated rats with 1,3,butanediol. They found that in adipose tissue, malic enzyme activity was greatly decreased in diabetic and increased in diabetic insulin treated animals. Phosphofructokinase and pyruvatekinase are decreased by starvation and diabetes and increased by insulin (Sharma et al 1963; Chang and Schneider 1971).

Though tolbutamide is a stimulator of insulin secretion it also has an action independent of insulin on carbohydrate metabolism. Tolbutamide increases the glucose uptake of rat diaphragm (less than insulin). Tolbutamide increases the phosphorylase activity of rat liver. Insulin has no effect. Glucose 6-phosphatase is inhibited by both. Triose phosphate isomerase is inhibited by only tolbutamide (Augusti and Kurup 1967).

Effect of insulin, tolbutamide and glucagon on activities of jejunal carbohydrate metabolizing enzymes was studied by Lufkin et al 1975 in normal young males and obese subjects. Insulin increased the activities of pyruvate kinase and hexokinase, decreased that of fructose 1-6-diphosphatase and had no effect on fructose 1-6-diphosphate aldolase. Glucagon had opposite effect. Tolbutamide significantly increased the activities of pyruvate kinase, hexokinase and fructose diphosphate aldolase and decreased the activity of fructose diphosphatase. These results suggest that some of these effects of tolbutamide are independent of endogenous insulin.

Effect of diabetes on cholesterol metabolism has been studied by Lawrence White (1970). His studies indicate that diabetes leads to decreased hepatic cholesterol synthesis chiefly as a result of inhibition of microsomal reduction of hydroxymethyl glutaryl CoA (H MG CoA) to mevalonic acid. Insulin increases the activity of HMG CoA reductase as has been mentioned earlier (Nepokroeff et al 1974). It seemed worthwhile to know whether the changes in the enzyme activity caused by the disturbance in the level of hormones are the first link in the series of events starting from

insulin treatment upto cardiovascular disease. Therefore, a study was planned to observe the long term effects of insulin and tolbutamide on activities of certain key enzymes involved in carbohydrate and lipid metabolism.

Present approach would help in evaluating the effectiveness of oral drugs and insulin in the management of diabetes and its complications.

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