CHAPTER VI

DISCUSSION

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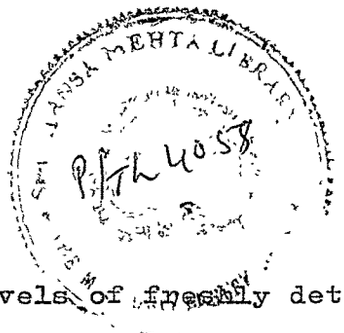
As observed from Table 1, incidence of cardiovascular disease was found to be lower in diet controlled group than in other treatment groups. It is difficult to make meaningful comment from the present data regarding cardiovascular complications in various groups as the number of patients in each group is not sufficiently large. However, some of the reports cited in the literature and UGDP studies have already shown beneficial effect of diet over drug treatment in controlling cardiovascular complications. Studies conducted by UGDP (Prout and Goldner 1970) report excessive cardiovascular mortality in patients treated with tolbutamide group. Insulin therapy was also found to exert no favourable effect on vascular complications of diabetes despite the fact that poor glucose control was present in only 11.6 percent of patients treated with insulin. However, it is of interest that in the latest publication of the UGDP, nonfatal cardiovascular events were not seen more frequently with tolbutamide since their mortality data strongly pointed out increased mortality due to cardiovascular diseases in tolbutamide group (VIth Supplementary Report - UGDP Series 1976). Joslin Clinic

has shown lowest number of cardiovascular deaths in diabetics treated with diet and highest in diabetics treated with sulfonylureas though severity of diabetes was a variable factor in the study. Amongst Chinese in Hongkong, 6.6 percent of diabetics were treated by diet therapy, 47.5 percent by oral drugs and 45.9 percent by insulin for the management of diabetes (MacFadzeon and Young 1968). In the same population prevalence of coronary artery disease was found in 4.7 percent, cerebrovascular disease in 7.4 percent, peripheral vascular disease in 1.4 percent, retinopathy in 14.4 percent and nephropathy in 10.3 percent. Findings of these studies would be of interest when one looks at the lipid profile of the drug treated groups in our human and experimental studies together.

Most of the diabetic patients showed normal blood sugar levels (Table 2) though few did show slight hyperglycaemia. This occasional hyperglycaemia occurred when the patient missed a tablet on previous night. Since these patients were regularly being checked up at the diabetic clinic, selection of well controlled diabetic patients was possible while referring to their past records and through occasional

rechecking of their blood sugar and glucose tolerance test. This avoided the possibility of including uncontrolled drug treated patients in the present study. Inspite of achieving good control of diabetes, patients showed varying lipid profile when classified according to drug treatment (Table 3). This is an important finding of the present study. All groups except diet and phenformin group showed significantly higher serum triglyceride levels as compared to normal group (P values are given in Table 3). When compared to diet control group, sulfonylurea groups and combination group showed significantly higher levels of serum triglycerides. . Similarly findings have been reported by Butterfield et al (1967) and Hulse and Gershberg (1969). Higher levels of serum triglycerides have been reported in sulfonylurea treated patients by Santen et al (1972). In present study phenformin group showed significantly lower levels of triglycerides than the tolbutamide group ($P < 0.05$). Lipid lowering effect of phenformin has been observed by many workers (Alterman and Lopez Gomez 1968 and Crawford et al 1969). This again indicates beneficial effect of diet control over sulfonylurea drugs as high levels of triglycerides are associated with greater risk for cardiovascular disease (Albrink et al 1963).



Serum cholesterol levels of freshly detected patients and tolbutamide treated patients were higher than normal group ($P < 0.001$ and $P < 0.005$ respectively). Tolbutamide group also showed significantly higher levels of cholesterol than diet controlled and phenformin group ($P < 0.001$ in both the cases). Importance of hypercholesterolaemia in development of cardiovascular disease is well established (Gofman and coworkers 1966, Westlund and Nicolaysen 1966). High levels of cholesterol have been reported with tolbutamide treatment by several other workers (Reinheimer et al 1967, Meenakshi et al 1969). However, reduction in serum cholesterol is reported with phenformin by Feldman (1977). He found no difference in mortality rates in tolbutamide, phenformin and placebo groups. Lipoprotein pattern is proved to be more informative rather than serum cholesterol and serum triglyceride concentration alone in assessing different metabolic processes leading to hyperlipidaemia. Such a system provides better understanding of the mechanism of production of high lipid values and their metabolic derangements and gives a better clue to selection of appropriate kind of prophylactic management. As seen from Table 4, type II

and type IV hyperlipidaemia were found to be more common in diabetes as a whole. More number of diabetic individuals were found to have type II and type IV serum abnormalities than nondiabetics (Thakur and Sinha 1977). In our studies, diet group had lesser number of abnormal cases as compared to freshly detected, tolbutamide and combination group (Table 4). All the groups except phenformin group had significantly higher pre-β lipoprotein levels (P values are given in Table 5). Phenformin group did not show high triglyceride levels which might have resulted in low pre-β lipoprotein levels in phenformin group. Sita Devi et al (1974) found 25 percent type II and 29 percent type IV and 46 percent normal lipoprotein patterns in maturity onset diabetics. They have also observed more patients with cardio-vascular diseases in type II and in type IV. Our studies though with small number of patients due to rigid criteria for selection coincidentally showed similar trend i.e. more number of cardiovascular diseased patients in tolbutamide treated group which showed more cases of type II abnormality. Hyperlipidaemia in tolbutamide treated patients have been reported by number of workers earlier but in their studies,

disbetes was not controlled. Results of the present study show hyperlipidaemia in tolbutamide treated Indian diabetic patients who are well controlled. This feature makes the study unique of its own kind particularly in Indian patients of low economic status. We have not come across reference of a comparable study in Indian literature because such large studies are only possible at teaching hospitals in India where while many poor patients apply for treatment, the drop-out rate from treatment is nearly 80 percent in chronic disease. Lipemia observed in tolbutamide therapy has been attributed to inhibition of lipoprotein lipase activity by the tolbutamide (Augusti and Kurup 1967). While discussing the hypolipidaemic effect of phenformin, Stout et al (1974) proposed that the predominant effect of phenformin was on the levels of very low density lipoproteins with no change in low density lipoproteins. The decrease in endogenous production of very low density lipoproteins may be due to reduction of basal insulin level and free fatty acid level by phenformin. Phenformin might be impairing triglyceride clearance from plasma (Shen and Bressler 1977). Phenformin is known to inhibit oxidative pathway of glucose metabolism. This may also cause reduction in the availability of reduced NADP used for lipid and cholesterol synthesis.

In the experimental study on rabbits only tolbutamide and insulin were used. Differences in lipid levels in tolbutamide and insulin treated animals together with differences without and with cholesterol feeding could be seen from Table 7 to 14. Serum cholesterol levels were significantly higher in group III (Table 10) than in group I and II ($P < 0.001$ in both cases). We did not find any effect of tolbutamide and insulin on serum proteins though marked reduction in proteins have been observed on tolbutamide therapy by Prasanna and Augusti (1973). Similar findings have been observed in experimental animals (Stamler et al 1955 and Hartal et al 1968). Present studies on controlled diabetic patients also showed hypercholesterolaemia after long term tolbutamide therapy. Total lipid levels were significantly higher in the livers of insulin treated group than control group ($P < 0.025$) (Table 12). Heavier livers observed in this group could be perhaps due to higher total lipids in the liver in the same group. Higher levels of total lipids in the liver could be explained by the lipogenic action of insulin. Free cholesterol levels in the liver (Table 12) were higher in tolbutamide treated group (163 mg%) as compared to control group (135 mg%).

In aorta, both cholesterol and total lipids were higher in case of insulin treated groups without cholesterol (107.5 mgms and 17.1 gms/100 gm tissue respectively as compared to control group (group I). No difference was found between groups in set 'B' i.e. groups fed cholesterol. The exact site of action of insulin on metabolism of cholesterol was not very clear so far. Recently Nepokroeff et al (1974) however, reported increase in hepatic β-hydroxy-β-methyl-glutaryl CoA reductase activity on insulin treatment in rats. Present studies did not show significant increase in cholesterol content of liver in experimental group, probably due to short duration and smaller doses of the drug. Decrease in the serum cholesterol was noted at the end of experiment (Table 8) in insulin treated group ($P < 0.025$). Serum lipid levels were found to be lowered in rats treated with insulin for 10 days (Dumaswala 1974).

Increase in cholesterol and total lipid content of aorta in insulin groups (Table 14) confirms the lipogenic action of insulin in aorta. Increased arterial cholesterol synthesis was found after insulin treatment by Stout (1971) and Mahler (1969). Insulin stimulated the incorporation of acetate into total lipids, cholesterol and phospholipids in aorta of rats. Earlier, Kendall

(1967) had pointed out the dependence of aortic wall metabolism on insulin.

It will be seen from the results of histopathological examinations that the atherogenic effect of cholesterol was exaggerated with both insulin and tolbutamide. Changes associated with lipemia were more marked in tolbutamide group. Fatty changes in the livers and myocardium were more extensive with tolbutamide whereas lesions in aorta were of greater severity in insulin group. Judging histopathologically, the effect of insulin is greater with respect to atherogenecity as compared to tolbutamide group. If the exposures are prolonged it would be difficult to draw comparative pictures between groups and groups. Therefore, in this study rabbits were sacrificed at an earlier stage. Biochemical parameters studied are somewhat in line with the histopathological changes observed. It should be noted that the dose of insulin and tolbutamide was important in these experiments. While tolbutamide is well tolerated by rabbits, it is not so with insulin. Slight overdose causes fatality in the animals. Hence, the doses were carefully worked out and administered in minute quantities so as to produce minimal hypoglycaemia (only about 10 mg fall in the blood sugar).

While observing these adverse effects of drugs which are used in large doses to treat diabetics, several clinical findings in diabetic patients are of interest. Obese insulin dependent diabetic patients commonly require larger than usual doses of insulin for establishing proper control as adipose tissue of these patients shows a diminished sensitivity to insulin (Boshell et al 1964). Higher incidence of atheroclerosis is found in people who have abnormally large amount of insulin (Stout and Vallance Owen 1969), and excessive insulin levels have been reported in vascular disease (Nikkila et al 1965; Peters and Hales 1965; Welborn et al 1966). The nearest parallel to the findings reported here occurs in obese hyperglycaemic spiny rat. This animal has both hyperglycaemia and hyperinsulinism, and it develops lipid infiltration of its coronary arteries at a much earlier age than its normal litter mates (Renold et al 1968). Excessive insulin promotes lipogenesis and arterial wall participates in it. This has been explained by the fact that insulin increases the oxygen consumption of human intimal cells and this may lead to localized hypoxia of the intima with subsequent increase in lipid accumulation (Robertson 1968). Mahler (1970) has shown decreased lipoprotein lipase activity in arterial tissue

after insulin administration in alloxan diabetic rats. He found greater accumulation of fat in the arteries and suggested that mechanism may be similar to that found in adipose tissue. Insulin supports the proliferation of arterial smooth muscle cells (Stout et al 1975). Altered platelet function in diabetes with hyperinsulinaemia leading to atherosclerosis has been reported by Szanto and Yudkin (1969). Importance of these observations is realised from the fact that initial arterial thrombus consists of platelet aggregates (Mustard and Packham 1970). Platelet thromboemboli may initiate disturbance in circulation. While considering this aspect, it is worth noting that phospholipids activate aggregation of platelets (Marcus et al 1972). All these observations are of value when we take into account higher levels of serum phospholipids and free cholesterol in liver of drug treated groups than the control group in our experiment.

Platelet aggregation as a factor for atherosclerosis is important and the effect of insulin and oral hypoglycaemic agents on platelet aggregation can not be ignored. Hellem (1968) has

shown increased platelet adhesiveness in diabetics treated with insulin. Sharma et al (1976) found that administration of phenformin and ethylestrenol with high cholesterol diet was found to increase the blood fibrinolytic activity and to prevent the increase in serum cholesterol and plasma fibrinogen. When given alone, both phenformin and ethylestrenol failed to produce sustained effect on blood fibrinolytic activity. In many ways platelet function and lipid metabolism are also found to be linked. In patients with raised levels of low density lipoprotein, this association is found stronger. Platelets contain increased phospholipids (Nordoy and Rodset 1971) and free cholesterol (Miettmen 1974). When human platelets are enriched in cholesterol by incubation with liposomes a striking increase in aggregability ensues (Shattil et al 1975). Ross and coworkers (1976) found decreased platelet survival in cholesterol fed monkeys probably because the platelets adhere to areas of endothelial damage. Smooth muscle cell hyperplasia, characteristic of atherosclerosis, follows experimental endothelial injury. This may be mediated by platelets. A nondialysable platelet component causes proliferation of smooth muscle cells and fibroblasts in tissue culture

(Rutherford and Ross 1976). Long term use of antidiabetic drugs either causes or aggrevates hyperlipemia and rise in low density lipoprotein levels in serum. This condition is closely linked with increased aggregation of platelets and in turn atherosclerosis. Stout (1970) has shown insulin induced atherosclerosis in chicks. In our studies plain insulin has been used and administered in very minute doses and yet we could find its deleterious effect. Present experiments report induction of lipid accumulation in the arteries of the experimental animals by insulin even without dietary manipulation in set 'A' (groups which did not receive cholesterol) of the experiment. Our observations discussed in the light of other findings mentioned above provide good evidence to support the hypothesis that insulin has atherogenic effect. No one has tried to prove the atherogenicity of insulin so far except Stout who used chicks as experimental animals (Stout 1970). We have done this in normal rabbits. This is an important contribution of our experimental work.

Table 16 shows that insulin group showed significant difference ($P < 0.01$) in malic enzyme activity as compared to tolbutamide group. Serum glutamate pyruvate transaminase was also higher in case of insulin group ($P < 0.005$) than tolbutamide group. Enzymes analysed in the present investigation were selected as the representative of various metabolic pathways associated with carbohydrate and lipid metabolism. Glucose-6-phosphodehydrogenase was selected as an index of hexose monophosphate shunt. Pathway and malic enzyme and citrate cleavage enzyme were selected because of their potential relationships to lipid metabolism. Pyruvate kinase reflects gluconeogenesis and glycolysis. Because of smaller doses and high biological variations, statistically significant increase in the activities of most of the enzymes could not be observed though insulin group did show significantly higher levels of malic enzyme than tolbutamide ($P < 0.01$). Therefore, results of experiment 2 could not be correlated well with enzyme studies in the same animals. However, it has been suggested that insulin functions as a major and perhaps the only factor in causing increased biosynthesis of the key glycolytic and lipogenic enzymes in

a selective fashion (Weber et al 1966, ~~1969,~~ 1971). They found decrease in pyruvate kinase activity in insulin deficiency and increase in G-6-P-D activity in presence of insulin. Present studies failed to reveal any significant difference in the pyruvate kinase, Glucose-6-P-D and citrate cleavage activity. However, long term administration of insulin in larger doses might be contributing to lipid synthesis by providing more NADPH for lipogenesis.

In present studies, cholesterol fed animals (set B) showed lower activities of most of the enzymes. Several investigators have reported that high cholesterol diet results in a decrease in the rate of protein synthesis (Saito and Fillios 1964, 1965). This decrease in the rate of protein synthesis in cholesterol fed animals has been attributed to the accumulation of the cholesterol in the endoplasmic reticulum of the cells of the liver. As enzymes are specific proteins, the decrease in the activities of liver enzymes may be due to decreased protein synthesis in the liver.

When the results of all the experiments (human studies and animal studies) are considered together, we find marked difference in the effect

caused by sulfonylureas (tolbutamide in particular), biguanides and insulin. These differences specially between action of insulin and tolbutamide can be explained in the light of the studies mentioned here and elsewhere as further clue to varied action of the drugs could only be obtained by applying radioisotopic techniques. It has been suggested that some of the antilipolytic effects of insulin differ from those of the hypoglycaemic agents. Insulin inhibits lipolysis by inhibiting the adenyl cyclase systems while the hypoglycaemic agents inhibit lipolysis by enhancing the phosphodiesterase activity. Tolbutamide has been found to inhibit lipoprotein lipase activity invitro whereas insulin has negligible effect. Hyperlipemia observed on tolbutamide therapy may possibly be due to this inhibition (Augusti and Kurup 1967). Another aspect that has been explored recently is decreased concentration of glycosamino glycans which might result into increased lipid accumulation in the arteries of tolbutamide treated rats. Similar process in human beings is likely to increase the incidence of cardiovascular complications in tolbutamide patients. This could be a future line of research for lipid chemists.

It may be pointed out that in our studies, uncontrolled diabetic patients (freshly detected) showed worse serum lipid profile than even tolbutamide treated patients. This finding suggests that primary need for prevention or delaying of the complications of diabetes is to control diabetes. But selection of treatment in a stabilized diabetes is then of due consideration and there is nothing better than a diet control. All drugs are involved in upsetting lipid metabolism of liver cell, arterial wall cell and the whole metabolic chain between them. Some like insulin upset more than the other as present work has shown. A very recent study by Ahuja et al (1978) on evaluation of the clinical and biochemical profile of recently diagnosed untreated diabetics reported significantly raised serum cholesterol i.e. above 250 mg percent in 35 percent, serum triglycerides above 130 mg percent in 26 percent and nonesterified free fatty acid levels more than 1000 mEq/L in 60 percent of diabetics. Pre-beta-lipoproteins were significantly high in 21 percent of diabetics. It was suggested that a derangement of endogenous lipid metabolism accounted for the observed hyperlipidaemia as dietary constituents (total calories and fats) were not quantitatively high in their study.

Recent reports on effect of sulfonylurea drugs on lipid profile of controlled and uncontrolled Indian diabetic patients shows interesting results. Serum cholesterol, triglycerides, free fatty acids (FFA) and prebetalipoproteins were found significantly higher in uncontrolled diabetic patients (255 mg percent, 310 mg percent, 268 µeq/L and 29.4 respectively) than in controlled diabetic patients who showed 248 mg percent cholesterol, 213 mg percent triglycerides, 249 µeq/L FFA and 18.7 of prebetalipoproteins (Kothari 1977). Kothari (1977) also found slight rise in mean cholesterol level and highly significant rise in level of serum triglycerides and phospholipids in cases with diabetes not associated with cardiovascular diseases. Same observations are found in diabetes associated with cardiovascular disease. Though the rise in serum triglycerides have been very nearly the same in both the groups, the lipoprotein pattern in diabetes not associated with cardiovascular disease showed significant rise in betalipoproteins and normal level of prebetalipoproteins with significant simultaneous fall in alphalipoproteins, while in diabetes associated with cardiovascular disease, lipoprotein pattern showed maximum significant rise in prebetalipoproteins with

simultaneous significant fall in beta and alpha-lipoproteins. This leads to the conclusion that the lipid transport in diabetes associated with cardio-vascular diseases is mainly through prebetalipoproteins, while in diabetes not associated with cardiovascular disease, the transport is mainly through betalipoproteins. Her study was conducted on patients of a private hospital belonging to high socioeconomic group whereas in present study patients belonged to low socioeconomic group attending a public general hospital. However, in both the studies deteriorated lipid profile in uncontrolled diabetes was a common observation. But, improved control of blood sugar can also present some other complications. When maturity onset diabetes is poorly controlled, there is increase in the hepatic lipid production. Although improved control reduces the concentration of plasma lipids, it appears to enhance the risk of supersaturated bile and cholesterol gall stones by decreasing the synthesis of bile acid (Bennion and Grundy 1977). It also appears from present studies that in diabetic patients, diet control has better effect on lipid metabolism than drug treat-ment. Amongst drug treated patients, phenformin has better effect than sulfonylurea compounds. Sulfonylureas

act by increasing the endogenous supply of insulin (other actions which are independent of insulin have also been reported) while phenformin causes greater utilization of available insulin. Excessive glucose and excessive insulin linked with increased platelet adhesiveness could initiate atherosclerosis. These effects were found to be reversed inspite of high sucrose diet if subjects were given phenformin (Szanto and Yudkin 1969) as phenformin reduces circulating insulin. Beneficial effect of phenformin on reducing fat synthesis and thereby body weight has been reported by Patel et al (1964). Role of insulin in the pathogenesis of atherosclerosis as reported by few studies earlier (Kendall 1967; Stout 1968,1969) further explains adverse effects observed in patients treated with sulfonylurea drugs as also in animals treated with tolbutamide and insulin in our experiments. As suggested by UGDP studies, by proper and wise combinations of hypoglycaemic drugs it may be possible to reduce complications of vascular diseases in diabetics. UGDP studies have been carefully scrutinized by many workers. The committee for assessment of biometric aspects of controlled trials of hypoglycaemic

agents (1975) has assessed the validity of the UGDP study. The probability that oral hypoglycaemic agents cause premature deaths due to cardiovascular disease remains valid. The UGDP is the most comprehensive study of oral hypoglycaemic agents published so far. Our results corroborate the UGDP study though with small number of patients in each group.

Excessive cardiovascular complications observed in patients treated with tolbutamide represents a real risk. Until it is possible to find the cause, this drug must be considered hazardous for long term use.

If a coherent picture is to be constructed on a logical and rational basis of the data analysed in the foregoing thesis, certain broad conclusions would emerge. The experiments are in animals which had no underlying pathology. Lesions of atheromatous type like lipid deposition in aorta could be induced by feeding cholesterol. If either tolbutamide or insulin is given, the atherogenesis in the vessel wall was more pronounced. If cholesterol was not given, the same drugs could not produce these very lesions though significant biochemical parameters

were disturbed. Thus, the underlying pathology of altered lipid metabolism of a diabetic is an important factor in producing the atherosclerosis. The two drugs are merely potentiators of a process or accelerators of a morbid change which is ushered by the disease itself. Evidence of hyperlipemia is seen in the clinically controlled drug treated cases. Even the same could be reduced in the normal animals treated with drugs. The analytical data of the lipid studies of the drug treated rabbits also support the abnormal tissue lipogenesis, lipid storage and lipid distribution being involved in the mechanism of atherosclerosis. If a drug accelerates or potentiates one morbid change that is already in progress while altering the other it is only an unmixed blessing. While drugs are helpful in controlling the blood sugar levels, they can be really dangerous in the long run as they also do harm. The treatment of hyperglycaemia does necessarily not treat the pathology of diabetes. Basic pathology relentlessly progresses over years but the controlled patients do better than the uncontrolled ones in any case. Search for a harmless yet more helpful remedy is still necessary. May be that studies like

this will provide a parameter for true evaluation of that harmlessness. If this be so, the present efforts are amply rewarded.

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