EFFECTS OF INSULIN OR THYROID TREATMENT
ON DIABETES-INDUCED MYOCARDIAL ABNORMALITIES

by

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We accept this thesis as conforming
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ABSTRACT

Diabetes induced in female Wistar rats by an intravenous injection of streptozotocin resulted in myocardial changes six weeks after injection of the drug. The left ventricular developed pressure (LVDP), the rate of pressure rise (positive dP/dt) and the rate of pressure decline (negative dP/dt) were depressed in diabetic rat hearts as compared to controls when measured using the working heart technique. The ability of myocardial SR to transport calcium was also depressed in diabetic animals and the level of long chain acylcarnitines was elevated in these animals.

Treatment of the diabetic animals with insulin (0.9 U/100g/day) immediately after the disease was detected prevented the deterioration of the physical condition of diabetic animals. The treated animals did not lose weight, had normal plasma glucose and insulin values and the degree of glycosylation of hemoglobin did not differ significantly from controls. Cardiac function as reflected by LVDP and positive and negative dP/dt was not depressed in insulin treated diabetic rats. In parallel to these results, it was found that the level of long chain acylcarnitines in SR of treated animals was not elevated. Calcium uptake activity of SR was also normal in diabetic animals treated with insulin. The data suggest that insulin treatment is capable of preventing changes in the myocardium of diabetic rats.

In the next set of experiments, diabetic animals were treated with insulin six weeks after the disease was induced. Four weeks of treatment normalized the physical features i.e. body weight, plasma.
glucose and plasma insulin values. However, glycosylated hemoglobin values were not completely reversed to normal. Studying the heart function, it was found that such treatment was effective in reversing the depressed cardiac function to normal. Calcium uptake activity and long chain acylcarnitine levels in SR were also reversed to normal. These experiments suggest that insulin treatment is capable of reversing as well as preventing diabetes-induced myocardial alterations.

The effect of insulin treatment on hearts of rats from five month diabetic animals was then examined. General features of the five month animals were similar to those described in the ten week reversal study. Heart function analysis revealed that while the LVDP and positive dP/dt were only partially reversed by insulin treatment, negative dP/dt was completely normalized. These studies suggest that the cardiac alterations induced by diabetes can be normalized by insulin if treatment is initiated soon after the onset of diabetes.

As diabetes results in hypothyroidism, we studied the effect of thyroid replacement therapy on diabetes-induced alterations. Treatment of diabetic animals with T3 (30 μg/kg/day) normalized the thyroid status of diabetic animals. However, cardiac function remained depressed in the T3 treated animals as did the calcium uptake in SR. Thus diabetes-induced myocardial alterations do not seem to be a result of the hypothyroidism. Our study also shows a correlation between the calcium uptake ability of SR and
levels of long chain acylcarnitines in SR with myocardial function. The correlationship, however, does not necessarily imply causality.

John H. McNeill, Ph.D.

Thesis Supervisor
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Arun G. Tahiliani
1.

INTRODUCTION

A. The disease diabetes mellitus

Diabetes mellitus has been defined as a disorder of metabolism in which deficiency of insulin, which may be absolute or relative, leads to a chronic state of hyperglycemia with or without glycosuria. The apparent deficiency of insulin action may be the result of inadequate secretion of insulin from the pancreas or a poor response to endogenous insulin in the major target tissues. Various factors have been suggested to be involved in the pathogenesis of the disease. Immunologically, circulating islet cell antibodies which are capable of mediating humoral and cellular responses that injure or destroy pancreatic β-cells are found in realtively high titres in the insulin dependent (IDDM) or type I diabetics (MacLaren, 1977; Lernmark et al., 1978). Epidemiological data from animal models has associated viruses with diabetes (Notkins, 1977), the one most closely associated being Coxsackie B4 virus (Yoon et al. 1979). There seems to be a positive correlation between the disease and histocompatibility antigen genotypes, particularly HLA-B8, BW15 and B18 (Cudworth and Woodrow, 1975). These antigens are determined by loci on the short arm of chromosome 6. It has been suggested that the antigens are determinants of the molecular nature of certain β-cell surface proteins, some of which may have certain unique defects that render the cell more susceptible to certain insults (Cudworth and Woodrow, 1975; Nerup, 1978).

On the other hand, in non-insulin dependent (NIDDM) or Type II diabetics (previously known as maturity onset diabetes mellitus), there seems to be a "down-regulation" of insulin receptors (Flier
et al., 1979) which may be a consequence of higher circulating levels of insulin (Kahn et al., 1977). Insulin resistance may be due to a decreased affinity of the receptors for insulin. For example, in rare types of diabetes mellitus which occur in association with acanthosis nigricans type B (in which there is a circulating antireceptor antibody) and with ataxia telangiectasia where no antibody is present but there is a diminished affinity of the receptors for insulin. Bar et al. (1978) suggested that insulin resistance could also be due to a "post receptor defect". Obesity seems to result in a state of peripheral insulin resistance (Ganda and Soeldener, 1977) and is an important determinant in NIDDM. In the NIDDM group of diabetics, a positive family history is much more frequent than in the IDDMs and the importance of heredity is further emphasized by studies of this disorder in monozygotic twins (Tattersall and Pyke, 1972). Some other etiological concepts involved in diabetes mellitus are summarized in Table I. A third type of diabetes mellitus, also known as gestational diabetes is associated with pregnancy. The term is restricted only to those women who become diabetic during pregnancy and who were not diabetic prior to it.

As a consequence of diabetes mellitus a variety of physiological responses are altered. On the basis of our knowledge about the effects of insulin on important target tissues (which are summarized in Table II) the abnormalities that occur due to insulin lack could be predicted. Clinically, the disease is characterized by considerable discomfort and fatigue, weight loss, marked thirst, excessive urination and/or severe skin irritations such as vaginitis in females and balanitis
**TABLE I. ETIOLOGICAL CONCEPTS IN DIABETES MELLITUS**

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>EFFECT</th>
<th>NET RESULT</th>
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<tbody>
<tr>
<td>1. HLA antigens B8, BW15, Bl8</td>
<td>Islet cell antibody production</td>
<td>Beta cell destruction</td>
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<td>2. Viral infections</td>
<td>&quot;</td>
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<tr>
<td>3. Heredity</td>
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<tr>
<td>4. Obesity</td>
<td>Hyperinsulinemia</td>
<td>&quot;Down regulation&quot; of insulin receptor</td>
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<tr>
<td>5. Altered insulin: glucagon ratio</td>
<td>Glucose metabolism</td>
<td>Hyperglycemia</td>
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<td></td>
<td>Glucose release</td>
<td></td>
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<tr>
<td>6. Presence of serum albumin - synalbumen antagonist (SAA)</td>
<td>Glucose uptake by muscle; Glucose uptake by adipose tissue</td>
<td>Obesity</td>
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<tr>
<td>7. Biological defective insulin</td>
<td>Less uptake of glucose</td>
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<td>8. Defective adipose lipase</td>
<td>Increased levels of free fatty acids</td>
<td>Insulin antagonism</td>
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<td>9. Accumulation of mucopolysaccharide in basement membrane of capillaries</td>
<td>Defective clearance of &quot;Down regulation&quot; of insulin from circulation insulin receptor leading to hyperinsulinemia</td>
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TABLE II.  EFFECTS OF INSULIN ON MAJOR TARGET TISSUES

<table>
<thead>
<tr>
<th>EFFECT</th>
<th>LIVER</th>
<th>MUSCLE</th>
<th>ADIPOSE</th>
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<tbody>
<tr>
<td>1. Glycogen synthesis</td>
<td>increased</td>
<td>increased</td>
<td>increased</td>
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<td>2. Glucose and amino acid uptake</td>
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<td>&quot;</td>
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<td>3. Protein synthesis</td>
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<td>4. RNA synthesis</td>
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<td>5. Fatty acids and triglyceride</td>
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<td>synthesis</td>
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<td>6. Glycogenolysis</td>
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<td>7. Gluconeogenesis</td>
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<td>8. Protein catabolism and amino acid</td>
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<td>output</td>
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<td>9. Lipolysis</td>
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<td>decreased</td>
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in males. The high blood glucose levels result in elevated levels of glycosylated hemoglobins. Since the modification of hemoglobin, which is due to nonenzymatic glycosylation of amino acids in the hemoglobin molecule, occurs continually during the life span of the erythrocyte, glycohemoglobin levels can provide a time averaged index of the degree of hyperglycemia during the previous few weeks depending on the species in consideration (MacDonald and Davis, 1979). In addition, there are a large number of secondary complications associated with the disease. In extreme forms, ketoacidosis results. This is probably due to a shift in the utilization of free fatty acids from synthesis of triglycerides and phospholipids to oxidation and generation of ketone bodies which may be due to increased activity of the carnitine acetyl transferase system in the liver (McGarry and Foster, 1976; 1977) (Fig. 1). Diabetic ketoacidosis may result in lactic acidosis (Watkins et al., 1969), alcoholic ketoacidosis (Fullop and Haberman, 1975) and rarely in cerebral edema (Young and Bradley, 1967).

Renal disease is a significant cause of death in diabetics (Lundbaek, 1965; Balodimos, 1971). The abnormalities so induced include decreased renal function, glomerular lesions, lesions in the renal vessels, tubulointerstitial lesions and basement membrane changes. Decreased adrenergic nervous activity may occur eventually resulting in the syndrome of hyporenemic hypoaldosteronism (Perez et al., 1977) which causes hyperkalemia and renal tubular acidosis.

Retinopathy is another common manifestation of diabetes. Proliferative diabetic retinopathy is a response of hypoxic retina resulting in the formation of additional blood vessels and accompanying
Free fatty acids which are normally converted to triglycerides and phospholipids, are metabolized to ketones in the mitochondria during diabetes. It has been suggested that the shift may partially be due to an increase in carnitine acyl transferase system.
fibrous tissue (Davis, 1974). Non-proliferative diabetic retinopathy, on the other hand, is characterized by increased vascular permeability, hemorrhages, microaneurysms, cotton wool spots, hard exudates, venous irregularities, macular edema and generalized ischemia of the retina (Davis, 1974). Eventually, ischemia of the macula and optic nerves occurs resulting in vision loss.

Other complications associated with diabetes include hyperosmolar coma without significant ketoacidosis (Arieff and Caroli, 1972) usually seen in IDDM in individuals with enough insulin to control ketoacidosis but not enough to control hyperglycemia. Diabetics seem to be more susceptible to urinary tract infections (Kass, 1956; Ooi et al., 1974); infections of foot ulcers (Bessman and Wagner, 1975) and to mucormycosis (Baker, 1971). The incidence of neuropathies is also much greater in diabetics and these may be either metabolic or ischemic and compression type (Sibley, 1982). Finally, another major complication of diabetes is the cardiovascular disease which will be discussed in detail at a later stage.

B. Regulation of myocardial contractility

Myocardial contractility is basically regulated by two mechanisms. One is a tonic form of control represented by changes in myosin ATPase activity. In this case, myocardial function changes evolve over long periods measured in hours, days or longer. The other, a phasic control mechanism, is represented by changes in calcium delivery which can vary from one beat to the next.

Evidence for the involvement of myosin ATPase in cardiac contrac-
tility is provided by different experimental settings. Changes in endocrine balance, e.g. hypophysectomy or chronic hypothyroidism, in which cardiac function is depressed have been correlated with low levels of cardiac myosin ATPase activity, whereas in experimental hyperthyroidism, myosin ATPase activity is high and myocardial activity is increased (Thyrum et al., 1970, Goodkind et al., 1974; Korecky et al., 1972). In the aging heart, myosin ATPase activity is decreased and this decrease is reported to parallel depressed cardiac function (Katz, 1977). Finally, hemodynamic overloading results in an alteration in myosin ATPase which is accompanied by decreased myocardial contractility (Swynghedauw et al., 1976). Thus alteration of cardiac function which occurs over longer periods of time seems to result, at least partially, from an alteration of myosin ATPase activity. The alteration could be a result of synthesis of a new type of myosin with altered ATPase activity or from synthesis of a protein which associates with myosin leading to enhanced or diminished ATPase activity depending on the circumstances (Goodkind et al., 1974; Yazaki and Raben, 1975).

The other regulatory factor, probably operating on a more acute basis, involves calcium. Changes in the availability of calcium available for binding to contractile proteins have the potential to modulate myocardial contractility on a beat-to-beat basis. Normally, as a result of excitation, calcium is released into the sarcoplasm and the concentration rises to about $10^{-5}$ M. Troponin C binds ionic calcium and changes are initiated in the troponin-tropomyosin system resulting in actin-myosin interaction (Perry, 1975).
Thus, changes in the intensity of interactions between contractile proteins can be brought about if the amount of calcium released is altered, if the binding ability of calcium to the troponin complex is altered or if there is a change in the intracellular environment which alters the interaction between contractile proteins (Katz, 1977).

The amount of calcium released may vary as a result of altered ability of the sarcotubular network to transport calcium (Figure 2). A number of investigators have shown that the calcium transporting ability of subcellular organelles is decreased during various types of heart failure (Sulakhe and Dhalla, 1971; Schwartz et al., 1973; Sordahl et al., 1973). A number of factors may affect the ability of the sarcotubular network to transport calcium, one of them being the levels of long chain acyl carnitines (L.C.A.C.). Elevated levels of palmitoyl carnitine, which is the most abundant of L.C.A.C. has been reported to be a potent time dependent inhibitor of calcium transport in the sarcoplasmic reticulum (SR) (Adam et al., 1978; Pitts et al., 1978).

Thus cardiac contractility seems to be dependent mainly on two parameters - the availability of calcium for excitation-contraction coupling and a myosin ATPase activity. However, this does not rule out the possibility of the presence of other biochemical factors.

C. Diabetes and the heart

As mentioned earlier, one of the major complications of diabetes is cardiovascular disease. There now exist a large number of studies
A hypothetical scheme of calcium fluxes in the cardiac cell, alterations in any of which may lead to altered myocardial contraction. (Modified from Katz, 1977)
which substantiate this statement. The Framingham study revealed the development of congestive cardiac disease in 17% of the diabetic population compared to 9% in the total population. Also, deaths due to cardiovascular problems in diabetics were thrice as many as those in the total population (Garcia et al., 1974). Similarly, the Rochester diabetes project (Palumbo et al., 1981), a study carried out by the University Group Diabetes Program (1976) and a report by Boyle et al. (1972) demonstrate the increased incidence of cardiovascular problems in diabetics.

It was thought earlier that diabetes resulted in atherosclerosis and that atherosclerosis so developed was the cause of cardiac problems in diabetics. Various studies support the hypothesis that diabetes augments atherosclerosis to some degree (Robertson and Strong, 1968; McGill, 1968; Sternby, 1968). However, it had been proposed long before that diabetes affects the small and large blood vessels leading to the concept of diabetic angiopathy (Lundbaek, 1954). It was then recognized that diabetes affected the small blood vessels and the term "diabetic microangiopathy" was introduced (Berkman and Rifkin, 1966). Later, the association of diabetes with large vessel disease was recognized, and thus "diabetic macroangiopathy" emerged (Lundbaek, 1971). The coexistence of diabetes and coronary artery disease is very well documented and it was thought that cardiac problems in diabetic patients occurred as a result of damaged coronary arteries. However, Vilhert et al. (1969) reported that the incidence of coronary heart disease was similar in diabetic and non-diabetic populations. Rubler et al. (1972) then demonstrated histologic abnormalities in
diabetic hearts with normal coronary arteries and introduced the concept of "diabetic cardiomyopathy". Further evidence showing that diabetes-induced cardiac problems were not exclusively the result of coronary abnormalities was provided by Hamby et al. (1974), where they showed the absence of coronary artery disease in a high percentage of diabetics with symptoms of heart disease. Since then, a considerable amount of work has been carried out towards a better understanding of diabetes-induced alterations in the heart in both clinical as well as experimental settings.

Another factor which was thought to be responsible for cardiac problems in diabetics was autonomic neuropathy. However, unlike coronary artery disease, the implications of this disorder are as yet controversial. The neuropathy results in tachycardia for prolonged periods (Keen, 1959; Wheeler and Watkins, 1973). Cyclical heart rate variation which depends on vagal innervation is reduced (Wheeler and Watkins, 1973; Gundersen and Neubauer, 1977). The most striking feature is postural hypotension which is probably due to lesions in the efferent limb of the baroreflex arc (Berner, 1952; Odel et al., 1955). Myocardial infarction in diabetes has been reported to be painless in an increasing number of patients and this has been attributed to autonomic neuropathy (Bradley and Schonfield, 1962; Ewing et al., 1976; Clarke et al., 1979).

There are a number of consequences resulting from such abnormalities but they are only speculative and remain to be proved. They include cerebral and possibly coronary hypoperfusion as a result of postural hypotension. Tachycardia may result in increased heart work, thereby
increasing susceptibility of the heart to cardiac disorders.
Damaged sympathetic supply might prevent a compensatory tachycardia in a patient with compromised cardiac output due to myocardial necrosis. Finally, a fixed heart rate may reduce exercise tolerance and might remove one of the mechanisms important to combat heart failure (Hampton et al., 1981).

As mentioned earlier, ample evidence exists supporting the concept of diabetic cardiomyopathy. Regan et al. (1974) studied ventricular function and compliance in diabetic dogs. They found that end diastolic volume and stroke volume in the diabetic hearts were significantly less than those in controls in response to increasing after loads suggesting a decreased compliance of these hearts. The diminished ventricular function was attributed to increased ventricular stiffness and was apparently due to accumulation of periodic acid-Schiff (PAS) positive glycoprotein in the myocardium and increased cell triglyceride levels. Abnormal left ventricular function in human diabetics has also been demonstrated (Ahmed et al., 1975; Seneviratne, 1977).

An experimental model that has been extensively studied with respect to diabetes-induced myocardial alterations is the diabetic rat. Diabetes is induced using either alloxan or streptozotocin (STZ). In a study on acute (3 day) alloxan diabetic rats, Miller (1979) reported a decreased ability of the isolated perfused working heart to respond to increased atrial filling pressures at a normal physiologic glucose concentration of 5 mM. However, when the glucose concentration was increased to 10 mM, cardiac function was normalized
suggesting that the abnormal function was due to a decreased uptake of glucose by diabetic hearts resulting in inadequate quantities of ATP being generated under working or stressful conditions. In another study, Fein et al. (1980) examined the effects of drug-induced diabetes on mechanics of the isolated ventricular papillary muscle. They observed abnormalities in the relaxation process which included a delayed onset of relaxation, a slower rate of relaxation and a delay in reaching peak relaxation rates. The shortening velocity was depressed at various loads, but the peak developed tension remained unchanged. Feuvray et al. (1979) reported that diabetic hearts were more susceptible to severe reductions in the oxygen supply: demand ratio as compared to controls. Using the isolated working heart preparation, various functional alterations have been observed (Penparkgul et al., 1980; Vadlamudi et al., 1982). Reduction of cardiac output, stroke work, peak left ventricular developed pressure and positive and negative dP/dt were seen in diabetic hearts when they are subjected to higher filling pressures. The abnormalities in cardiac function were reported to occur at a time point between four weeks and three months of diabetes (Vadlamudi et al., 1982). Similarly, Fein et al. (1980) observed that the depression in papillary muscle mechanics occurred about five weeks after onset of the diabetic state. In a recent study, Ku and Sellers (1982) reported that the basal force of contraction of isolated electrically driven atria was lower than the controls and that the magnitude of the inotropic response to ouabain was higher in diabetic atria. It thus seems clear that cardiac function is significantly depressed in diabetic rats—the depression occurring four to eight weeks after the disease
is induced.

Using the same model for diabetes, a number of biochemical studies have been carried out for a better understanding of the abnormalities at a more basic cellular level. Modrak (1980) studied the collagen content in diabetic rats but did not observe any significant change in twenty-six week diabetic animals. This is in contrast to other studies where increases in collagen content have been reported (Regan et al., 1974; Yeh et al., 1978) in other species. The apparent discrepancy has been suggested to be due to differences in species, length of diabetes and the type of diabetes produced (Modrak, 1980). As mentioned earlier, the sarcoplasmic reticulum appears to be the most active subcellular organelle involved with sequestration of activator calcium. Penpargkul et al. (1979) reported that the calcium binding and uptake were depressed in diabetic rat hearts and this has since been confirmed by other workers (Lopaschuk et al., 1983; Ganguly et al., 1983). The depression could be a result of elevated long chain acyl carnitine levels in the diabetic cardiac cytosol (Feuvray et al., 1979) which are known to depress Ca\textsuperscript{2+} uptake by the SR \cite{(Cohen et al., 1978; Adam et al., 1978)}. The elevation of long-chain acyl carnitines in cardiac SR has recently been confirmed (Lopaschuk et al., 1983).

High levels of long-chain acyl carnitines are also known to inhibit Na\textsuperscript{+}, K\textsuperscript{+} - ATPase (Wood et al., 1977) and Ca\textsuperscript{2+}-ATPase in the SR (Cohen et al., 1978). Biochemical studies in diabetic rats have demonstrated that actomyosin ATPase is depressed in the myocardium from these animals (Malhotra et al., 1981). Myofibrillar basal (Mg\textsuperscript{2+} dependent)
and Ca\textsuperscript{2+}-stimulated ATPase activities in diabetic rat hearts are reported to be significantly lower than controls (Pierce and Dhalla, 1981). Similarly, Dillman (1980) and Malhotra et al. (1981) have observed a depression of cardiac myosin ATPase activity in the diabetic rat heart. In a recent study, Ku and Sellers (1982) reported that Na\textsuperscript{+} K\textsuperscript{+} ATPase activity, estimated by specific ouabain-sensitive \textsuperscript{86}Rb uptake was significantly decreased in four to six week diabetic myocardial ventricular slices. It thus appears that a number of biochemical parameters which are apparently important for maintenance of normal myocardial performance are altered by the diabetic state. Furthermore, there also seems to be a close parallel between these parameters and the functional and mechanical parameters with respect to the duration of diabetes after which the alterations are seen.

Although a specific cardiomyopathy associated with a lack of insulin and a reasonable amount of biochemical evidence supporting the presence of the disorder was established, little was known about the prevention or reversal of these abnormalities. Wu et al. (1977) studied the effects of chronic tolbutamide administration on the altered myocardial function in diabetic dogs. They found that the treatment caused a further reduction in left ventricular function and enhanced the ventricular stiffness. However, in a later study, Pogatsa et al. (1978) found that hypoglycemic therapy in more severely diabetic dogs could, at least partially, normalize the ventricular stiffness. Fein et al. (1981) used insulin to control hyperglycemia in diabetic rats and to study its ability to reverse the abnormalities
induced by diabetes on papillary muscle mechanics. They found that insulin treatment could completely reverse the diabetes-induced alterations when it was initiated six to eight weeks after induction of the disease.

Miller (1979) and Penparkgul et al. (1980) studied the effects of insulin perfusion in vitro on diabetic rat hearts. Miller (1979) used three day diabetic rats and was able to reverse the depressed myocardial function by perfusing insulin through the isolated working hearts. However, in contrast, Penparkgul et al. (1980) perfused hearts from chronically diabetic rats with insulin and were unable to reverse the cardiac abnormalities. This suggests that the initial cause of diabetes-induced myocardial alterations is probably due to insufficient glucose transport, while chronic hyperglycemia results in changes at more basic levels, such as the subcellular level. These latter changes could be reversed only by chronic insulin therapy as suggested by the study of Fein et al. (1981).

A number of studies have been carried out in attempts to study the effects of insulin treatment on the biochemical and subcellular parameters. Dillman (1980) studied the effect of insulin treatment on myosin ATPase from hearts of four week diabetic rats. Insulin was initiated four weeks after induction of diabetes and was continued for another four weeks. This resulted in complete reversal of depressed myosin ATPase. Similarly, Fein et al. (1981) studied the effect of similar insulin therapy on Ca\(^{2+}\)-actomyosin ATPase and Ca\(^{2+}\)-myosin ATPase activities. While only trends of reversal were seen six and ten days after initiating insulin therapy, complete reversal
was seen by twenty-eight days of treatment. Fein et al. (1981) also found that insulin therapy was capable of reversing diabetes-induced depression of actomyosin and myosin ATPase activities. In a recent study, Ku and Sellers (1982) reported the ability of insulin to reverse depressed cardiac Na\(^+\), K\(^+\)-ATPase activity as well as myocardial force of contraction. Histological studies have demonstrated that insulin treatment can prevent the diabetes-induced increase in connective tissue (Baandrup et al., 1980).

It thus appears that diabetes induces a specific type of cardiomyopathy which results in altered cardiac muscle mechanics and eventually decreases cardiac function. The biochemical basis for such alterations could be depressed enzyme systems and altered subcellular organelle function. The abnormal enzyme activities could either be a result of synthesis of enzymes with lower activities, or a result of an altered environment in which the enzymes cannot function properly. Furthermore, insulin treatment seems to be effective in normalizing the mechanical and some biochemical parameters. This could again be a result of either normal function due to a normal environment or due to synthesis of enzymes with normal activities. The latter seems to be a more probable hypothesis as insulin therapy could reverse the alterations only after about four weeks of treatment.

D. Thyroid hormones: Their association with diabetes mellitus and heart function.

Thyroid hormones are synthesized in the thyroid gland via a series of complex steps. 3, 5, 3'-triiodothyronine (T\(_3\)) is generally
estimated to be nearly four times as potent as thyroxine and the latter usually predominates by a factor of several fold. However, in case of iodide deficiency, the ratio is nearly reversed (Greer et al., 1968). Thyroxine is degraded via the monodeiodination pathway to give rise to either T₃ or 3', 3', 5'-triiodothyronine ('reverse' or 'R' T₃) (Braverman et al., 1970; Gavin et al., 1977). Different debilitating disorders are associated with reduced serum T₃ and elevated RT₃ levels due to changes in thyroxine metabolism (Suda et al., 1977; Chopra and Smith, 1975; Burger et al. 1976).

Pittman et al. (1979) have shown that production of T₃ is significantly impaired in diabetes mellitus. Various suggestions regarding the mechanism of such reduction have been forwarded. Deficient thyrotropin (TSH) secretion has been demonstrated in diabetic animals during a low iodine diet regime (Pericas and Jolin, 1977). More recently, Wilber et al. (1981) demonstrated that diabetes mellitus is associated with a reduction in circulating thyrotropin releasing hormone (TRH). Reduction of T₃ levels during diabetes has also been attributed to reduced extrathyroidal conversion of thyronine to T₃ (Pittman et al., 1979; Balsam et al., 1978). This may be due to diminution in hepatic 5'-monodeiodinase resulting in depressed deiodination reactions (Chopra et al., 1981).

While it is clear from the above discussion that diabetes causes thyroid hormone alterations, the converse too seems to be true. Hypothyroidism results in impairment of early phase insulin release similar to that seen in NIDDM (Shah and Cerchio, 1973). Besides this carbohydrate homeostasis is influenced by thyroid hormones
The correlation of diabetes with thyroid status is further supported by studies which demonstrate that diabetes-induced reduction of thyroid hormones can be normalized by insulin administration (Serif and Sihotang, 1962; Kumaresan and Turner, 1966).

Thyroid hormones have a profound effect on the mammalian cardiovascular system. Various parameters are affected by altered thyroid hormone levels resulting in cardiac complications in otherwise healthy hearts (Sandler and Wilson, 1959). Different myocardial enzyme systems seem to be affected and this probably leads to altered cardiac contractility. Addition of thyroxine to a particulate preparation of the cat left ventricle resulted in stimulation of adenylate cyclase (Levey and Epstein, 1968). However, a number of other reports suggest that the adenylate cyclase-cyclic AMP system may not be very crucial in determining the cardiotonic effects of thyroid hormones (Sobel et al., 1969; McNeill et al., 1969; McNeill, 1978). Another enzyme system which is activated by the hormones and could be important for cardiac contractility is the phosphorylase system. The enhanced activity of cardiac phosphorylase a in hyperthyroidism is not accompanied by increases in cyclic AMP levels (McNeill et al., 1969) or by enhanced phosphorylase b kinase activity (Frazer et al., 1969) It is not a result of increased sensitivity of adenylate cyclase (McNeill et al., 1969) and is independent of increased coronary blood flow observed during hyperthyroidism (Longhurst and McNeill, 1979). It is not clear as yet as to how thyroid hormone excess results in an activation of phosphorylase.
The positive inotropy associated with hyperthyroidism could also, at least in part, be due to the effects of thyroid hormones on myocardial ATPase such as myosin and actomyosin ATPases, Na\(^+\), K\(^+\)-ATPase and Mg\(^2+\) dependent Ca\(^2+\) ATPase. The association of some of these ATPases with cardiac contractility is discussed at a later stage. Evidence suggests that cardiac contractility depends on the state of the ATPases. Calcium stimulated myosin ATPase has been shown to be elevated during hyperthyroidism (Thyrum et al., 1970). Various studies suggest that the enhanced activity seen is probably a result of synthesis of a new type of myosin, or synthesis of a new protein which associates with myosin leading to greater actin activated ATPase activity (Goodkind et al., 1974; Yazaki and Raben, 1975; Thyrum et al., 1970). However, the activity of this enzyme is extremely species dependent with rabbits and guinea pigs exhibiting low basal activity. Rats, on the other hand have high basal activity in the euthyroid state and this may explain why studies in rats have been unable to demonstrate augmentation of myosin ATPase in hyperthyroid animals (Rovetto et al., 1972; Yazaki and Raben, 1975).

The effect of thyroid hormone on calcium ATPase has been substantiated in a number of studies. An enhancement of calcium ATPase activity has been reported (Limas, 1978). McConnaughey et al. (1978), however, were unable to show any increase in activity of this enzyme in thyroxine treated rats. Most studies, in which thyroxine treatment was carried out for longer periods of time (about 10 days) demonstrate increased activity, while most others in which the treatment was for shorter periods (3-5 days) were unable to
demonstrate significant increases in the activity of Ca\textsuperscript{2+} ATPase. This suggests that the enhanced activity is probably due to synthesis of either a new, more active enzyme or of greater amounts of the same enzyme which is apparent only after chronic treatment with thyroid hormone.

It thus appears that myocardial contractility depends, at least in part, on the thyroid status of the animal. As myocardial contractility and thyroid hormone levels are depressed in diabetes, we looked at myocardial contractility in diabetic rats after treating them with thyroid hormone in an attempt to make them euthyroid.
SPECIFIC GOALS OF THE PRESENT INVESTIGATION

This study was designed on the following lines:

1. To locate a time point at which myocardial alterations are induced by experimental diabetes.

2. To study the ability of insulin to prevent the diabetes-induced myocardial changes, both functional and biochemical, from occurring.

3. To study the ability of insulin to reverse the depression of myocardial function and the calcium uptake in SR as well as the ability of insulin treatment to reverse elevation of long chain acylcarnitines in SR. The reversal study was carried out six weeks and five months after induction of diabetes, in an attempt to locate a point by which the diabetes-induced changes become irreversible.

4. To examine whether the diabetes-induced myocardial changes are direct or whether they are a result of diabetes-induced hypothyroidism. This was done by treating the diabetic animals with T3 and then studying the function. Treatment was initiated as soon as diabetes was detected i.e. about 3 days after the injection of streptozotocin.
MATERIALS AND METHODS

A) MATERIALS

(i) Radioisotopes:

\[ ^{45}\text{CaCl}_2 \text{ (10 Ci/}\mu\text{mole)}, \alpha^{32}\text{P}-\text{ATP} \text{ (16.4 Ci/}\mu\text{mole)} \text{ and (1 - }^{14}\text{C)} \text{ acetyl CoA were purchased from Amersham Radiochemicals (Toronto, Ontario).} \]

(ii) Enzyme:

Carnitine acetyltransferase was purchased from Sigma Chemical Co.

(iii) Chemicals:

The following chemicals were purchased from Sigma Chemical Co.:

Trizma adenosine triphosphate (Tris-ATP), \(1 \times 8-400\) Dowex - 1 Anion Exchange Resin, Bovine Serum Albumin, L-histidine free base, EGTA, EDTA, Imidazole, magnesium chloride, potassium chloride, sodium chloride, sodium azide, sucrose, Trizma base, Trizma HCl, Triton X-100, Trizma maleate, dithiotheretol, D,L-carnitine, streptozotocin, acetyl coenzyme A, 2-thiobarbituric acid, 5-hydroxymethylfurfuraldehyde, trichloroacetic acid, sodium fluoride, potassium hydronide, copper sulfate, deoxycholate, potassium phosphate, 3, 5, 3' triiodothyronine.

Calcium chloride dihydrate, L-ascorbate, Trizma oxalate, sodium carbonate, D-glucose, sodium bicarbonate and citric acid were purchased from Avalar Chemicals.

Sodium tetrathionante was purchased from Pierce Chemical Co.

Aquasol scintillation fluid was purchased from New England Nuclear.

PZI insulin (100 \(\mu\text{g/ml})\) was purchased from Connaught Laboratories.
(iv) Apparatus:
A Glucometer™ and Dextrostix® were purchased from Ames Co.,
(Elkhart, Indiana).

(v) Assay kits:
Glucose assay kits were purchased from Sigma Chemicals.
Insulin assay kits were obtained from Becton Dickinson.
Thyroid assay kits were obtained from Abbott Laboratories.

(vi) Animals:
Female Wistar rats (Canadian Breeding Farms, Montreal) weighing
150-175 g were used throughout the study. They received food and
water ad libitum.

B) METHODS

I Preparation of animals:
a) Induction of diabetes: The rats were made diabetic by a
single intravenous injection of streptozotocin (STZ) (55 mg/kg)
dissolved in 0.1 M citrate buffer (pH 4.5). Control rats were
injected with the buffer alone. The onset of diabetes was assessed
by testing for urine glucose using TesTape®, and severity of the
disease was estimated by measuring non-fasting blood glucose concentrations
(blood being obtained from the tail vein during ether anesthesia)
using Dextrostix and a Glucometer.

b) Treatment protocols:
(i) six week study: Having ascertained that the animals
were diabetic, their blood glucose levels were measured on the third
day after injecting them with STZ. Those with blood glucose values >300 mg % were subdivided into two weight-matched groups. In one study, one group of animals received protamine zinc insulin (PZI) subcutaneously once daily, while the other was left untreated. Blood glucose was monitored using a Dextrostix and a Glucometer once every two days in the first week, after which it was monitored every week. The dose of insulin was adjusted in order to achieve blood glucose levels in the range of 100-200 mg %. The approximate dose of insulin used was 0.9 U/100 g body weight/day. In another study, one group of diabetic animals was treated with triiodothyronine (T3) in alkaline solution (30 μg/kg/day s.c.). In this latter study, one group of controls was also treated with T3. Treatment in either case was carried out for six weeks after which period the animals were sacrificed. Hearts were excised and either perfused for function studies or used for the preparation of sarcoplasmic reticulum (SR). Blood was collected and the plasma was analyzed for glucose, insulin, glycosylated hemoglobin and T3 levels.

(ii) Ten week study: In this case, the diabetic animals were subdivided into two groups six weeks after injecting STZ. One group received PZI as in the above protocol for four weeks. Blood glucose determinations were made three times in the first week of treatment and once each week thereafter. In effect, the animals were sacrificed ten weeks after induction of diabetes. The hearts were used for either function or biochemical studies as previously described and blood was collected and used for the above mentioned analyses.

(iii) Six month study: Diabetic animals were subdivided five months after they were made diabetic. One subgroup was treated with
PZI in a manner identical to the protocol described in the "ten week study". In this study the rats were sacrificed six months after they were made diabetic and the hearts perfused for function studies.

II Isolated working perfused heart preparation.

The method used for perfusing hearts is a modification of Neely's working heart preparation, as described by Rodgers et al. (1981). The perfusion fluid used was Chenoweth-Koelle (1946) buffer which has the following composition (mM concentrations of solutes): NaCl, 120; KCl, 5.6; CaCl$_2$, 2.18; MgCl$_2$, 2.1; NaHCO$_3$, 19; glucose, 10 and EDTA 0.03. The buffer was oxygenated continuously with a 95% O$_2$ and 5% CO$_2$ mixture and was maintained at 37°±1°C (pH 7.4). A 3 cm piece of PE 90 tubing was attached on one side to a Statham P23AA transducer (Gould Statham Instruments) and to a 20 gauge needle on the other. Left ventricular developed pressure (LVDP) was measured by inserting the needle through the apex of the heart into the left ventricle. Intraaortic pressure was measured by means of another Statham P23AA transducer connected to a side arm of the aortic outflow system. LVDP, intraaortic pressure and the first derivative of LVDP were recorded on a Grass Model 79D polygraph. The hearts were stimulated by means of a platinum electrode placed on the left atrium at twice the threshold voltage with square pulses of 5 msec duration from a Grass Model SD9D stimulator to give a rate of 300 beats/min. The data were collected and analyzed with a microcomputer according to a method by Harris et al. (1983) (Apple II+ with a Mountain hardware board). The pressure transducer signal from the polygraph was sampled at 667 Hz over 1.5
seconds at each point where data were recorded. Six complete cardiac pulses were thus obtained and analyzed using various curve fitting techniques to determine pulse height, slopes of the curves, area, start, finish, etc. Values from three pulses were averaged to produce data values at each point.

Function curves were performed on each heart after it had equilibrated for about 5-7 minutes at an atrial filling pressure of 15 cm H$_2$O. Studies were performed by varying the height of the left atrial filling reservoir (and thus varying the left atrial filling pressure) from 5 cm H$_2$O to 22.5 cm H$_2$O in 2.5 cm steps. Reservoir height was altered stepwise from 15 cm H$_2$O to 5 cm H$_2$O, increased to 22.5 cm H$_2$O and finally reduced to 15 cm H$_2$O, data being recorded at each point after pressure development stabilized. A complete function curve took about 30 mins to perform, the total perfusion time being 35 to 45 minutes. At the end of the function curve, wet ventricle weight was recorded.

III Preparation of cardiac microsomes enriched with sarcoplasmic Reticulum.

This preparation was made by a modification of the method of Sumida et al. (1978). Hearts were excised and placed in cold 10 mM Tris maleate buffer (pH 6.8). The ventricles were separated, weighed and homogenized in about 15 ml of the above buffer with a teflon pestle for 15 seconds at 1500 rpm. The resulting homogenate was placed in a Korex$^R$ tube and centrifuged at 4000 times g for 10 minutes.
The supernatent was passed through four layers of cheesecloth and centrifuged at 9000 times g for twenty minutes. Supernatent was again passed through four layers of cheese cloth and centrifuged at 40,000 times g for 100 minutes. The resulting pellet was resuspended in a 10 mM Tris maleate buffer containing 0.6 M KCl and centrifuged at 40,000 times g for 100 minutes. The supernatent was discarded and the pellet gently washed with 10 mM Tris maleate buffer. It was then resuspended in 10 mM Tris Maleate buffer containing 40% sucrose, quick frozen in acetone on dry ice and stored at -70°C until further use.

IV Measurement of calcium uptake by cardiac microsomes enriched in sarcoplasmic reticulum vesicles.

a) Measurement of calcium transport activity in the SR:

ATP-dependent calcium uptake activity was measured by the method of Tada et al. (1974) with a few modifications. The incubation medium contained 40 mM histidine hydrochloride, pH 6.8, 5 mM MgCl₂, 110 mM KCl, 5 mM Tris-ATP, 2.5 mM Tris-oxalate and different concentrations of CaCl₂ containing ⁴⁵CaCl₂. About 50 µg of microsomal sarcoplasmic reticulum was used at each calcium concentration. The desired free calcium concentrations were obtained by adding ethylene glycol Tris (-aminoethyl ether) - N,N'-tetraacetate (EGTA) to a CaCl₂ solution, the free calcium being calculated by the equation of Katz et al. (1970). The reaction was terminated by filtering an aliquot of the reaction mixture through a Millipore filter (HA 45, Millipore Co.). The filter was then washed with about 20 ml of 40 mM Tris chloride, pH 7.2, dried and counted for radioactivity in Aquasol⁸ using standard
liquid scintillation counting techniques. The rate of Ca^{2+} uptake by the microsomal preparation is expressed as nmoles Ca^{2+} taken up per mg protein per minute.

V Determination of long chain acylcarnitine levels in SR.

An aliquot of the microsomal suspension containing about 0.5 mg protein was diluted to 4 ml with 10 mM Tris maleate and centrifuged at 40,000 x g for 45 minutes. The pellet was resuspended in 0.5 ml of cold 6% (0.6 ml) perchloric acid. The suspension was centrifuged at 12,000 x g for 10 minutes. To a 200 μl aliquot of the supernatent, 150 μl of a 2 M Tris base solution was added. The pellet was washed with 6% perchloric acid solution, suspended in 100 μl distilled water and subjected to alkaline hydrolysis by incubating it with 100 μl of 1 M Tris base and 50 μl of 0.4N KOH at 70°C for 1 hour. Following incubation, 0.575N HCl (200 μl) was added to neutralize the base.

The actual determination of carnitine was done according to a radioisotopic procedure developed by McGarry and Foster (1976). A medium containing 120 μM Tris chloride (pH 7.3), 2 μM sodium tetra-thionate and 25 nmoles (0.025 μCi) of 1-^{14}C acetyl coenzyme A was prepared and 1.05 ml added to each of the supernatent or hydrolyzed samples containing carnitine. Free carnitine in the sample was converted to labelled acetyl carnitine by the addition of 0.5 U of the enzyme carnitine acetyltransferase. The reaction mixture was incubated at room temperature for 30 minutes. To this was added 0.3 ml of Dower 1x8-400 anion exchange resin (200-400 mesh in chloride form) suspension.
(containing 0.08 ml of the resin and 0.22 ml of water) and the sample was placed on ice. The sample was vortexed twice at 10 minute intervals and centrifuged at 3000 x g for 5 minutes. To an 0.7 ml aliquot of the supernatent, 10 ml of aquasol was added and the sample counted using standard scintillation techniques. Levels of free carnitine and long chain acylcarnitines were expressed as nmoles/mg protein sarcoplasmic reticulum.

VI Protein assay.

To a 20 μl aliquot of the microsomal sample, distilled water was added. Ten μl of 2% deoxycholate solution were added to the suspension and after 10 minutes of incubation, 0.5 ml 24% trichloroacetic acid were added to precipitate the proteins. The tubes were centrifuged at 3000 x g for 30 minutes and the precipitated protein assayed using the standard Lowry (1951) protein assay. Bovine serum albumin was used as a standard.

VII Blood analysis.

(i) Glycosylated hemoglobin in blood was assayed by the method of Subramanian et al. (1980). Cells obtained after separation of plasma were washed thrice with saline at 4°C and were then hemolysed with distilled water. To the resultant supernatent, acidified acetone was added to separate out the globin. The globin pellet obtained by centrifuging the suspension was washed once with acidified acetone and then with acetone to remove the acid. Finally, the pellet was suspended in diethyl ether and dried under nitrogen. Globin thus
obtained was stored at -20°C until the assay was carried out.

Globin was assayed by digesting 75 mg with 4 ml 10 M acetic acid for 16 hours at 80°C. To a 1 ml aliquot of the digest, 1 ml of 2% trichloroacetic acid was added and the mixture allowed to sit for 5 minutes. The suspension was centrifuged and thiobarbiturate reagent was added to the pellet. The reaction mixture was incubated for 50 minutes at 40°C and the absorbance measured at 443 nM. Hydroxymethylfurfuraldehyde (HMF) was used as a standard. The degree of glycosylation is expressed as μmol HMF/g globin protein.

(ii) Plasma immunoreactive insulin was determined by the radio-immunoassay method of Herbert et al. (1965). Reagents for the assay were obtained from Becton Dickinson and Co. The insulin assay reagents consisted of human insulin standards, [125I] porcine insulin and an insulin antibody raised against porcine insulin, for which human insulin exhibits 100% and rat insulin 90% cross reactivity. Human insulin standards were used since the error made in the estimation of rat insulin levels was minimal owing to the very high cross reactivity with rat insulin.

(iii) Plasma glucose levels were determined by the glucose oxidase method using a Sigma glucose kit.

(iv) Thyroid status of the rats was determined by measuring their T3B index which shows how much excess binding capacity the serum thyroxine binding globulin has available. The T3B index was determined using a Triobead - 125 T3 uptake kit which was obtained from Abbott Laboratories.
VII Statistical analyses:

All results for any particular group were averaged and the standard error of the mean (S.E.M.) determined at each experimental point. One way analysis of variance (ANOVA) followed by Newman-Keuls test was used for comparing results from a given set of groups. A probability of less than 0.05 (p<0.05) was used as the level of significance.
RESULTS

Effect of diabetes on myocardial function, calcium uptake activity of cardiac SR and carnitine levels of cardiac SR in rats.

The general features of diabetic rats were quite characteristic of the disease and are summarized in Tables III-IX. Diabetic animals weighed significantly less than the buffer injected controls and the plasma glucose levels of diabetic rats were exceedingly high (between 300% and 500% over control). Other features exhibited by diabetic rats were low insulin levels (in most cases less than 50% of control) and elevated glycosylated hemoglobin levels. Upon sacrifice, it was found that diabetic rat hearts were smaller and weighed less than controls, but in most cases the heart weight/body weight ratios were significantly greater than controls. In addition, it was observed that diabetic animals exhibited polydipsia and polyuria. It was thus ascertained that the animals were indeed moderately to severely diabetic. Mortality rate within the diabetic groups was very low (about 2%).

When the hearts were isolated and perfused in the working heart mode, it was observed that by six weeks of diabetes, the functional parameters measured were significantly depressed in relation to controls. Left ventricular developed pressure (LVDP), rate of pressure development (+dP/dt) and rate of pressure decline (-dP/dt) were found to be significantly lower than controls at filling pressures greater than 17.5 cm H₂O (Figures 3, 4, 5). Previous studies have suggested that myocardial changes induced by streptozotocin diabetes in rats occurred between four weeks and twelve weeks after injection of the drug (Vadlamudi et al.)
From our study it seems that changes in myocardial function set in by six weeks of diabetes.

Results similar to these were obtained when longer durations of diabetes were studied. Hearts obtained from rats diabetic for ten weeks also exhibited depressed cardiac function. The LVDP was significantly depressed at higher filling pressures, namely, those above 17.5 cm H$_2$O (Figure 9). Similarly, positive dP/dt was depressed in the diabetic hearts at filling pressures greater than 12.5 cm H$_2$O (Figure 10). Negative dP/dt was also depressed at higher filling pressures (Figure 11). The depression of these parameters in ten week diabetic animals did not appear to be any worse than the depression seen in six week diabetic animals. However, in the ten week diabetic rat hearts, depression of positive and negative dP/dt seemed to occur at relatively lower filling pressures.

In another set of experiments we studied myocardial function of rats six months after they were injected with streptozotocin. As in the above studies, depression of the parameters studied was observed. LVDP was depressed at filling pressures greater than 17.5 cm H$_2$O (Figure 15). Positive dP/dt was also depressed at filling pressures greater than 17.5 cm H$_2$O (Figure 16) while negative dP/dt was depressed at filling pressures greater than 15 cm H$_2$O (Figure 17). The values of LVDP, positive dP/dt and negative dP/dt obtained in six month control hearts were lower than those obtained in either six week or ten week controls. However, function of six month diabetic hearts did not appear to be more depressed than the six week or ten week diabetic hearts.
Calcium uptake activity in cardiac SR was affected significantly by diabetes. Six weeks and ten weeks of diabetes affected the calcium transporting ability of SR at all calcium concentrations used (Figures 6 and 12). A significant elevation of long chain acylcarnitine levels in the SR which paralleled the depression of calcium uptake was seen both six weeks (Figure 7) and ten weeks (Figure 13) after the induction of diabetes. However, the free carnitine levels were not affected by diabetes at either of the time points studies (Figures 8 and 14).

Effects of insulin treatment on diabetes-induced alteration of myocardial function and biochemistry.

a) Six week prevention study. Insulin treatment in a subgroup of diabetic rats was initiated three days after STZ injection. These rats did not lose weight, had plasma glucose values not significantly higher than controls, and were normoinsulinemic (Tables III and IV). Glycosylation of hemoglobin was normal and they did not exhibit polydipsia or polyuria. There were no mortalities during the course of treatment. It thus seemed that this group of diabetic rats was receiving adequate insulin treatment. As mentioned above, six weeks of diabetes were found to be sufficient to induce changes in the rat myocardium. By treating a subgroup of diabetic rats with insulin immediately after the onset of diabetes, it appeared that the abnormalities could be prevented from occurring. The LVDP, positive dP/dt and negative dP/dt were not significantly different from control
values (Figures 3, 4 and 5) and were significantly higher than
values obtained from the diabetic group. It was also seen that at
all filling pressures these parameters were slightly, though not
significantly higher in the treated diabetics than in normal controls.

Similarly, insulin treatment was effective in preventing biochemi-
cal changes from occurring in diabetic rats. Calcium transporting
activity of cardiac SR, which was depressed in diabetic animals, was
found to be normal in treated diabetics (Figure 6). Levels of long
chain acyl carnitines in cardiac SR were also found to be normal in
insulin treated rats (Figure 7). Free carnitine levels in SR remained
unaffected (Figure 8).

b) Ten week reversal study: In this study, insulin treatment
was initiated six weeks after the rats were made diabetic with STZ
- a period by which the myocardial abnormalities supposedly occur.
The diabetic animals weighed less than controls when treatment was
initiated but gained weight rapidly during the course of insulin
treatment. At the time of sacrifice, the treated diabetics weighed
significantly more than the untreated diabetics, had normal plasma
glucose and plasma insulin levels and did not exhibit polydipsia and
polyuria. However, though glycosylated hemoglobin levels were signifi-
cantly lower than untreated diabetics, the levels were significantly
elevated as compared to controls (Tables V and VI).

As is evident from Figure 9, LVDP could be reversed to normal
by treating the diabetic animals with insulin even after the myocardial
abnormalities had occurred. Similarly positive dP/dt (Figure 10) and
negative dP/dt (Figure 11) could be brought back to normal. It thus
seems that myocardial function changes induced by diabetes can be normalized if insulin treatment is initiated soon after the changes occur. This view is further supported by the biochemical observations. Calcium uptake activity in cardiac SR could be reversed to normal by four weeks of insulin treatment (Figure 12). Long chain acyl carnitine levels in the SR seemed to parallel the normalization of function and calcium uptake activity as they were brought back to normal levels in the cardiac SR of treated diabetics (Figure 13). However, as in the previous study, free carnitine levels remained unaffected (Figure 14).

c) Six month reversal study: In this study, diabetic rats were treated with insulin for four weeks, five months after they were made diabetic. While diabetic animals weighed significantly less than controls, the treated diabetics gained weight and weighed nearly the same as controls. Plasma glucose and plasma insulin in the insulin treated group were normal but the glycosylated hemoglobin showed only a partial recovery towards normal (Table VII). Figure 15 demonstrates the effect of insulin treatment on LVDP in diabetic hearts. Though a trend towards normalization was seen, LVDP in treated diabetics was not significantly different either from normal controls or from diabetics at all filling pressures. In the case of positive dP/dt, a similar trend was seen. However, here the values for treated diabetics were significantly lower than controls but significantly greater than the untreated diabetics (Figure 16). The only parameter that seemed to be appreciably reversed to normal was negative dP/dt (Figure 17). At all filling pressures above 17.5 cm H₂O, there was no difference between controls and treated diabetics while values for untreated diabetics were
significantly depressed.

Effect of T₃ treatment on diabetes-induced myocardial alterations.

₃ treatment was initiated in control and diabetic rats 3 days after injecting them with buffer or with STZ. While the diabetics, treated and untreated lost weight rapidly, the treated controls did not lose weight significantly. Both the diabetic groups exhibited hyperglycemia and hypoinsulinemia. Glycosylated hemoglobin values were elevated in both the diabetic groups (Tables VIII and IX). It thus seemed that T₃ treatment did not alter the diabetic status of rats. However, when thyroid status was studied, untreated diabetics exhibited hypothyroidism and T₃ treated diabetic rats had normal thyroid status. Thus is appeared that the T₃ treatment regimen used was sufficient to normalize the diabetes-induced hypothyroidism when the heart function of these animals was studied. T₃ treatment did not appear to have any significant effect on function. Figure 18 shows the LVDP and untreated diabetics showed depressed LVDP. In the T₃ treated controls or diabetics no significant elevation or depression was seen as compared to untreated controls or diabetics respectively. Similar results were obtained for positive dP/dt (Figure 19) and negative dP/dt (Figure 20).

Finally, we studied the effect of T₃ treatment on calcium uptake activity in diabetic rats. As is seen in Figure 21, the diabetic cardiac SR was significantly depressed as compared to control. As in the function studies, T₃ treatment did not significantly affect the SR activity in either control or diabetic hearts.
Thus it appears that correcting the thyroid status in diabetic rats is not beneficial with respect to myocardial changes while insulin seems to be very effective at least in the initial phases of the disease.
### TABLE III

**GENERAL FEATURES OF ANIMALS USED FOR HEART FUNCTION ANALYSIS**

(SIX WEEK INSULIN PREVENTION STUDY)

<table>
<thead>
<tr>
<th></th>
<th>BODY WEIGHT (g)</th>
<th>PLASMA GLUCOSE (mg %)</th>
<th>GLYCOXYLATED HEMOGLOBIN (µmol HMF/g globin)</th>
<th>PLASMA INSULIN (µu/ml)</th>
<th>HEART WT/ BODY WT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CONTROL</td>
<td>220±5</td>
<td>129±8</td>
<td>1.38±0.21</td>
<td>17.2±3.2</td>
<td>0.69±0.01</td>
</tr>
<tr>
<td>2. DIABETIC</td>
<td>162±7*</td>
<td>549±14*</td>
<td>2.98±0.14*</td>
<td>1.4±0.2*</td>
<td>0.50±0.02*</td>
</tr>
<tr>
<td>3. DIABETIC TREATED</td>
<td>237±7</td>
<td>175±20</td>
<td>1.51±0.19</td>
<td>13.8±4.6</td>
<td>0.66±0.02</td>
</tr>
</tbody>
</table>

*<p<0.05

### TABLE IV

**GENERAL FEATURES OF ANIMALS USED FOR CARDIAC BIOCHEMICAL ANALYSIS**

(SIX WEEK INSULIN PREVENTION STUDY)

<table>
<thead>
<tr>
<th></th>
<th>BODY WEIGHT (g)</th>
<th>PLASMA GLUCOSE (mg %)</th>
<th>GLYCOXYLATED HEMOGLOBIN (µmol HMF/g globin)</th>
<th>PLASMA INSULIN (µu/ml)</th>
<th>HEART WT/ BODY WT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CONTROL</td>
<td>218±3</td>
<td>130±6</td>
<td>1.36±0.11</td>
<td>29.3±0.6</td>
<td>0.59±0.01</td>
</tr>
<tr>
<td>2. DIABETIC</td>
<td>176±10*</td>
<td>388±19*</td>
<td>2.89±0.13*</td>
<td>14.0±0.5*</td>
<td>0.50±0.02</td>
</tr>
<tr>
<td>3. DIABETIC TREATED</td>
<td>218±2</td>
<td>190±7</td>
<td>1.56±0.09</td>
<td>23.5±0.7</td>
<td>0.64±0.02</td>
</tr>
</tbody>
</table>

*<p<0.05
FIGURE 3:

Effect of insulin treatment on myocardial function in six week animals. Left ventricular developed pressure (LVDP) is plotted against left atrial filling pressure. Insulin treatment was initiated three days after injection of streptozotocin (STZ) and continued for six weeks. The depression of LVDP seen in diabetic rats could be prevented by insulin treatment. Results are expressed as mean ± S.E.M. (*p<0.05).
SIX WEEK STUDY

(*) CONTROL (N=6)
(o) DIABETIC (N=7)
(*) INSULIN TREATED (N=5)
Rate of pressure development (positive dP/dt) is plotted against left atrial filling pressure. Conditions were similar to those in Figure 3. A depression was seen at filling pressure greater than 15.0 cm H₂O in diabetic animals and the depression could be prevented by insulin treatment (*p<0.05).
SIX WEEK STUDY

(×) CONTROL (N=6)
(o) DIABETIC (N=7)
(*) INSULIN TREATED (N=5)
FIGURE 5:

Changes in the rate of relaxation are shown. Insulin treatment proved to be effective in preventing the depression of negative $dP/dt$ seen in diabetic hearts at higher filling pressures (*$p<0.05$).
SIX WEEK STUDY
(*) CONTROL (N=6)
(○) DIABETIC (N=7)
(●) INSULIN TREATED (N=5)
FIGURE 6:

The figure demonstrates the effect of six weeks of diabetes on calcium uptake activity in the cardiac sarcoplasmic reticulum (SR). The activity was depressed over the entire range of calcium concentrations used in diabetic rats. Insulin treatment prevented such a depression from occurring in diabetic rats (*p<0.05).
SIX WEEK CA UPTAKE STUDY

(x) CONTROL (N=8)
(o) DIABETIC (N=8)
(o) DIABETIC (INSULIN TREATED) (N=8)

FREE CA LOG CONC (UM)

CA UPTAKE ACTIVITY (NMOL/ME PROTEIN/MIN)
FIGURE 7:

The levels of long-chain acylcarnitines in SR of control (CON), diabetic (DIAB) and diabetic animals treated with insulin for six weeks (DIAB + INS) are shown. Though the levels were elevated in six week diabetic rats, the elevation could be prevented by insulin treatment (*p<0.05).
SIX WEEK STUDY (N=8)

LONG CHAIN ACYL CARNITINES (NMOL/MG S.R.)

CON  DIAB  DIAB+INS
FIGURE 8:

Free carnitine levels in the SR in six week diabetic animals (DIAB) did not appear to be elevated as compared to control (CON). Insulin treatment did not affect the free carnitine levels.
SIX WEEK STUDY (N=8)

FREE CARNITINE (NMOL/MG S.R.)

CON  DIAB  DIAB+INS
# TABLE V

GENERAL FEATURES OF ANIMALS USED FOR HEART FUNCTION ANALYSIS  
(TEN WEEK INSULIN REVERSAL STUDY)

<table>
<thead>
<tr>
<th></th>
<th>BODY WEIGHT (g)</th>
<th>PLASMA GLUCOSE (mg %)</th>
<th>GLYCOSYLATED HEMOGLOBIN (μmol HMF/g globin)</th>
<th>PLASMA INSULIN (μu/ml)</th>
<th>HEART WEIGHT (g)</th>
<th>HEART WT/ BODY WT (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CONTROL</td>
<td>263±5</td>
<td>117±11</td>
<td>1.15±0.09</td>
<td>14.8±3.2</td>
<td>0.72±0.02</td>
<td>3.57±0.10</td>
</tr>
<tr>
<td>2. DIABETIC</td>
<td>206±5*</td>
<td>584±26*</td>
<td>2.60±0.22*</td>
<td>2.8±0.7*</td>
<td>0.61±0.01*</td>
<td>4.12±0.08*</td>
</tr>
<tr>
<td>3. DIABETIC TREATED</td>
<td>271±6.0</td>
<td>168±17</td>
<td>1.72±0.10*</td>
<td>18.4±3.9</td>
<td>0.76±0.02</td>
<td>3.71±0.15</td>
</tr>
</tbody>
</table>

*p<0.05

# TABLE VI

GENERAL FEATURES OF ANIMALS USED FOR CARDIAC BIOCHEMICAL ANALYSIS  
(TEN WEEK INSULIN REVERSAL STUDY)

<table>
<thead>
<tr>
<th></th>
<th>BODY WEIGHT (g)</th>
<th>PLASMA GLUCOSE (mg %)</th>
<th>GLYCOSYLATED HEMOGLOBIN (μmol HMF/g globin)</th>
<th>PLASMA INSULIN (μu/ml)</th>
<th>HEART WEIGHT (g)</th>
<th>HEART WT/ BODY WT (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CONTROL</td>
<td>225±2</td>
<td>133±9</td>
<td>1.38±0.09</td>
<td>34.1±0.3</td>
<td>0.61±0.01</td>
<td>2.73±0.06</td>
</tr>
<tr>
<td>2. DIABETIC</td>
<td>154±10*</td>
<td>366±28*</td>
<td>2.99±0.08*</td>
<td>19.4±0.46*</td>
<td>0.49±0.02*</td>
<td>3.21±0.09</td>
</tr>
<tr>
<td>3. DIABETIC TREATED</td>
<td>232±4</td>
<td>193±16</td>
<td>2.15±0.07</td>
<td>32.5±0.99</td>
<td>0.64±0.02</td>
<td>2.71±0.04</td>
</tr>
</tbody>
</table>

*p<0.05
FIGURE 9:

The figure demonstrates the effectiveness of insulin treatment in reversing diabetes-induced depression of LVDP. Treatment with insulin was initiated six weeks after injection of STZ, and was continued for four weeks. The depression was reversed by insulin treatment. Values are expressed as mean ± S.E.M. (*p<0.05).
TEN WEEK STUDY

(x) TEN WEEK CONTROL (N=7)
(o) TEN WEEK DIABETIC (N=6)
() FOUR WEEK TREATED (N=6)
FIGURE 10:

Rate of pressure development at various left atrial filling pressure is shown. The condition of treatment were similar to those in Figure 9. Insulin treatment was capable of reversing the depression of positive dP/dt induced by six weeks of diabetes (*p<0.05).
TEN WEEK STUDY

(×) CONTROL (N=7)
(○) DIABETIC (N=6)
(★) INSULIN TREATED (N=6)

FILLING PRESSURE (CM H2O)

POSITIVE DP/DT (MM HG/SEC)

2.5 5 7.5 10 12.5 15 17.5 20 22.5

FILLING PRESSURE (CM H2O)
FIGURE 11:

Rate of pressure decline (negative dP/dt) was depressed in diabetic hearts at filling pressures greater than 12.5 cm H$_2$O. Insulin treatment seemed to reverse the depression and the values in treated animals were similar to control (p<0.05).
TEN WEEK STUDY

(x) TEN WEEK CONTROL (N=7)
(o) TEN WEEK DIABETIC (N=6)
(n) FOUR WEEK TREATED (N=6)
FIGURE 12:

Calcium uptake activity in the SR is demonstrated in this figure. As in six week diabetic rats, the activity was depressed in ten week diabetics over almost the entire concentration range used. By treating the animals with insulin, it was possible to reverse the depression (p<0.05).
TEN WEEK CALCIUM UPTAKE (INSULIN & DIABETES)

(×) CONTROL (N=10)
(○) DIABETIC (N=9)
(○) DIABETIC TREATED (N=9)
FIGURE 13:

The histogram demonstrates the ability of insulin to reverse the diabetes-induced elevation of long chain acylcarnitine levels in the SR. Each bar represents mean ± S.E.M. of a group of animals (*p<0.05).
TEN WEEK REVERSAL STUDY (N=5-8)

LONG CHAIN ACYL CARNITINES (NMOL/MG S.R.)

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>DIAB</th>
<th>DIAB+INS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels</td>
<td>0.8</td>
<td>3.2</td>
<td>1.6</td>
</tr>
</tbody>
</table>
FIGURE 14:

Free carnitine levels in the SR of control, diabetic and insulin treated animals are shown. Neither diabetes nor insulin treatment in diabetic rats affected these levels.
TEN WEEK REVERSAL STUDY (N=5-8)
### Table VII

**General Features of Animals Used for Heart Function Analysis**  
*(Six Month Insulin Reversal Study)*

<table>
<thead>
<tr>
<th></th>
<th>Body Weight (g)</th>
<th>Plasma Glucose (mg %)</th>
<th>Glycosylated Hemoglobin (µmol HMF/g globin)</th>
<th>Plasma Insulin (µu/ml)</th>
<th>Heart Weight (g)</th>
<th>Heart WT/Body WT (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>297±8</td>
<td>130±6</td>
<td>1.63±0.09</td>
<td>23.8±0.7</td>
<td>1.06±0.04</td>
<td>3.49±0.12*</td>
</tr>
<tr>
<td>2. Diabetic</td>
<td>234±10*</td>
<td>281±19*</td>
<td>3.32±0.11*</td>
<td>13.25±0.18</td>
<td>0.98±0.03</td>
<td>4.21±0.13</td>
</tr>
<tr>
<td>3. Diabetic Treated</td>
<td>301±6</td>
<td>182±12</td>
<td>2.72±0.14*</td>
<td>24.5±0.3</td>
<td>1.24±0.05*</td>
<td>4.09±0.10</td>
</tr>
</tbody>
</table>

*P<0.05
FIGURE 15:

Effect of chronic (six month) diabetes on LVDP is shown. At atrial filling pressures greater than 17.5 cm H₂O, the LVDP was depressed in diabetic rats. Four weeks of insulin treatment initiated five months after induction of diabetes did not significantly reverse the depression. However, the depression was affected to an extent that it was no longer significantly depressed as compared to controls. This suggests that insulin treatment at this stage showed only a trend towards reversal (*p<0.05).
FUNCTION STUDIES

(*) SIX MONTH CONTROL (N=7)
(o) SIX MONTH DIABETIC (N=6)
(•) FOUR WEEK INSULIN TREATED (N=7)
FIGURE 16:

Positive dP/dt is plotted against left atrial filling pressure. In contrast to the results obtained in LVDP, positive dP/dt depression which was significant in six month diabetics was significantly reversed by insulin treatment. However, it remained significantly depressed as compared to controls suggesting a partial reversal towards normal (*p<0.05).
FUNCTION STUDIES

(*) SIX MONTH CONTROL (N=7)
(S) SIX MONTH DIABETIC (N=6)
(•) FOUR WEEK INSULIN TREATED (N=7)
Six months of diabetes induced significant reduction in the negative dP/dt. Unlike the effect of insulin treatment on LVDP and positive dP/dt, the depression of negative dP/dt was completely reversed in six month diabetic animals by four weeks of insulin treatment (*p<0.05).
FUNCTION STUDIES

(*) SIX MONTH CONTROL (N=7)
(O) SIX MONTH DIABETIC (N=6)
(+) FOUR WEEK INSULIN TREATED (N=7)
TABLE VIII

GENERAL FEATURES OF ANIMALS USED FOR HEART FUNCTION ANALYSIS
(SIX WEEK THYROID PREVENTION STUDY)

<table>
<thead>
<tr>
<th></th>
<th>BODY WEIGHT (g)</th>
<th>PLASMA GLUCOSE (mg %)</th>
<th>GLYCOSYLATED HEMOGLOBIN (µmol HMF/g globin)</th>
<th>PLASMA INSULIN (µu/ml)</th>
<th>HEART WEIGHT (g)</th>
<th>HEART WT/ BODY WT (mg/g)</th>
<th>T₃ B INDEX (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CONTROL</td>
<td>218±6</td>
<td>129±8</td>
<td>1.59±0.12</td>
<td>18.7±0.6</td>
<td>0.6±0.005</td>
<td>2.73±0.03</td>
<td>53.3±2.7</td>
</tr>
<tr>
<td>2. CONTROL TREATED</td>
<td>211±4</td>
<td>119±8</td>
<td>1.33±0.09</td>
<td>19.1±0.7</td>
<td>0.66±0.01</td>
<td>2.97±0.03</td>
<td>68.8±6.0</td>
</tr>
<tr>
<td>3. DIABETIC</td>
<td>170±7*</td>
<td>403±25*</td>
<td>3.04±0.17</td>
<td>5.6±0.4*</td>
<td>0.55±0.02*</td>
<td>3.25±0.07</td>
<td>45.9±0.8</td>
</tr>
<tr>
<td>4. DIABETIC TREATED</td>
<td>176±7*</td>
<td>339±29</td>
<td>3.32±0.11*</td>
<td>7.12±0.6*</td>
<td>0.59±0.02</td>
<td>3.35±0.06*</td>
<td>54.7±4.1</td>
</tr>
</tbody>
</table>

TABLE IX

GENERAL FEATURES OF ANIMALS USED FOR CARDiac BIOCHEMICAL ANALYSIS
(SIX WEEK THYROID PREVENTION STUDY)

<table>
<thead>
<tr>
<th></th>
<th>BODY WEIGHT (g)</th>
<th>PLASMA GLUCOSE (mg %)</th>
<th>GLYCOSYLATED HEMOGLOBIN (µmol HMF/g globin)</th>
<th>PLASMA INSULIN (µu/ml)</th>
<th>HEART WEIGHT (g)</th>
<th>HEART WT/ BODY WT (mg/g)</th>
<th>T₃ B INDEX (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CONTROL</td>
<td>214±5</td>
<td>120±5</td>
<td>1.55±0.13</td>
<td>31.1±4.1</td>
<td>0.8±0.02</td>
<td>3.79±0.05</td>
<td>53.3±2.6</td>
</tr>
<tr>
<td>2. CONTROL TREATED</td>
<td>217±4</td>
<td>121±6</td>
<td>1.47±0.11</td>
<td>31.1±5.7</td>
<td>0.83±0.01</td>
<td>3.82±0.31</td>
<td>68.7±6.0*</td>
</tr>
<tr>
<td>3. DIABETIC</td>
<td>158±11*</td>
<td>385±22*</td>
<td>3.08±0.11*</td>
<td>11.5±2.9*</td>
<td>0.66±0.05</td>
<td>4.14±0.5*</td>
<td>45.8±0.8*</td>
</tr>
<tr>
<td>4. DIABETIC TREATED</td>
<td>158±6*</td>
<td>376±35*</td>
<td>3.26±0.05*</td>
<td>10.8±1.9*</td>
<td>0.76±0.04</td>
<td>4.81±0.5*</td>
<td>54.69±4.15*</td>
</tr>
</tbody>
</table>

*p<0.05
FIGURE 18:

Effect of triiodothyronine (T₃) on myocardial function of six week animals. T₃ treatment (30 μg/kg/day s.c.) was initiated three days after the animals were made diabetic and was continued for six weeks. LVDP, which was depressed in six week diabetic rats was not significantly affected by the treatment (*p<0.05).
FUNCTION DATA (T3 & DIABETES)

(x) CONTROL (N=8)
(a) CONTROL (T3 TREATED) (N=7)
(o) DIABETIC (N=7)
(♦) DIABETIC (T3 TREATED) (N=6)
FIGURE 19:

Positive dP/dt is plotted against various left atrial filling pressures. The parameter was depressed in diabetic animals and the depression could not be prevented by T₃ treatment (*p<0.05).
FUNCTION DATA (T3 & DIABETES)

(×) CONTROL (N=8)
(o) CONTROL (T3 TREATED) (N=7)
(⋄) DIABETIC (N=7)
(<) DIABETIC (T3 TREATED) (N=6)
FIGURE 20:

The changes in negative dP/dt are shown. T₃ treatment did not prove to be effective in preventing the depression of the rate of relaxation in diabetic rats (*p<0.05).
FIGURE 21:

The Figure demonstrates the effect of six weeks of diabetes on calcium uptake activity in the cardiac SR. The depression of activity was seen over the entire concentration range in diabetic animals and was not significantly affected by T$_3$ treatment (*p<0.05).
T3 TREATMENT & CALCIUM UPTAKE IN DIABETIC RATS

- CONTROL (n=7)
- CONTROL TREATED (n=7)
- DIABETIC (n=7)
- DIABETIC TREATED (n=7)

FREE CA CONC (LOG UM)

CA UPTAKE (NMOL/MG/MIN)
DISCUSSION

The present series of experiments was designed to locate a point in the duration of diabetes at which myocardial alterations are seen and to investigate the effect of various treatments on such alterations. Previous studies carried out on myocardial function as well as biochemistry were carried out at various times following the onset of diabetes. Miller (1979) reported that myocardial function changes could be seen three days after rats were injected with alloxan. However, Vadlamudi et al. (1982) failed to confirm this observation and suggested that changes in myocardial function of diabetic rats occur between thirty days and three months after rats are injected with STZ. Similarly, Fein et al. (1980) studied the effect of STZ-disease on rat papillary mechanics and reported that the changes were seen five weeks after the onset of diabetes. Results from our study are in general agreement with the studies of Vadlamudi et al. and Fein et al. as depression of heart function was seen after six weeks of diabetes (Figures 3 and 4). The diabetes-induced depression did not appear to worsen perceptibly with progression of the disease. The values for LVDP, positive $dP/dt$ and negative $dP/dt$ did not appear to be depressed any further in the ten week (Figures 9, 10, 11) or six month (Figures 14, 15, 16) diabetic rats as compared to the six week diabetic rats. This observation is supported by Fein et al. (1980) where they studied the papillary muscle mechanics abnormalities at five, ten and thirty weeks after inducing diabetes. They found that the changes did not deteriorate further as the disease progressed. It thus seems that depression of heart function occurs by about six weeks and the depression
apparently plateaus soon after it sets in. This is in agreement with the study by Vadlamudi et al. (1982) where a plateauing phenomenon with respect to the depression of heart function was seen. Severity of diabetes may play a role in the depression of heart function as well. The model we used was a moderately diabetic one while that used by Fein et al. (1980) appeared to be severely hyperglycemic which may be why they observed depression at an earlier time point in rats of same strain, sex and age as were used in our study. However, it should be pointed out that we did not study the function at five weeks and the possibility that we would have seen depressed function at five weeks cannot be ruled out. Secondly, we used the whole working heart and changes in overall cardiac function may not necessarily coincide with changes in individual components of the heart such as the papillary muscle.

From our studies, it is also apparent that calcium uptake in cardiac SR is depressed by six weeks of diabetes (Figure 6). Lopaschuk et al. (1983) reported that calcium uptake activity in diabetic cardiac SR is depressed between four weeks and three months of diabetes. This observation is in agreement with our results. There also seems to be a close parallel between the calcium uptake activity and heart function. Diminished accumulation of calcium from the cytoplasmic space by SR has been suggested to be a detrimental factor in the decreased ability of the heart to relax (Dhalla et al. 1982). This suggestion could explain the depression of negative dP/dt which we observe in diabetic hearts. Regarding the time course of altered function and biochemistry, the altered calcium uptake activity seems to precede the altered
contractility. Such a conclusion could be drawn on the basis of findings by Vadlamudi et al. (1982) and Ganguly et al. (1983). While Vadlamudi et al. (1982) did not find a depression of negative dP/dt four weeks after the onset of diabetes, Ganguly et al. (1983) reported altered calcium uptake in SR by four weeks of diabetes. On the other hand we found that cardiac function was depressed by six weeks of diabetes and Fein et al. (1980) reported that papillary muscle mechanics were altered by five weeks. Dillman (1980) and Malhotra et al. (1981) have demonstrated a depression in Ca\(^{2+}\) stimulated cardiac ATPase activity due to diabetes. This finding has been confirmed in a number of other studies (Fein et al. 1981; Dillman, 1982; Ganguly et al., 1983). It may thus be hypothesized that diabetes induces a depression of Ca\(^{2+}\) ATPase activity which in turn causes a depression of calcium uptake activity in the SR. Such depression of calcium transport may then contribute towards an altered myocardial function.

One of the factors that have been implicated as a possible cause of depressed calcium uptake by the SR is elevated levels of long chain acyl carnitines. Various workers have shown that long chain acyl carnitines are potent inhibitors of membrane transport proteins (Woods et al., 1977; Adams et al., 1979; Pitts et al., 1978). Our results (Figure 7) as well as studies from other laboratories demonstrate that long chain acyl carnitines are significantly elevated during diabetes (Feuvray et al., 1979). Moreover, the elevation of long chain acyl carnitines in cardiac SR seems to parallel the depression of calcium transport as well as altered myocardial function. Studies carried out under other experimental conditions such as ischemia have also demonstrated significant elevations of long chain acylcarni-
tines in cytosol of heart cells (Idell-Wenger et al., 1979). Such observations support the suggestion that altered long chain acyl carnitine levels may indeed be involved with changes in myocardial contractility.

This suggestion is supported by the work of Lopaschuk et al. (1983) which was carried out in parallel with the experiment of Vadlamudi et al. (1982). Lopaschuk et al. (1983) found that the depression of calcium transporting activity of SR and the elevation of long chain carnitines in SR was apparent three months after diabetes was induced and was not seen at four weeks of diabetes. Thus there seems to be a close correlation between cardiac function and these biochemical parameters. However, in a very recent study, Lopaschuk et al. (1983) have presented evidence dissociating the cardiac function from calcium uptake activity and levels of long chain acyl carnitines and the results of that study are discussed at a later stage.

Besides the biochemical factors which we have studied, other biochemical factors have also been suggested to play a role in diabetes-induced alterations of myocardial contractility. Regan et al. (1979) have suggested that altered myocardial contractility during diabetes could be a result of increased ventricular stiffness, which was attributed to an increase in interstitial glycoprotein in the myocardium. However, this study was carried out in dogs and no such findings were reported when diabetic rat myocardium was histologically examined (Bhan et al., 1978). Myocardial collagen has been shown to be increased in diabetic rhesus monkeys eighteen months after induction of alloxan diabetes (Yeh et al., 1978). However, Modrak (1980) was unable to
demonstrate significant increases in myocardial collagen content in twenty-six week STZ diabetic rats. The contrasting results have been suggested to be due either to the difference in species or the difference in duration of diabetes in the two studies (Modrak, 1980).

Contractile proteins in the heart may also be responsible for altered myocardial contractility. In rat, myosin ATPase has three isoenzymes which differ in intrinsic activity (Hoff et al., 1977). Diabetes seems to cause a redistribution of the isoenzymes and this results in an overall decrease of myosin ATPase activity (Dillman, 1980; Malhotra et al. 1981). Sodium pump activity was also found to be depressed four to six weeks after induction of diabetes (Ku and Sellers, 1982). Depression of the sodium pump was indicated by a decrease in the ouabain-sensitive $^{86}$Rb uptake by the sarcolemma as well as a decrease in production of inorganic phosphate.

It seems unlikely that depression of myocardial function by diabetes is a result of the depression of a single biochemical parameter but is probably the net result of a number of factors acting together. In the diabetic state which is an abnormal environment, the preformed normal systems may not function as well. It could also be that the systems which are altered in the diabetic state - such as certain enzymes - have lower efficiency than normal and thus the heart as a whole cannot function as well.

Lack of insulin is the characteristic feature of diabetes. There is now a growing interest regarding the ability of insulin to normalize diabetes-induced myocardial changes. We attempted to study the ability of insulin to prevent and reverse the altered myocardial function as
well as calcium uptake activity and levels of long chain acylcarnitines in the cardiac SR. By treating diabetic animals with a long acting porcine insulin (PZI) (duration of action about 36 h) daily, we were able to control the diabetes quite effectively. The treated animals did not lose any weight and at the time of sacrifice, exhibited normal plasma glucose and insulin levels. The heart weight to body weight ratios which were higher in diabetic rats were normal in the treated group (Tables III and IV). However, the most convincing evidence suggesting effective control of diabetes was the degree of glycosylation of hemoglobin. In the prevention study, glycosylated hemoglobin levels did not increase as compared to normal while the diabetic levels increased significantly after a period of six weeks. It was thus apparent that blood glucose levels in the treated group were not elevated to levels high enough to increase glycosylation for any length of time at any point during treatment. We observed a large variation of insulin values between groups (Tables III and IV) which may be due to the non-fasted state of the rats.

When heart function was studied, we found that the depression in cardiac function did not occur in the treated group of diabetics (Figures 3, 4 and 5). This suggests that by correcting the lack of insulin, the myocardial changes may be prevented. The observation also suggests that myocardial alterations resulting from STZ-induced diabetes are not due to a direct toxic effect of STZ but result from hyperglycemia or other metabolic changes induced by the drug. Such a view is supported by the observation that myocardial changes do not occur in rats treated with 3-O-methyl glucose prior to administration of STZ (Fein et al., 1981). 3-O-methyl glucose is a non-metabolizable
analog of glucose and prevents development of diabetes in STZ treated rats. Penparkgul et al. (1981) also found that treatment with the analog could prevent depression of cardiac SR function by preventing the development of diabetes. In close parallel to the function studies we found that the calcium uptake activity in cardiac SR was normal in the treated group of diabetic rats (Figure 6). Similarly, long chain acyl carnitine levels were not significantly elevated in the treated diabetics (Figure 7). Baandrup et al. (1981) studied the ability of insulin to prevent histological changes in the myocardium of diabetic rats. They found that proper control of hyperglycemia with insulin could prevent the changes such as increases in the amount of connective tissue. It thus seems that insulin treatment is an effective way of preventing diabetes induced changes in function, biochemistry and structure of the rat myocardium.

In the reversal study, we treated diabetic rats six weeks after induction of diabetes i.e. a period by which the alterations had supposedly occurred. Treatment of the diabetic animals for four weeks with insulin resulted in a dramatic change in their physical condition. They regained weight significantly, their plasma glucose levels were lowered to normal and plasma insulin levels elevated to normal (Tables V and VI). However, the glycosylation of hemoglobin was not completely reversed by such treatment. The values for glycosylated hemoglobin were between normal and diabetic values and significantly different from both. Glycosylation of hemoglobin occurs throughout the life span of the erythrocyte (McDonald and Davis, 1979), and by six weeks of diabetes there probably is a high level of glycosylation of
hemoglobin. Four weeks of insulin treatment did not reverse the glycosylation completely and this can be explained on the basis of the life span of the mammalian erythrocyte which is about 120 days. Insulin treatment would effectively prevent further glycosylation of cells but the treatment would have to be continued longer in order to allow for total replacement by unglycosylated erythrocytes.

Studying the heart function, we found that four weeks of insulin treatment was capable of reversing the diabetes-induced abnormalities completely (Figures 9, 10, 11). Our study is in agreement with a study carried out by Fein et al. (1981) where they examined the effect of insulin treated diabetes-induced alterations in myocardial muscle mechanics. They initiated insulin treatment about eight weeks after induction of diabetes and studied the ability of insulin to reverse the abnormalities at various time points during the course of treatment. Complete reversal of altered papillary muscle mechanics was seen only after four weeks of insulin as was observed in our case. It could be argued that heart function improvement could simply be the result of elevated insulin levels in blood and addition of insulin to the perfusion medium could normalize heart function as well. This view would be supported by studies in which addition of insulin to the perfusate has been shown to result in improved function (Miller, 1979). However, it should be pointed out that Miller's study was carried out three days after injection of alloxan, at which stage a depression of cardiac function was observed when 5.5 mM glucose was used and the depression was overcome either by increasing the glucose concentration to 10 mM or by including insulin in the medium. The defects observed by Miller
could be the result of acute metabolic derangements causing defects in energy utilization due to lack of insulin and at this stage, the cardiac function abnormalities could be overcome simply by including insulin or increasing the substrate concentration. Chronic diabetic states such as the model we used may involve altered biochemical changes. Moreover, Penparkgul et al. (1980) have demonstrated that perfusion of working hearts with insulin-containing buffer did not improve function of eight week diabetic hearts. Similarly, Fein et al. (1981) were unable to reverse depression of papillary muscles by including insulin in the bathing solution.

We also found that insulin treatment could reverse the depression of calcium uptake activity in SR (Figure 12) and normalized the elevated levels of long chain acyl carnitines in SR (Figure 13). Once again we were able to demonstrate a parallel between the cardiac contractility and biochemistry of the heart. Studies from other laboratories have also indicated that insulin treatment is capable of reverting cardiac SR function to normal (Penparkgul et al., 1981). Myosin ATPase has also been reported to be normalized by four weeks of insulin in diabetic rats (Dillman, 1980; Fein et al., 1981). It thus appears that insulin treatment can reverse all the diabetes-induced myocardial changes that have been studied. The reversal is gradual but definite and is complete by four weeks of treatment as pointed out by Fein et al.; (1981) and by our study. These findings are of significant importance but before these results are applied more widely, the features of this model should be considered. The model we have used, as have others who reported results similar to ours, is a moderate to severe
diabetic rat which has diabetes for only six weeks (and up to
ten weeks in other studies) before insulin treatment is initiated.
The duration of diabetes would obviously have an influence on the
results obtained. It is possible that at longer durations, the
abnormalities become more pronounced and after a particular stage,
they probably become irreversible. Pogatsa et al. (1979) reported
that insulin therapy could not completely reverse myocardial alterations
in chronically diabetic dogs.

In order to examine the above problem we studied the effect of
insulin treatment in rats diabetic for a longer duration of time.
Diabetic rats were treated with insulin for four weeks five months
after diabetes was induced with STZ. The animals treated with insulin
gained weight, had plasma glucose and insulin levels similar to those
of controls but the glycosylated hemoglobin levels did not come back
to control values (Table VII). When the heart function was studied,
the results obtained were not as promising as in the earlier studies.
The LVDP in diabetic treated rats was not completely reversed to
normal. Although it was higher than in the diabetic animals and
lower than in the control animals it did not differ significantly from
either (Figure 15). On the other hand, positive dP/dt in the treated
group of animals was higher than the diabetics and lower than the
controls, and the difference was significant in both cases (Figure 16).
This suggests that even at this stage the changes are not completely
irreversible and it may be possible to normalize the changes by using
a longer duration of insulin treatment. The view that changes are not
irreversible even at six months of diabetes is supported by our results
obtained on the rate of relaxation. Negative dP/dt was completely reversed to normal by insulin treatment (Figure 17). Studies have not previously been carried out on diabetic rats treated with insulin at this stage of diabetes and it would be interesting to see whether or not insulin is capable of reversing the abnormalities to normal. From our study it would seem that insulin treatment does affect myocardial function even at this stage and perhaps a longer treatment regimen would effectively normalize the function.

As mentioned in the Introduction, diabetes causes a significant depression of thyroid hormone levels. Hypothyroidism resulting from diabetes has been suggested to be the cause for diabetes-induced myocardial changes because hypothyroidism is known to slow relaxation and to depress the force-velocity relation in papillary muscles (Buccino et al., 1967). In a recent study, Dillman (1982) studied the effect of thyroid replacement therapy or diabetes-induced depression of myosin ATPase. The treatment effectively restored depressed cardiac myosin ATPase activity to normal in diabetic rats and when the treatment was initiated early enough, the depression could be prevented. However, results from our study indicate that diabetes-induced myocardial functional alterations are unlikely to be a result of depressed thyroid status as replacement with T3 was unable to cause any improvement in the myocardial function (Figures 18, 19, 20).

Again, in parallel to the function study, we found that depression of calcium uptake activity in the SR could not be prevented by T3 treatment (Figure 21). Fein et al. (1981) also found that thyroid replacement therapy was unable to reverse diabetes-induced alterations
of papillary muscle mechanics. In a very recent report, Ganguly et al. (1983) were unable to normalize the calcium uptake in the SR of diabetic hearts by treating the diabetic rats with thyroid hormone. It thus seems that hypothyroidism does not mediate the diabetes-induced myocardial alteration. It also appears that myosin ATPase is not solely responsible for the depression of heart function because while the Ca\(^{2+}\) stimulated myosin ATPase can be normalized by thyroid replacement (Dillman, 1982) muscle function cannot be normalized. From our studies, it seems like there is a close parallel between the levels of long chain acyl carnitines, the calcium uptake activity of SR as well as the myocardial function. However, in a recent study from our laboratory, Lopaschuk et al. (1983) have demonstrated a dissociation between calcium uptake activity in SR and cardiac function. By treating diabetic animals with carnitine in an attempt to decrease long chain acyl carnitine levels in the SR, they were able to normalize the calcium uptake activity in SR to normal. However, when cardiac function in these animals was studied, the myocardial function remained depressed in the carnitine treated rats indicating a possible dissociation between the calcium uptake in SR and the myocardial function. To gain a clearer perspective as to the significance of biochemical parameters in myocardial function, it would be essential to study all these parameters under a series of different conditions. At present, it seems more likely that myocardial contractility is the end result of a number of biochemical factors put together rather than any one factor and that most or all of these biochemical factors have to be functioning normally for the heart to function normally.
Finally, the sarcolemmal calcium pump may also be an important contributor towards cardiac contractility (Caroni and Carifoli, 1980; Trumble et al., 1980). This parameter remains to be studied in the diabetic state. However, studies in our laboratory carried out in conjunction with D. Godin (unpublished results) indicate the sarcolemmal Na\(^+\),K\(^+\) ATPase is not affected by diabetes. Similarly p-nitro phenyl phosphate (PNPP) ATPase which is an indicator of Na\(^+\), K\(^+\) ATPase activity was not affected by diabetes. It is obvious that myocardial contractility, even though depressed in diabetes, is compatible with life to a certain degree. Probably, the sarcolemmal pumps are more resistant to the effects of diabetes and perhaps when these pumps are affected, the heart becomes severely damaged. A similar hypothesis could be forwarded for mitochondrial enzymes as the mitochondrial Ca\(^{2+}\)-Mg\(^{2+}\) ATPase was unaffected by four weeks of diabetes (unpublished results from our laboratory).
SUMMARY AND CONCLUSIONS

1. Streptozotocin-induced diabetes caused a depression of the left ventricular developed pressure (LVDP), rate of pressure rise and the rate of pressure decline in the rat isolated working heart. The depression was apparent by six weeks and did not worsen perceptibly with progression of the disease.

2. In close parallel to the depression of heart function, a depression of the ability of cardiac sarcoplasmic reticulum (SR) to transport calcium was seen. An accompanying elevation of long chain acyl carnitines in the SR was seen. However, the levels of free carnitines were not significantly affected by diabetes.

3. By treating diabetic rats with insulin immediately after diabetes was detected, the depression of heart function could be prevented. The treatment was also effective in preventing depression of calcium uptake activity in the SR as well as elevation of long chain acyl carnitines in the SR.

4. In the reversal study, i.e. when insulin treatment was initiated six weeks after induction of diabetes, the myocardial function could be reversed to normal. Similarly, calcium uptake depression and elevation of long chain acyl carnitines in SR could be normalized by insulin treatment.

5. When diabetes was allowed to progress for five months before initiating insulin treatment, the reversibility was not seen as readily. Though the rate of relaxation was completely normalized, the reversibility was not seen as readily. Though the rate of
relaxation was completely normalized, rate of contraction and LVDP showed only trends towards normal.

6. Replacement of thyroid hormone with $T_3$ effectively elevated the thyroid index to normal. However, neither function nor calcium uptake were normal in the treated group of diabetics suggesting that the changes observed during diabetes do not occur as a result of hypothyroidism.

In conclusion, it is apparent that insulin treatment is an effective method to prevent as well as reverse cardiac abnormalities induced by diabetes. However, it is critical that the therapy be initiated without allowing the disease to progress for very long periods of time as the effectiveness of insulin treatment to reverse the myocardial abnormalities seems to decrease as the disease progresses. While diabetes does cause hypothyroidism, the lowered thyroid status does not seem to be responsible for the myocardial changes. This view is based on our findings that replacement therapy with $T_3$ does not prevent the myocardial damage from occurring in diabetic rats.
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