STUDIES ON DIABETES - INDUCED MYOCARDIAL ALTERATIONS IN STREPTOZOTOCIN DIABETIC RATS

by

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in

THE FACULTY OF GRADUATE STUDIES
Division of Pharmacology and Toxicology of the Faculty of Pharmaceutical Sciences

We accept this thesis as conforming to the required standard:

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July 1985
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ABSTRACT

Diabetes is known to result in a large number of alterations which affect various systems and organs. One of the more prominent disorders associated with diabetes is that of cardiac disease. Clinically, diabetics suffer from morbidity and mortality of cardiac origin to a greater extent than the nondiabetic population. Various functional studies have also revealed that the efficiency of diabetic hearts to function as pumps is lower than that of normal hearts. Experimentally, myocardial function of either rats or dogs made diabetic with either streptozotocin (STZ) or alloxan has been studied and a depression clearly demonstrated in both the species. The abnormalities of cardiac function in experimental diabetes are accompanied by depression of various enzyme systems in the heart. These include the ability of the sarcoplasmic reticulum (SR) to take up calcium; the myosin and actomyosin ATPase activities; and the \( \text{Na}^+ \), \( \text{K}^+ \) ATPase activity. All these changes can be prevented and reversed by insulin treatment suggesting that the myocardial problems seen in STZ or alloxan diabetic animals are due to diabetes and not direct toxicities of the drugs.

It is not known whether the beneficial effects of \textit{in vivo} insulin treatment are due to its direct myocardial effects or whether they are secondary to its effects mediated via normalisation of metabolism in diabetic animals. Thus, in the first part of the present investigation, we examined the direct effects of insulin on hearts from either control or diabetic rats using the isolated working heart preparation. Rats made diabetic with STZ (55 mg/kg) were sacrificed either 3 days or 6 weeks
after induction of the disease and their hearts isolated and perfused in the working heart mode. Glucose concentrations varying from 5mM to 20mM were used in the perfusion medium, either in the presence or absence of insulin (5mU/mL). Left ventricular function was expressed as left ventricular developed pressure (LVDP) and the rates of contraction and relaxation (positive and negative dP/dt respectively) at various left atrial filling pressures. Three days after injecting STZ into rats, the animals exhibited hypoinsulinemia, hyperglycemia and their body weights although not significantly different from those of control animals, tended to be lower than the body weights of controls. Animals treated in this manner did not exhibit depression of cardiac function when compared with the myocardial function of control rats. Hearts from control rats exposed to regular insulin in the presence of 5mM glucose exhibited values of contractility which were significantly greater as compared with those obtained from control rat hearts not exposed to the hormone. When insulin was perfused along with a higher concentration of glucose (10mM), function of control rat hearts was affected to a significant extent. As opposed to the effects on control rat hearts, insulin failed to increase contractility in hearts from 3 day diabetic rats when either 5 or 10mM glucose was used in the perfusion medium.

The study was then repeated using animals which had been diabetic for six weeks. At the time of sacrifice, these animals were hypoinsulinemic, hyperglycemic and weighed significantly less than their age-matched controls. Analysis of cardiac function revealed a significant depression in diabetic rats as compared with controls. Increasing glucose concentrations from 5 to 20mM in the perfusion medium did not affect the function of either control or diabetic rat hearts. Perfusion
with regular insulin increased contractility in control rat hearts; the increase in contractility was not affected by increasing the glucose concentration from 5 to 10mM. However, contractility of diabetic rat hearts was not affected by insulin perfusion when either 5 or 10mM glucose was used in the perfusion medium. In order to eliminate the possibility of involvement of glucagon (which may contaminate commercial insulin preparations) in the effects of insulin on control rat hearts, part of the study was repeated using glucagon-free insulin. While the glucagon-free insulin increased contractility in control rat hearts, diabetic rat hearts were not affected. These results are identical to those obtained with regular insulin, suggesting that the effects of insulin observed were due to insulin itself.

Although insulin treatment prevents and reverses diabetes-induced myocardial alterations in the rats, due to its widespread metabolic effects, it is not a good tool for investigating the specific factors which cause the cardiac abnormalities. In addition, a major problem with insulin treatment clinically is the fact that hypoglycemia can be associated with it, inadequate control occurs in some diabetics and secondary complications, such as myocardial problems, occur despite insulin treatment. It is thus desirable to have treatments which selectively affect certain aspects of diabetes so that the suspected underlying causes can be corrected specifically and their significance in causing the myocardial problems assessed. It would also be useful to have drug treatments which could either substitute for insulin or could be used in addition to the peptide. We have thus studied the effectiveness of certain treatments in preventing diabetes-induced myocardial alterations. The first one used was methyl palmoxirate, a fatty acid
analog which is reported to reduce blood glucose levels in diabetic rats and dogs. The glucose-lowering effect is mediated via inhibition of fatty acid metabolism due to inhibition of carnitine acyl transferase resulting in inhibition of acyl carnitine formation and eventually inhibition of fatty acid transport across the mitochondrial membrane. Rats were treated with the drug (25mg/kg/day p.o.) three days after they were injected with either STZ or buffer. The treatment was carried out for 6 weeks and cardiac performance was then assessed. Untreated and treated diabetic rats were hypoinsulinemic, hyperglycemic and hyperlipidemic at the time of sacrifice. Cardiac function, which was depressed in diabetic animals, was still depressed despite the methyl palmoxirate treatment. However, the ability of the myocardial sarcoplasmic reticulum (SR) to take up calcium, which was depressed in diabetic rats, was normal in treated diabetic rats. Also, the levels of long chain acyl carnitines (LCAC) in the myocardial SR were normalised by methyl palmoxirate treatment in diabetic rats.

In an effort to normalise diabetes-induced myocardial alterations in rats, we then attempted a combination of either methyl palmoxirate or carnitine (as both can prevent the depression of SR calcium uptake) with thyroid hormone treatment (as it can normalise myosin ATPase depression in diabetic rat hearts). The treatment protocol was identical to that described above (30μg/kg/day s.c. T3 was used). Although the general features of both control and diabetic animals were not affected by either of the combination treatments, cardiac dysfunction in diabetic rats was prevented by methyl palmoxirate and T3 treatment. Carnitine and T3 treatment, on the other hand, affected the function of diabetic rat hearts only at the lower left atrial filling pressures.
These results suggest that the combination treatment of methyl palmoxirate and T₃ affect parameters besides SR calcium uptake and myosin ATPase. This is because the combination of carnitine and T₃, which also supposedly affects same parameters as the other combination, could not prevent the myocardial alterations. One of the possible reasons for the effectiveness of the combination of methyl palmoxirate and T₃ could be that animals treated with methyl palmoxirate derived at least part of their metabolic energy (especially at higher left atrial filling pressures) from glucose and thus reduced the oxygen demand at higher filling pressures as opposed to the untreated diabetic rat hearts which depended completely on fatty acids for their metabolic energy demands.

John H. McNeill, Ph.D.
Thesis Supervisor
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<tr>
<td>LVDP</td>
<td>Left ventricular developed pressure</td>
</tr>
<tr>
<td>+dP/dt, Positive dP/dt</td>
<td>Rate of left ventricular pressure development</td>
</tr>
<tr>
<td>-dP/dt, negative dP/dt</td>
<td>Rate of left ventricular pressure decline</td>
</tr>
<tr>
<td>STZ</td>
<td>Streptozotocin</td>
</tr>
<tr>
<td>LCAC</td>
<td>Long chain acyl carnitines</td>
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<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3,5,3' triiodothyronine</td>
</tr>
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<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>3,5,3',5' tetraiodothyronine</td>
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INTRODUCTION

I. THE CLASSIFICATION, PATHOGENESIS AND ETIOLOGY OF DIABETES MELLITUS

Diabetes mellitus consists of a variety of syndromes with different etiologies. It is characterized by fasting hyperglycemia and altered lipid metabolism, the underlying cause of which is a relative or absolute lack of insulin secretion or action. The syndromes which make up diabetes can be broadly divided into two groups: those associated with insulin deficiency, which account for about 20% of all cases, and those associated with insulin resistance, which account for nearly 80% of cases (Grunberger et al., 1983). Accordingly, primary diabetes has been defined as either insulin-dependent (IDDM) or non-insulin-dependent (NIDDM) (NDDG, 1979). Besides these primary syndromes, there are other types of diabetes which include gestational diabetes (GDM), impaired glucose tolerance (Craig, 1980) and diabetes resulting from other diseases (Table I).

IDDM, previously referred to as juvenile onset diabetes, can occur at any age, but the pathological changes resulting from diabetes are more severe in the pancreas of young diabetics than in that of patients who develop it at a later stage in life. These changes include reduction in the size of islet cells, lymphocytic infiltration or "insulitis" (the occurrence of which is not always seen), reduction in the number and size of islets, and islet regeneration and hypertrophy especially in young diabetics (Gepts, 1981). Although it was thought that the beta cells completely disappear from the pancreas of juvenile diabetics, more recent
# Table 1

## The Classification of Diabetes

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<th>Clinical Characteristics</th>
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<td>Type I insulin-dependent type (IDDM)</td>
<td>Genetic and environmental; association with HLA-type and abnormal immune responses, including the presence of islet-cell antibodies</td>
<td>Dependence on injected insulin to prevent ketosis and preserve life. There may be a noninsulin dependent phase in this disease. Onset usually in youth, but may occur at any age. Insulinopenia.</td>
</tr>
<tr>
<td>Type II noninsulin-dependent type</td>
<td>Genetic factors; a subgroup shows autosomal-dominant inheritance. Environmental factors are also important, and obesity is suspected as an etiologic factor</td>
<td>Persons are not ketosis prone, may use insulin for correction of hyperglycemia. However, ketosis may develop under circumstances of stress or infection. Serum insulin may be normal, depressed or elevated. Onset usually after 40, but may occur at all ages. 60–90% are obese.</td>
</tr>
<tr>
<td>Other syndromes associated with diabetes</td>
<td></td>
<td>Requires two diagnostic determinations: diabetes mellitus and presence of the associated syndrome.</td>
</tr>
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- Pancreatic disease including hemochromatosis
- Hormonal: Cushing's syndrome, acromegaly, pheochromocytoma, glucagonoma
- Drug- or chemical-induced
- Insulin receptor abnormalities
- Certain genetic syndromes
- Other
studies using C-peptide radioimmunoassay have shown evidence of residual insulin secretion 10 years after the clinical onset of the disease (Heding and Rasmussen, 1975). Immunocytochemical studies have also shown the presence of beta cells (in much smaller numbers than controls) in 50% of juvenile diabetics with a disease of less than 10 years and in 18% of juvenile diabetics with a longer duration of the disease (Gepts and DeMey, 1978).

Heterogeneity, which implies that different genetic and/or environmental factors are involved, has been established for the etiology of diabetes. Studies on monozygotic twins have suggested that there is a large group of individuals with insulin dependent diabetes in whom non-genetic factors are of major importance, although the actual predisposition to diabetes is genetically determined (Tattersall and Pyke, 1972; Pyke, 1979). Studies on histocompatibility complexes (HLA) had shown an increase in the HLA antigens B8 and B15 in insulin-dependent diabetics (Cudworth and Woodrow, 1975; 1976). However, more recent studies suggest that the risk for insulin-dependent diabetes is stronger with HLA-Dw3 and Dw4 alleles (Christy et al., 1979; Nerup et al., 1977). The HLA-B8 form of insulin-dependent diabetes mellitus appears to be an autoimmune disease characterized by an increased prevalence of the Dw3 allele, an increased persistence of pancreatic islet cell antibodies, antipancreatic cell-mediated immunity and lack of antibody response to exogenous insulin (Irvin et al., 1977; 1978). The other form, associated with HLA-B15, appears to be associated with the Cw3 and Dw4 alleles, is not associated with islet cell antibodies and is accompanied by increased antibody response to exogenous insulin (Rotter and Rimoin, 1981). Islet cell antibodies which have been found in insulin-dependent diabetics
(McLaren et al., 1975; Lernmark et al., 1979) may be either cytoplasmic and/or cell surface antibodies (Freedman et al., 1979). However, the significance of these antibodies in the production of IDDM is not clear. Also, it is not known whether these antibodies are the result or the cause of beta cell destruction and/or dysfunction.

Another factor that has been implicated in the pathogenesis of IDDM is the presence of viruses. Mumps, rubella, cytomegal-, reo- and Cocksackie- viruses are capable of multiplying in and causing lesions of the pancreatic tissue (Craighead, 1975). In recent years, a correlation between coxsackie B4 virus and IDDM has been suggested (Yoon et al., 1979). Various studies have also been carried out in animal models and the evidence seems to implicate viruses in the pathogenesis of diabetes (Craighead, 1978). Insulin resistance, which is often associated with NIDDM, is also seen in a significant number of insulin-dependent diabetics (DeFronzo et al., 1982). Although this may be due to formation of antibodies to exogenously administered insulin (Gorden et al., 1982), a majority of the resistant patients do not have high titres of circulating insulin antibodies (DeFronzo et al., 1982). The cellular mechanism of the insulin resistance thus remains to be determined.

Non-insulin-dependent diabetes mellitus, on the other hand, presents a different picture. The pathology of the pancreas of patients in this group is not altered as dramatically as that of patients with IDDM. As a group, there is some diminution of beta cell number; hyaline deposition is not infrequent but insulitis is not present. Fatty infiltration, which is often focal, is frequent and is associated with atrophy and inter-acinar fibrosis; atherosclerosis and arteriosclerosis is seen in about two-thirds of diabetic pancreases examined (Volk and Wellman, 1979).
A large number (between 60 and 90%) of those having NIDDM are obese and they often exhibit hyperinsulinism and associated insulin resistance. The basis of NIDDM is not very well understood, but two factors that are thought to be of significance are those of insulin secretion and insulin resistance. An attractive hypothesis forwarded by Ward et al., (1984) suggests that the disease may be initiated by a primary beta cell lesion which results in decreased insulin output. As a result, plasma glucose levels rise and thus insulin secretion is stimulated to normal levels. If insulin resistance were to develop under these circumstances, glucose levels would rise even further and the net result of this cycle could be development of diabetes. This hypothesis is summarized in figure 1. The degree of hyperglycemia in NIDDM patients is determined by an interaction between the patients beta cell sensitivity to glucose and the degree of tissue sensitivity to insulin. Mathematical modelling has predicted that significant hyperglycemia would result from a 50% decrease of beta cell function if there were marked insulin resistance (Turner et al., 1979). In clinical studies, first-phase insulin release i.e. the immediate release of insulin in response to a challenge with intravenous glucose (which probably involves release of preformed insulin) is virtually absent (Brunzell et al., 1976). However, the delayed phase of glucose-induced insulin release is usually retained in diabetic patients and appears to be normal in patients with fasting plasma glucose levels of <200 mg% (Pfeifer et al., 1981).

With respect to the beta cell lesions (which, Ward et al. have suggested to be the initial event in NIDDM), a number of theories have been proposed. Recent investigations using DNA hybridization techniques have shown that diabetic patients of certain racial groups have an
Figure 1: A hypothesis for the pathogenesis of NIDDM forwarded by Ward et al. (1984). (A) Normal basal feedback loop for insulin and glucose. (B) Hypothetical initial beta-cell lesion of NIDDM. (C) Effect of hyperglycemia to compensate for the beta-cell defect in NIDDM. (D) Control of beta-cell function in NIDDM accompanied by tissue insensitivity to insulin. (From Ward et al., 1984).
increased incidence of a 1.6 kilobase insertion in the 5' flanking region of the insulin gene on chromosome 11 (Rotwein et al., 1983). Since this region is often associated with regulation of the structural genes, it is postulated that such an insertion could be associated with a reduced ability to synthesize insulin. Metabolic features of NIDDM were also mimicked in animal and human studies in which either the beta cell number (by pancreatectomy in rats: Bonner-Weir et al., 1983) or function (by administration of somatostatin and glucagon to normal humans: Ward et al., 1983) had been altered. Other studies have suggested that increased sensitivity of beta cells to the inhibitory effects of endogenous prostaglandins may be partially responsible for diminished responsiveness to glucose since the latter could be increased by sodium salicylate, a prostaglandin synthesis inhibitor (Robertson and Chen, 1977). The findings that responsiveness to glucose could also be partially restored by phentolamine and that some non-insulin-dependent diabetics had slightly elevated levels of catecholamines suggest a role for the alpha adrenergic system in the pathogenesis for NIDDM (Robertson et al., 1976). Similarly, an increase in insulin responsiveness has been demonstrated by administration of naloxone to diabetics suggesting the participation of endogenous opiate-like peptides in the abnormal islet function of NIDDM (Guigliano et al., 1982). All these studies suggest that considerable heterogeneity exists in the production of beta cell dysfunction in NIDDM.

With respect to the mechanism of insulin resistance in NIDDM, the precise mechanisms are largely unknown. Ciaraldi et al. (1982) have shown a rightward shift in the dose-dependent uptake of glucose induced by insulin in Type II diabetics, as well as a marked decrease in the
maximal response to insulin. They have thus suggested that defects in both receptor binding and in the post-receptor events exist. Other studies also support this view (Hissin et al., 1982; Bolinder et al., 1982). The post-receptor defect is thought to be due to an alteration in the intrinsic activity of the glucose transporter system (Ciaraldi et al., 1982). However, whether this is due to a decrease in number of transporting units (Hissin et al., 1982) or due to a decrease in the accessibility of the carrier to the cell surface (Cushman and Wardzala, 1980) is not definitely known. It should also be pointed out that the post-receptor defects in NIDDM may also include more distal events occurring within the cell.

Besides insulin, other hormones may also be involved in the pathogenesis of diabetes. Glucagon, a polypeptide secreted by the alpha-cells of the pancreas, is known to cause hyperglycemia and its levels are known to be elevated in diabetics (Pek, 1977; Lefebvre and Luyckx, 1979). Diabetes has been referred to by some workers as a bihormonal disorder as disturbances in glucagon secretion have been known to accompany insulin deficiency (Unger and Orci, 1975). Also, the inhibitory effect of hyperglycemia on glucagon secretion is lost in diabetes, and in some cases, hyperglycemia may even increase the secretion of glucagon, thus adding to pre-existing hyperglycemia. Glucagon has also been known to induce glycogenolysis and gluconeogenesis in the liver and thus hyperglucagonemia (such as that seen in some diabetics) may well cause hyperglycemia via this mechanism (Pek, 1977).

II. CARDIAC DERANGEMENTS INDUCED BY DIABETES

Biochemical Features

Diabetes is known to cause a number of disorders in various systems.
The most obvious of these is the alteration of overall metabolism as well as altered metabolism in individual tissues including the heart. Other complications of diabetes include retinopathy, which leads to blindness in patients with long-standing diabetes (Davis, 1974), nephropathy, which is the major cause of deaths in insulin-dependent diabetics (Lundbaek, 1965), ketoacidosis, which could progress to lactic acidosis, alcoholic acidosis and, rarely, cerebral edema (Watkins et al., 1969; Young and Bradley, 1967) as well as hyperosmolar coma without significant ketoacidosis (Arieff and Caroll, 1972). Another significant problem frequently encountered in diabetic patients is an alteration of the cardiovascular system. This topic is dealt with in the following sections.

The metabolic disorders of diabetes have been a topic of vigorous investigation as a result of which these changes are well characterized. As a result of the insulin lack, glucose uptake into muscle cells is severely restricted and hyperglycemia develops. Also, in adipose tissue, insulin-suppressible tissue-bound lipase becomes activated in the absence of insulin and the result is cleavage of triglycerides into fatty acids and glycerol both of which are released into the plasma and the net result is hyperlipidemia.

In the heart, energy to support mechanical function is derived almost completely from aerobic metabolism by the process of oxidative phosphorylation in the mitochondria. In the fed state, a partial source of energy is glucose due to high levels of glucose and insulin and low levels of free fatty acids. However, in diabetes and starvation, due to a lack of insulin, glucose transport across the cell membrane is limited, and due to stimulation of lipolysis, free fatty acids in the plasma are
elevated. The net result is a shift of metabolism from glucose to fatty acid utilisation. Glucose and fatty acid metabolism seem to be related to each other and the relation has been described by Randle's glucose-fatty acid cycle (Randle et al., 1966) which is discussed in the following text. Two enzymes which are of major significance in the metabolism of glucose are phosphofructokinase and pyruvate dehydrogenase. Phosphofructokinase is responsible for the phosphorylation of fructose phosphate and is inhibited by high concentrations of ATP and citrate (Parmeggiani and Bowman, 1963). The enzyme is inhibited in diabetes, perhaps because of an increase in citrate in diabetic muscle, concomitant with an increased oxidation of fatty acids (Parmeggiani and Bowman, 1963). Pyruvate dehydrogenase, on the other hand, is responsible for the mitochondrial breakdown of pyruvate to acetyl-CoA. While the total amount of this enzyme is not affected in diabetes, the fraction present in the active form is significantly reduced from 16-20% found normally to 1-6% in diabetes (Henning et al., 1975). Also, activation of the enzyme during muscle contraction is significantly affected in diabetes (from 41% in controls to 13% in diabetics). The mechanism for this depression seems to be multifaceted. The enzyme is activated by dephosphorylation, so one of the reasons for its low activity in diabetic hearts during contraction could be impaired dephosphorylation. The enzyme is also regulated by NADH and acetyl-CoA, high concentrations of either being inhibitory. Since fatty acids are utilised as the major source of metabolic fuel during diabetes and concentrations of acetyl-CoA and NADH are high, this has been suggested to be one of the mechanisms by which pyruvate dehydrogenase is inhibited (Batenburg and Olson, 1976).

Myocardial pyruvate dehydrogenase (PDH) enzyme inactivation has been
shown in diabetes (Kobayashi and Neely, 1983). As a result, increased cardiac work and elevated levels of pyruvate do not activate PDH in diabetic rat hearts. Thus the utilization of glucose from endogenous and exogenous sources is severely limited in diabetic rat hearts. A direct consequence of this inactivation is that the diabetic rat heart becomes dependent on lipid metabolism for energy to a very significant extent. The major lipids utilised are fatty acids and ketone bodies since lipoprotein-bound triglyceride utilisation is decreased due to a fall in the activity of lipoprotein lipase in diabetes. In a recent study, Pieper et al. (1984) showed that fatty acid perfusion of diabetic hearts caused a time and concentration-dependent decrease in the levels of ATP. Furthermore, utilisation of ketone bodies by rat hearts is restricted in the TCA cycle at the level of 2-oxoglutarate dehydrogenase reaction. It is probable that the myocardial alterations seen in diabetic rats may be the result of these biochemical alterations. This can be tested by using agents which will reduce the oxidation of fatty acids and thereby normalise the metabolic events of diabetes in the heart.

Fatty acid metabolism, on the other hand, is also controlled by certain factors. Normally, free fatty acids are used in preference over glucose since the metabolic substrate as the glycolytic pathway is more sensitive to regulatory factors (such as the ATP/ADP, acetyl-CoA/CoA and NADH/NAD ratios) than is the beta oxidation pathway. This is beneficial in view of the metabolic demands of muscular contraction since the oxidation of a single mole of palmitic acid generates three times the amount of energy provided by a mole of glucose. However, it should be pointed out that this could be detrimental under conditions of limited oxygen supply as the ratio of ATP generated/oxygen consumed per mole of
substrate is greater for glucose than it is for fatty acids; thus under hypoxic conditions, glucose will be utilised preferentially. With respect to the metabolism of fatty acids in the myocardium, they diffuse freely across the cell membrane and are bound to a cytoplasmic protein, now referred to as the Z protein, which prevents back diffusion of fatty acids out of the cell. Fatty acids are then metabolized according to the scheme in figure 2. Increase in the activity of carnitine acyl transferase has been observed in the liver during diabetes (Harano et al., 1972) and the levels of long chain acyl carnitines were also elevated in diabetic hearts (Feuvray et al., 1979; Lopaschuk et al., 1983).

Studies by Randle and coworkers have shown that alterations of carbohydrate metabolism which are characteristic of the diabetic state could be reproduced almost completely in normal muscle by an increased oxidation of free fatty acids and ketones (Randle et al., 1963; 1964; 1966). It was thus suggested that fatty acids and glucose metabolism are interdependent and that alterations of fatty acid metabolism may be the primary event in diabetes (Randle et al., 1963; 1966). If this hypothesis were true, then decreasing the oxidation of free fatty acids would be one approach to the treatment of diabetes mellitus. The finding that a number of agents including hypoglycin, 4-pentenoic acid, (+)-decanoyl-carnitine, and alpha-bromo fatty acids that inhibit fatty acid oxidation produced hypoglycemia supported the above view (Randle, 1969; 1970; Stewart and Hanley, 1969; Tutwiler, 1973; Burges et al., 1968). However, the toxicity of the compounds and the findings that some of them were not orally effective (alpha-bromo fatty acids) or inhibited enzymes not related to fatty acid oxidation (Randle, 1970; Stewart and Hanley, 1969; McGarry and Foster, 1973; Williamson et al., 1973; Tanka, 1975)
SCHEMATIC REPRESENTATION OF METABOLIC EVENTS IN THE MYOCARDIUM

* inhibited by methyl paloxirate
led to a search for newer hypoglycemic agents. In the process, McNeil Laboratories have synthesized a number of compounds, two of which (McN-3802, 2-tetradecylglycidic acid or palmoxiric acid and McN-3716, (methyl 2-tetradecylglycidate or methyl palmoxirate) (Fig. 3) have been investigated to some extent.

Both palmoxiric acid and methyl palmoxirate exhibited dose-dependent hypoglycemic activity in fasted rats (the dose range was 5-40 mg/kg, p.o. and the blood glucose lowering obtained was up to an 80% decrease in fasting blood glucose) (Tutwiler et al., 1978). In non-fasted diabetic rats, a modest lowering of blood glucose was obtained with methyl palmoxirate (25 mg/kg) but lowering was much greater in fasted diabetic rats. The most impressive results of methyl palmoxirate were seen in diabetic (depancreatized) dogs where a normalisation of blood glucose was seen up to 24 hr after administration of the drug (25 mg/kg, p.o.) (Tutwiler et al., 1978).

Various lines of evidence suggest that the mechanism of action of methyl palmoxirate is indeed via the inhibition of fatty acid oxidation. A direct inhibition of CO₂ production from palmitate has been shown in diaphragms from rats pretreated with methyl palmoxirate (Tutwiler et al., 1979). However, oxidation of short chain fatty acids, palmityl carnitine, glucose, glycolytic intermediates, beta-hydroxybutyrylate, succinate or citrate was not inhibited. On the other hand, the oxidation of glucose to 14CO₂ was stimulated in hemidiaphragms from rats treated with methyl palmoxirate, the stimulation being secondary to the inhibition of fatty acid metabolism (Tutwiler et al., 1979). The mechanism by which methyl palmoxirate inhibits fatty acid oxidation has also been studied and it appears to be through irreversible inhibition of
Figure 3: Palmoxiric acid, methyl palmoxirate and other fatty acid analogs synthesized by McNeill laboratories in the search for newer hypoglycemic agents.
carnitine palmitoyl transferase-A (Tutwiler et al., 1981; Tutwiler and Ryzlak, 1980). A recent report has shown that inhibition of the enzyme is via formation of the CoA ester of methyl palmoxirate (Kiorpes et al., 1984). Finally, although most of the studies reported were carried out in either diaphragm or in the liver, inhibition of fatty acid oxidation by palmoxiric acid has also been shown in the isolated perfused rat heart (Pearce et al., 1979).

**Clinical Features of Diabetes-Induced Myocardial Alterations**

Myocardial abnormalities occurring as a result of diabetes have been described in various clinical and experimental settings. A number of clinical studies have shown that the incidence of coronary and congestive heart diseases is much greater among diabetics. Amongst other studies, the Framingham study showed that the morbidity in diabetics was almost three times greater than in the general population. Mortality due to cardiovascular causes in diabetic women was five times greater and in diabetic men two times greater than in nondiabetics of the corresponding sex (Kannel et al., 1974). Similarly, in the Rochester project, the observed cases of coronary heart disease (angina pectoris, myocardial infarction or sudden unexpected death) and of congestive heart failure without antecedent heart disease in patients with maturity-onset diabetes exceeded those expected from the general population (Palumbo et al., 1981). The pathogenesis of heart failure in diabetes in uncertain, but various causes such as coronary microangiopathy and macroangiopathy have been implicated.

Macroangiopathy, or large vessel disease, is known to occur in diabetics. Early histologic studies on coronary arteries revealed a thickening of the intima in diabetics (Lefkovits, 1937) which was confirmed in a
later study (Goodale et al., 1980). More recently, a reduction of the lumen of extramural coronary arteries of young juvenile-onset diabetics was shown (Crall and Roberts, 1975). Besides these changes, histochemical studies have revealed an accumulation of hyaline substances along with deposition of cholesterol crystals, calcium and increased amounts of glycoproteins (Ledet, 1968). Such a deposition, which may result in hardened arteries and a narrowed arterial lumen, superimposed on atherosclerosis could easily account for the angina and myocardial infarction associated with diabetes. Microangiopathy, or small vessel disease, is also known to occur in diabetics especially in the kidney and the eye. In the heart, a greater percentage of diabetics exhibit proliferative lesions of the smaller intramural coronary arteries as compared with nondiabetics (Blumenthal et al., 1960; Hamby et al., 1964). The lesions include endothelial cellular proliferation and deposition of PAS-positive material in the vessels (Bumenthal et al., 1960). Similar accumulations of PAS-positive material in small intramural coronary arteries have been reported in other studies (Crall and Roberts, 1978; Ledet, 1976). However, a number of studies were unable to demonstrate obstructive lesions of left ventricular intramural vessels (Roberts, 1972; Regan et al., 1975). Depression of cardiac function of diabetic patients was reported although the coronary arteriograms were normal (Ahmed et al., 1975). Similarly, other studies have suggested the presence of heart disease in spite of normal coronary arteries in diabetic patients (Hamby et al., 1974; Zoneraich et al., 1978; Okada et al., 1983). Hence, although large coronary artery disease may contribute to diabetes-induced cardiac disorders, small vessel involvement is yet to be proven. Whether or not diabetes and coronary artery disease are causally
related has recently been reviewed by Jarret (1984).

Some diabetic patients who do not show symptoms of cardiac disease exhibit changes in cardiac function. Elevation of left ventricular end diastolic pressure and a decrease in end diastolic and stroke volumes were seen. However, no change in the ejection fraction or in the rate of contractile element shortening was observed. These changes were not accompanied by increased wall thickness (Regan et al., 1981). However, accumulation of glycoproteins (Regan et al., 1981; Sohar et al., 1970) and collagen (Wu et al., 1977) has been observed in human diabetic hearts. Relatively severe perimysial fibrosis has also been reported in autopsied diabetic hearts (Okada et al., 1983). Since extracellular structures are major determinants of elasticity (Brady, 1968), perimysial fibrosis resulting from diabetes may reduce compliance, elevate end diastolic pressure and eventually induce heart failure. A number of degenerative changes are seen in the diabetic myocardium. These include small areas of infarction or necrosis, fresh infarcts and large scars (Pearce et al., 1983; Soler et al., 1975; Ingelfinger et al., 1976; Regan et al., 1975). Preclinical abnormalities of the diabetic state include a prolongation of the pre-ejection period (PEP), and shortening of the left ventricular ejection time (LVET) (Ahmed et al., 1975). An elevated PEP/LVET ratio which is commonly seen during heart failure (Ahmed et al., 1972) was also seen in diabetics although the elevation was not as severe as that seen in heart failure (Ahmed et al., 1975). Besides functional changes, abnormalities in rhythm were also seen (Riff and Riff, 1974). These changes were reflected in the ECG as changes in the T wave and ST segment (Burch, 1981).

Another common abnormality occurring as a result of diabetes is that
of autonomic neuropathy involving the cardiovascular system (Ewing et al., 1980; Clarke et al., 1979). Both the parasympathetic and the sympathetic systems are affected with the parasympathetic being affected first. Due to loss of parasympathetic control, an increase in resting heart rate was seen in diabetic patients (Ewing et al., 1981; Weiling et al., 1983). Furthermore, heart rate in diabetic patients does not respond as well as that in nondiabetics to maneuvers such as forced breathing and standing, suggesting a loss of sympathetic function (Weiling et al., 1983; Lloyd-Mostyn and Watkins, 1976). A high incidence of postural hypotension, a smaller increment in noradrenaline release in response to standing or tyramine and an exaggerated increase in blood pressure to exogenous norepinephrine were noted in diabetics (Tomeh et al., 1979). The postural hypotension may be due to sympathetic dysfunction, but other factors such as blunted renin and aldosterone responses (Bretta-Picoli et al., 1981) or exogenously administered insulin (Page and Watkins, 1976) may be involved. Although the presence of neuropathy in diabetic patients is well documented, its significance in the production of cardiac disease is not known. A possibility is that, due to an increased heart rate, inadequate left ventricular filling occurs. Combined with other factors, such as increased wall stiffness and an altered contractile apparatus (as discussed later), inadequate left ventricular filling superimposed on diseased vasculature may result in a reduced compliance.

Myocardial Alterations in Experimental Diabetes

In order to characterize cardiovascular problems associated with diabetes and their underlying causes, a number of studies have been carried out using diabetic models such as streptozotocin (STZ)- or
alloxan-treated rats or dogs. Animals so treated develop myocardial abnormalities which have now been extensively studied. Hemodynamic studies carried out in alloxan-treated dogs eleven months after induction of diabetes showed that increments in end diastolic and stroke volumes were smaller in diabetic hearts as compared to controls in response to increased afterloads (Regan et al., 1974). Similarly, in eighteen month alloxan-diabetic monkeys, increasing the preload resulted in increased stroke volume and end diastolic volume but this increase was significantly lower than in controls (Haider et al., 1981). In both studies, end diastolic pressure increased to a greater extent in diabetics than in controls suggesting increased wall stiffness in the diabetic hearts. The increased stiffness was suggested to be due to accumulation of PAS-positive material and collagen in the interstitium (Regan et al., 1974; Haider et al., 1981).

In a different model, the alloxan-diabetic rat, depression of cardiac function has been shown in the isolated working heart (Miller, 1979; Penpargkul et al., 1980; Vadlamudi et al., 1982). In these studies, various parameters of cardiac function such as left ventricular developed pressure, rates of contraction and relaxation, cardiac output and aortic output were recorded at increasing left atrial filling pressures. One study showed a depression three days after the induction of diabetes which was accompanied by depressed glucose uptake and lower ATP levels. The depression of function, ATP levels and of glucose uptake could be reversed either by increasing the glucose concentration (from 5mM to 10mM) or by including insulin in the buffer (Miller, 1979). Another study reported a depression of cardiac function thirty days after alloxan treatment (Vadlamudi et al., 1982). In the latter study no
depression was seen seven days after the induction of diabetes and the discrepance was attributed to a higher glucose concentration (10mM) used.

In a study by Penpargkul et al., (1980), eight week STZ-diabetic rats exhibited depressed cardiac function which was not accompanied by depressed coronary flow or by decreased myocardial oxygenation. It thus seems that at early time points in diabetes, depressed cardiac function may be the result of altered glucose transport which results in decreased ATP levels (Miller, 1979). A time course study on isolated perfused hearts revealed that the onset of depression of function in STZ-diabetic rat hearts was between thirty days and three months of diabetes, the precise time point of which was later determined to be around six weeks (Tahiliani et al., 1983). Furthermore, the depression did not seem to worsen with the progression of diabetes (Vadlamudi et al., 1982). It should be pointed out that the decreased response to increasing filling pressures is seen only at the higher filling pressures in both alloxan- and STZ-diabetic rats (Miller, 1979; Penpargkul et al., 1980; Vadlamudi et al., 1982) These results suggest that diabetic hearts have a lower cardiac reserve and, although capable of functioning like controls under normal conditions, may not be able to tolerate situations of high stress and increased venous return.

Mechanical studies on isolated papillary muscles from diabetic hearts revealed abnormalities in relaxation (Fein et al., 1980; 1981). While resting tension of these muscles was unaffected, the onset of relaxation was delayed, the rate of relaxation was slower and there was a delay in reaching the peak relaxation rate (Fein et al., 1980). However the peak tension developed and peak shortening were not affected by diabetes. It was suggested that altered relaxation might impair diastolic
filling of ventricles at physiological heart rates and eventually contribute to impaired function (Fein et al., 1980). However, it should be pointed out that STZ-diabetic rats exhibit significantly lower heart rates than do normal rats. It is not known whether ventricular filling is impaired in diabetic rats. It is possible that relaxation may only be a result of the altered biochemical changes occurring as a result of diabetes (as discussed later) and may not be responsible for the functional alterations seen. As in the studies on whole heart function, the alterations of papillary muscle mechanics induced by diabetes seem to occur about five weeks after the induction of STZ-diabetes. Further, they do not seem to worsen as the disease progresses. Myocardial contractile abnormalities induced by diabetes are also seen in rabbits. In a recent study, 10 week alloxan-diabetic rabbits exhibited a depression of left ventricular developed pressure and the rate of pressure development. Left ventricular end diastolic pressure was elevated in these animals as compared with controls (Bhimji et al., 1985).

Hearts from six to nine day diabetic rats have been shown to be more susceptible to ischemia as compared to controls (Hearse et al., 1975). The increased vulnerability could be abolished by including insulin (10 mU/mL) in the buffer. In other studies, while mild ischemia affected control and diabetic rat hearts to the same extent, severe ischemia caused damage in diabetic hearts more rapidly than it did in controls (Feuvray et al., 1979; Ingebretsen et al., 1980). Similarly, in the dog, ischemia was shown to reduce function to a greater extent in diabetic animals than in controls (Haider et al., 1977).

Rat myocardial ultrastructure, on the other hand, does not show signs of damage at the earlier time points in diabetes. Seven days and
six weeks after the induction of diabetes, electron microscopic analysis did not reveal significant damage (Renila and Akerblom, 1984; Jackson et al., 1985). However, significant damage was seen after three months of diabetes. Among the changes seen were an accumulation of lipids and glycogen accompanied by myelin formation. There was a loss of myofilaments and a loss of mitochondrial cristae. After six months of diabetes, the degree of damage was greater. In addition to the above mentioned changes myocytolysis and damage to the mitochondrial membrane were also seen (Jackson et al., 1985).

On the other hand, a number of alterations in various myocardial enzyme systems are seen as a result of diabetes. Amongst others, two of the cardiac enzyme systems studied during diabetes are myosin and actomyosin ATPases. A number of studies have shown a diabetes-induced depression of these enzymes in rat hearts (Dillmann, 1980; Malhotra et al., 1981; Garber et al., 1983). Similarly, myofibrillar ATPase from diabetic rat hearts is also depressed (Pierce and Dhalla, 1981). This depression was suggested to be due to altered myosin isoenzyme distribution. In nondiabetic control rats, myosin ATPase is present predominantly in the most active $V_1$ form (about 72% of the total) and about 13% in the $V_3$ form. The $V_3$ form is thought to be the slowest of the myosin isoenzymes with regard to ATP hydrolysis as well as cross-bridge formation and is seen in high concentrations in diabetic rat hearts while the $V_1$ content of these hearts is low (Dillmann, 1980). The mechanism by which diabetes induces a shift of myosin ATPase isoenzymes is thought to occur as a result of diabetes-induced hypothyroidism. Depression of myosin ATPase activity has also been demonstrated in the alloxan-diabetic rabbit (Bhimji et al., 1985). However, in normal
rabbits the $V_3$ form of myosin ATPase predominates, so the mechanism for
depression of myosin ATPase would be different from that seen in rats.
Various studies have demonstrated the presence of hypothyroidism in
diabetic patients and animals (Fein et al., 1980; Dillmann, 1980;
Pittman et al., 1979; Balsam et al., 1978). Thyroid hormones, in turn,
have a profound effect on the cardiovascular system. While thyroid
hormone administration in rabbits and guinea pigs results in increased
synthesis of cardiac myosin isoenzyme (Goodkind et al., 1977; Flink
et al., 1979), thyroidectomy in rats decreased activity of the enzyme
in the heart (Yazaki and Raben, 1975). Further evidence suggesting the
role of hypothyroidism in diabetes-induced depression of myosin ATPase
is provided by studies in which depression of the enzyme in diabetic rats
could be prevented by thyroid replacement therapy (Dillmann, 1982). Since
activity of myosin ATPase is known to correlate closely with contracti-
licity (Scheuer and Bhan, 1979; Barany, 1967), it can be hypothesized that
diabetes induces depression of myosin ATPase in the heart which then
leads to altered contractility. However, evidence discussed below
suggests that depression of myosin ATPase is not the only factor
responsible for diabetes-induced myocardial depression.

Another system in the heart which is affected by diabetes is the
sarcoplasmic reticulum (SR). Studies on the uptake of calcium into SR
have shown a depression of calcium transport in diabetic hearts
(Penpargkul et al., 1981; Lopaschuk et al., 1983). Since the amount
of calcium taken up by the SR is lower than normal, the quantity of
calcium available for release and thus the amount of calcium actually
released during subsequent beats may be altered and contractility may
thus be affected. Though this hypothesis is attractive, certain data from
our laboratory (described later) do not support the view that abnormal calcium uptake is entirely responsible for abnormal contractility. The depression of calcium uptake is accompanied by an elevation of long chain acyl carnitines (LCAC) in the SR (Lopaschuk et al., 1983). As mentioned in the previous sections of the text, myocardial LCAC levels are elevated during diabetes (Feuvray et al., 1979). LCAC have been shown to depress SR calcium uptake in vitro (Pitts et al., 1978) and may thus be responsible for the depression of calcium transport. Elevated levels of LCAC in rat myocardial SR can be lowered by carnitine treatment but the mechanism for this normalization is not clear. The suggestion that LCAC may be causally related to the depression of SR calcium uptake is supported by various studies in which an elevation of LCAC is seen to occur simultaneously with a depression of the SR. These studies are described in the sections that follow.

III. NORMALIZATION OF DIABETES-INDUCED MYOCARDIAL ALTERATIONS

Effects of Lowering Blood Glucose

By far the most successful treatment in preventing and reversing cardiac abnormalities associated with diabetes is that of insulin. Doses of insulin which have been used effectively are around 10U/kg/day. The effectiveness of insulin is not totally unexpected since insulin lack is the mainstay of diabetes. At a relatively early (six weeks) stage of diabetes, all the abnormalities studied were prevented when treatment was initiated as soon as diabetes was detected. The changes studied included hyperglycemia, weight loss, glycosylation of hemoglobin and depressed cardiac function (Tahiliani et al., 1983). Also, myocardial, mechanical and functional alterations and hypothyroidism have been reversed by four
weeks of insulin treatment when the treatment was started after the abnormalities had set in i.e. about six weeks after the induction of diabetes (Tahiliani et al., 1983; Garber and Neely, 1983; Fein et al. 1981). However, although these studies are a starting point, they do not resemble the clinical picture. Hence, it would be interesting and probably more relevant clinically if the effectiveness of chronic insulin treatment was studied in more chronic diabetic models. In one study, treatment was initiated in 5 month diabetic rats and continued for 4 weeks (Tahiliani et al., 1984). The results of this study indicated that the cardiac changes were only partially reversible using that particular treatment protocol. Whether the changes are irreversible at this stage of diabetes or whether insulin therapy utilized was insufficient has not been determined.

However, whether the beneficial effects of insulin therapy on the functional alterations in diabetic rat hearts are due to its direct myocardial effects or are secondary to its effects on metabolism is not known. As mentioned in the preceding section on the diabetes-induced myocardial alterations, myocardial impairment induced by acute alloxan diabetes has been suggested to arise as a result of impaired glucose uptake (Miller, 1979), since the impairment could be normalized by increased perfusate glucose concentrations as well as by inclusion of insulin in the buffer. Insulin perfusion has been shown to enhance myocardial contractility (Downing et al., 1977; Downing and Lee, 1979) but the mechanism is not clear (for review, see Farah and Alousi 1981). Also, not much is known about the effect of insulin perfusion on myocardial alterations induced by chronic diabetes. Chain et al., (1969) reported that insulin perfusion in the working heart enhanced glucose uptake and
conversion of glucose to CO₂ in control hearts whereas in 7 day diabetic hearts, only the uptake of glucose was stimulated while CO₂ production was 50% less than that in control hearts. Fein et al., (1981) were unable to demonstrate any effect of insulin in vitro on control or diabetic papillary muscle mechanics. Similarly, Penpargkul et al., (1980) found that insulin did not reverse the abnormalities in ventricular function induced by chronic diabetes.

The effectiveness of insulin treatment has also been studied in preventing or reversing various diabetes - induced biochemical parameters. The change in myosin ATPase activity and the shift in isoenzyme composition was reversed by four weeks of insulin treatment (Dillmann, 1980). Similar results were obtained in a different study in which 6-10 week diabetic rats were treated with insulin for periods between 2 and 28 days. While the animals treated for 2 or 6 days exhibited actomyosin and myosin ATPase activities similar to those in untreated diabetics and those treated for 10 days showed only trends towards recovery, rats treated for 28 days had values similar to controls (Fein et al., 1981). Reversibility of diminished myosin ATPase activities has also been reported by Garber et al. (1983). In a series of recent reports, responsiveness to different doses of insulin was studied (Fein et al., 1984; Rubinstein et al., 1984). An inverse correlation between the blood glucose concentrations and either actomyosin ATPase activities, myosin isoenzyme composition, myocardial function or myocardial mechanics was found. These results suggest that although the abnormalities induced by diabetes may be independent of the duration of diabetes (Fein et al., 1981; Vadlamudi et al., 1982), they depend on the severity of the disease.
The ability of insulin to prevent and reverse the SR calcium uptake abnormalities in the heart has also been studied. Ganguly et al. (1983) showed that the depression of SR calcium uptake could be reversed by 2 weeks of insulin treatment. Similarly, studies from our laboratory have shown that the defect can be prevented and reversed by 4 weeks of insulin treatment (Lopaschuk et al., 1984). As mentioned previously, the defect in SR calcium uptake activity may be due to the elevation of LCAC in the SR. The levels of the latter have also been shown to be normalized by insulin treatment (Lopaschuk et al., 1984). Another factor that may be responsible for the SR depression observed in diabetes is the microenvironment surrounding the Ca$^{2+}$-stimulated ATPase protein. The altered cholesterol / phospholipid ratio which has been used as a crude gauge of membrane fluidity and could affect the hydrophilic environment (Cooper, 1977) is altered in diabetes and, moreover, can be reversed by insulin treatment (Ganguly et al., 1983).

Besides the biochemical and functional parameters, insulin therapy is also effective in preventing the diabetes-induced ultrastructural alterations in the myocardium (Reinla and Akerblom, 1984; Baandrup, 1980). In one study, insulin treatment was carried out for 9 months starting from the second day of induction of the disease and the diabetes-induced increase in the amount of myocardial connective tissue could be prevented (Baandrup, 1980). In another study, morphological damage characterized by enlarged mitochondria and dilated sarcoplasmic reticular tubules was evident 7 days after the induction of diabetes. This defect could be completely normalized by insulin treatment (Reinla and Akerblom, 1984).

In contrast to the beneficial effects of insulin, chronic tolbut-
amide therapy in alloxan-diabetic dogs did not normalize the effects of diabetes. Instead, further reduction of left ventricular function and a worsening of myocardial morphology was seen despite improved glucose tolerance (Wu et al., 1977). These effects are probably drug-related as hearts from control animals treated with the drug were also adversely affected (Wu et al., 1977).

Clinically, a major problem with insulin treatment is the hypoglycemia that can be associated with it as well as the lack of adequate control in some diabetics. Moreover, the myocardial abnormalities seen in various clinical studies exist despite insulin treatment. Experimentally, a disadvantage with using insulin as a tool to investigate mechanisms of the cardiomyopathy is that it is nonspecific since it reverses most known problems associated with diabetes or prevents them from occurring, at least in the more acute stages of the disease. It is thus desirable to have treatments which selectively affect only certain aspects of diabetes. By doing so, it would be possible to correct these problems individually and estimate the significance of each in causing myocardial alterations. These treatments are described in the following sections.

One of the most impressive of such treatments has been that of vanadate treatment. This treatment was selected as vanadate had been reported to have an insulin-like effect (Degani et al., 1981; Tamura et al., 1983; Scheter et al., 1980) and moreover, vanadate has also been shown to have positive inotropic effects (Schwartz et al., 1980; Scholz et al., 1980). In a study from our laboratory, diabetic rats administered vanadate did not exhibit any glycosuria and on sacrifice, had blood glucose levels not different from controls (Heyliger et al., 1985). The mechanism of the blood glucose-lowering effect of vanadate is as yet
speculative but the net result is an insulin like effect. Recent studies have shown that vanadate induces phosphorylation of the insulin receptor which is mediated via stimulation of tyrosine kinase (Tamura et al., 1984). Cardiac function of diabetic rats treated with vanadate was not different from that of controls. This finding confirms the previous observation of a correlation between the severity of diabetes and the myocardial alterations (Tahiliani et al., 1983; Fein et al., 1981). Biochemical studies are currently in progress in our laboratory and no data are available as yet. In view of the outstanding success of vanadate in controlling all of the symptoms and abnormalities of diabetes studied (Heyliger et al., 1985), normalization of the biochemical parameters would not be unexpected. Thus, although the discovery of vanadate as a hypoglycemic agent does not resolve the problem of the mechanism by which diabetes induces myocardial abnormalities, the finding is obviously significant.

Other treatments

As mentioned above, one of the features of diabetes is the presence of a hypothyroid state. Since hypothyroidism is known to affect myocardial myosin ATPase, Dillmann (1982) attempted to normalize the altered myosin ATPase with T3 treatment. While a lower dose (3μg/kg/day) did not affect the isoenzyme composition, a larger pharmacological dose (30μg/kg/day) normalized the myosin ATPase. In that study, the thyroid hormone levels of diabetic animals treated with T3 were not reported and so the effectiveness of the lower dosage protocol in correcting the thyroid deficiency in diabetic animals is not known. The decrease in heart rate occurring as a result of diabetes is also correctable by thyroid hormone treatment (Garber et al., 1983) but myocardial
function is depressed despite the treatment (Tahiliani and McNeill, 1984). The suggestion that diabetes may induce a decrease in T₃ binding in the heart (Garber et al., 1983) is possible, but a more likely explanation for the inability of thyroid hormone to correct the diabetes-induced functional alterations could be the fact that systems besides myosin ATPase which are known to regulate cardiac contractility (such as the ability of SR to take up calcium) are altered by diabetes and not corrected by T₃ treatment (Ganguly et al., 1983; Tahiliani and McNeill, 1984).

Various treatments have also been tried in attempts to correct the abnormality of calcium uptake in the SR. As mentioned above, the depression seems to be a result of elevated levels of LCAC in the SR. There are two lines of evidence which support this view. First, the two events occur simultaneously under various conditions such as diabetes or euthyroid diabetes (Tahiliani and McNeill, 1984). Insulin treatment which prevents and reverses the elevation of LCAC also prevents and reverses the depression of SR calcium uptake in diabetic rats (Lopaschuk et al., 1984). Second, in vitro studies have shown that palmityl carnitine is a potent inhibitor of calcium transport in the SR (Adam et al., 1978). Thus, attempts to correct the calcium uptake have been made by correcting the elevation of LCAC in the SR. One of the treatments tried was the use of carnitine. Although the mechanism for the carnitine-induced lowering of cardiac LCAC is not understood, a definite lowering is obtained in rats (Lopaschuk et al., 1984). In keeping with the hypothesis that LCAC are responsible for the depression of cardiac SR, carnitine treatment also reversed the SR depression. However, the myocardial functional depression was not affected by carnitine treatment (Lopaschuk et al.,
1984). The study suggested that, like the depression of myosin ATPase, depressed SR calcium uptake is not the only factor contributing towards altered function in diabetes.

IV. THE STREPTOZOTOCIN-DIABETIC RAT MODEL

Diabetes can be induced experimentally in animals using either streptozotocin (STZ) (Rakieten et al., 1963) or alloxan (Dunn and McLetchie, 1943). The diabetic syndrome produced by either drug is similar but the mechanisms by which they produce diabetes are not as well understood. There appears to be some debate as to which kind of human diabetes is mimicked by the syndrome produced by either alloxan or STZ. On the one hand, both definitely cause beta cell damage which would suggest that the diabetes is of the Type I form. However, insulin levels are reduced by only 50% as compared with controls and STZ-treated animals can survive without administration of exogenous insulin suggesting that the condition is not insulin-dependent. Recently, Schaffer et al., (1985) have developed a model of NIDDM by injecting 3 day old rats with STZ. These rats had marked glucose intolerance, as determined by intraperitoneal glucose tolerance tests, but fasting and non fasting plasma glucose levels were near normal up to 12 months after the injection.

Alloxan-induced diabetes is preceded by a characteristic triphasic response in blood glucose levels (Lundquist and Rerup, 1967). A similar response to STZ has also been reported (Junod et al., 1969). The variations in blood glucose with alloxan are as follows:

1. An initial short lasting hyperglycemia, which is probably due to increased glycogenolysis in the liver,
2. A transient hypoglycemia which is probably due to availability of large amounts of insulin released by beta cell damage; this phase lasts
about 48 hours, and

3. A permanent hyperglycemia.

With respect to the mechanisms of diabetogenicity, only hypotheses are available. Alloxan is taken up by pancreatic cells and may be reduced to dialuric acid. These two compounds then form an oxidation-reduction cycle, the net result of which is generation of highly reactive oxygen-containing free radicals which could then damage the beta cells (MaLaissie, 1982). STZ, on the other hand, which has a glucose moiety (2-deoxy D-glucose) coupled to N-nitrosomethylurea may be taken up via the glucose transport system in the pancreas, which would explain its accumulation in the pancreatic islets (Johanson and Tjalve, 1978). How it produces its diabetogenic effect beyond this step is not known. A recent study has suggested the involvement of immune responses, at least in the production of multiple dose STZ (40 mg/day for 5 days)-induced diabetes (Kim and Stienberg, 1984). The evidence suggesting this conclusion was that diabetes produced using this dosage protocol could not be produced in T-cell-deficient mice and secondly, low dose-STZ-pretreated mice were rendered diabetic by the transfer of spleen cells from STZ diabetic mice. It is thus possible that STZ may modify the beta cell and thus trigger an autoimmune response which leads to their destruction. If this theory is correct, then it would add additional weight to the suggestion that STZ-induced diabetes is Type I as an autoimmune component is thought to be of importance in Type I diabetes. However, it should be noted that while the study by Kim and Steinberg (1984) may explain the diabetogenic effects of multiple dosing, acute injections of larger amounts of STZ can also produce diabetes in a much shorter time span in which an autoimmune response likely would not occur.
PURPOSE OF THE PRESENT INVESTIGATION

Although insulin treatment in vivo has been shown to have beneficial effects on diabetes-induced myocardial dysfunction, it is not known whether these beneficial effects of insulin therapy are due to its direct myocardial effects or are secondary to its effects on metabolism. As mentioned in the preceding section on the diabetes-induced myocardial alterations, myocardial impairment induced by acute alloxan diabetes has been suggested to arise as a result of impaired glucose uptake (Miller, 1979) since the impairment could be normalized by an increase in perfusate glucose concentrations as well as by the inclusion of insulin in the buffer. However, no such impairment was seen in the study by Vadlamudi et al., (1982) until 30 days after the induction of diabetes with alloxan.

Hence, the present investigation was undertaken to re-evaluate the effects of 3 days of diabetes on myocardial performance and the effects of in vitro insulin perfusion on the cardiac function of acutely STZ-diabetic rats. We were also interested in the effects of increasing perfusate glucose concentrations on myocardial function in diabetic and control animals. We were also interested in determining whether the beneficial effects of insulin treatment on diabetes induced myocardial abnormalities were due to the direct myocardial effects of insulin or were due to the secondary effects of insulin in chronic (6 week) diabetics. The rationale for studying the effects of Humulin® (which is glucagon-free) was to eliminate the possibility that the effects of insulin we were observing were due to glucagon as the latter is known to be present in
In the other part of the present investigation, we have attempted to normalise the diabetes-induced myocardial functional alterations by using a number of interventions with different proposed mechanisms of action. The first one used was methyl palmoxirate (also known as methyl 2-tetradecylglycidate), a compound synthesized by McNeil Laboratories in a search for newer hypoglycemic agents. It is a fatty acid analog and the structure is shown in figure 3. The drug has been reported to have a blood glucose-lowering effect which is most pronounced in animals which depend on fatty acids as their source for metabolic fuel such as diabetic and/or starved animals (Tutwiler et al., 1979). The mechanism of action of methyl palmoxirate is suggested to be via inhibition of fatty acid metabolism which is brought about by inhibition of carnitine acyl transferase, the latter being responsible for the formation of fatty acyl carnitines and thus transfer of fatty acids from the cytosol to the mitochondria (see figure 2). In this way, the utilisation of glucose is increased and blood glucose is lowered, an observation which is consistent with Randle's glucose-fatty acid cycle (Randle et al., 1966). The compound was thus interesting to us for two reasons. First, it decreased the formation of LCAC and thus would be expected to normalise the depression of calcium uptake in the SR. Second, it was shown to control hyperglycemia in diabetic animals and so could be used as a tool for studying the contribution of elevated glucose levels to diabetes - induced myocardial alterations.

The results of experiments with methyl palmoxirate proved to be partially successful, in that the drug prevented the elevation of LCAC and the resulting depression of calcium uptake in the SR, but it was
unable to control either the hyperglycemia or the functional depression induced by diabetes. Hence, we tested a combination therapy of methyl palmoxirate with $T_3$ treatment in STZ-diabetic rats, as the latter has been shown to prevent the depression of myosin ATPase in the hearts of diabetic rats (Dillmann, 1982; Garber et al., 1983). Since the treatment successfully prevented the functional alterations, thereby suggesting that the functional alteration was due to a combination of depressed myocardial SR calcium uptake and depressed myocardial myosin ATPase, we carried out treatment with another drug combination which would be expected to have similar results. This was the rationale for using a combination of $T_3$ and carnitine, as the latter also prevents the elevation of LCAC in the myocardium and prevents the depression of myocardial SR calcium uptake (Lopaschuk et al., 1984).
MATERIALS AND METHODS

A) MATERIALS

I Radioisotopes :

\[ ^{45}\text{CaCl}_2 (10\mu\text{Ci/mole}) \text{ and } (1-^{14}\text{C}) \text{ acetyl CoA} \]

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 X 8-400 - 1 Dowex anion exchange resin
- Bovine Serum Albumin
- L-histidine free base
- EGTA, EDTA
- magnesium chloride
- potassium chloride
- sodium chloride
- sodium azide
- sucrose
- Trizma base
- Trizma HCl
- Trizma maleate
- D,L-carnitine
- L-carnitine
- acetyl coenzyme A
- copper sulfate
- deoxyccholate
- 3,5,3'-triiodothyronine
- citric acid
- trisodium salt of citric acid
- Calcium chloride dihydrate
- Trizma oxalate
- D-glucose
- sodium bicarbonate
- Sodium tetrathionate
- Aquasol®
- Regular insulin (100U/mL)
- Streptozotocin
- Methyl palmoxirate

were purchased from Avalar Chemicals.

Sodium tetrathionate was purchased from Pierce Chemical Co.

Aquasol® was purchased from New England Nuclear.

Regular insulin (100U/mL) was purchased from Connaught Laboratories and Humulin® from Eli Lilly and Co.

Streptozotocin was generously donated by Upjohn Pharmaceuticals.

Methyl palmoxirate was a generous gift from McNeil Laboratories.
Assay kits:
Assay kits for the following substances were purchased from Boehringer Mannheim Canada: glucose, triglycerides, cholesterol, and total lipids. Insulin assay kits were purchased from Amersham. T₃ and T₄ assay kits were purchased from Abbott Laboratories.

Animals:
Female Wistar rats (Canadian Breeding Farms, Montreal) weighing between 150 and 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) (55mg/kg) dissolved in 0.1M citrate buffer. The buffer was made by mixing 0.1M citric acid and 0.1M sodium citrate until a pH of 4.5 was obtained. Rats were anaesthetized lightly with diethyl ether and their tail veins dilated by inserting into warm water. Control rats were injected with the buffer alone. The onset of diabetes was assessed by testing for urine glucose using TesTape® (Lilly). When animals were to be used three days after the induction of diabetes, they were sacrificed about 72 hours after the injection of STZ.
   b) Treatment protocols:
      GENERAL: Various treatments have been used in the course of the study. In general, after diabetes was ascertained as mentioned above, the animals were divided into equal groups. This was usually done three days after they were injected with STZ. One subgroup in each group received the treatment with the drug while the other subgroup served as a control.
Animals were weighed daily at the time of drug or vehicle administration and where a change in glucose levels was expected, the urine glucose was monitored daily. Drug administration was usually done around 3 p.m. and efforts were made to keep this time constant throughout the study.

i) Methyl palmoxirate study: Three days after injection of STZ, both controls and diabetics were randomly divided into two subgroups. While one subgroup from each received 1 mL/kg of 1.5% tragacanth suspension via an intragastric tube, the other subgroup of each received 25 mg/kg methyl palmoxirate p.o. as a suspension in 1.5% tragacanth. Daily treatment with the drug was continued for six weeks at which point the animals were sacrificed.

ii) T₃ and methyl palmoxirate treatment: Three days after STZ treatment, controls and diabetic animals were divided into four subgroups each. One subgroup from each received 1 mL/kg of a 1.5% tragacanth suspension p.o. and 1mL/kg alkaline saline s.c. daily. These groups served as the control groups. Another set of subgroups (one control and one diabetic) received 30 μg/kg T₃ dissolved in alkaline saline s.c. daily for six weeks. The third set of subgroups received a daily dose of methyl palmoxirate (25 mg/kg in 1.5% tragacanth p.o.) for six weeks while the last set received both T₃ and methyl palmoxirate in the above mentioned doses daily also for six weeks.

After completion of the treatment or when the desired time point was achieved, the rats were sacrificed by decapitation and blood was collected in heparinized tubes. Hearts were excised immediately and used for either perfusion, as described below for function studies, or for the preparation of sarcoplasmic reticulum (SR). Blood was centrifuged at 1500 rpm for 10 min. and the plasma separated and frozen at -20° C. At a later
time (usually within 3 weeks) it was thawed and used for estimation of insulin, glucose, total lipids, triglycerides, cholesterol, T₃ and T₄ levels.

II. Isolated perfused working heart preparation

The method used for perfusing hearts is a modification of Neely's working heart preparation as described by Rodgers et al (1981), a schematic of which is shown in figure 4. The perfusion fluid used was that described by Chenoweth and Koelle (1946) and contained the following (in mM): NaCl, 120; KCl, 5.6, MgCl₂, 2.1; CaCl₂, 2.18 and NaHCO₃, 19. Glucose concentrations varying between 5 and 20mM were used for part of the study. When not specified, the concentration of glucose used was 10mM. Regular insulin or Humulin® (5mU/mL) were used in part of the study. Each heart was perfused either in the presence or absence of insulin. The buffer was constantly oxygenated with 95% oxygen and 5% carbon dioxide and the temperature maintained with the aid of water jackets 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) via a 20 gauge needle and to another 20 gauge needle on the other side. Left ventricular developed pressure (LVDP) was measured by inserting the latter needle through the apex of the heart into the left ventricle. Intraaortic pressure was measured by connecting another Statham transducer to the side arm of the aortic outflow system. The hearts were subjected to a 75 cm water column afterload and the ejection from the heart was buffered by 2-3 mL of air in the aortic outflow system. LVDP, intraaortic pressure, first derivative of the LVDP and the heart rate were recorded on a Grass Model 79D polygraph. The hearts were stimulated
Figure 4: A schematic representation of the working heart apparatus. Rat hearts are first perfused in the retrograde mode by way of the aorta with buffer from the jacketed reservoir A. The heart is then perfused by way of the left atrium with buffer from reservoir B. Constant level fluid reservoirs C and D are used for maintaining constant perfusion pressure which can be changed by raising or lowering the reservoirs. The heart pumps against an afterload of 75 cm H₂O (resistance unit is shown as E above). F is the capacitance unit containing 2-3 ml of air and is used to provide elasticity to the system. Transducers G and H record the pressures in the ventricle and the aortic outflow system respectively. Platinum electrodes are placed on the left atrium to pace the heart at 300 beats per minute.
by means of platinum electrodes 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 the method of Harris et al. (1983) using an Apple II Plus™ computer with a Mountain hardware board. The pressure transducer signal from the polygraph was sampled at 667 Hz over a 1.5 second duration at each point where the 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, areas under the curves, times for contraction, relaxation and the total time of the pulse. 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 10 minutes at a left atrial filling pressure of 15 cm H₂O. Studies were performed by varying the height of the left atrial filling reservoir (and thus varying left atrial filling pressure) from 5 to 22.5 cm H₂O in 2.5 cm steps. The height was first lowered from 15 cm to 5 cm; increased to 22.5 cm and finally lowered to 15 cm, data being recorded at each point after it had stabilized (usually between 45 secs and 1 min). A complete function curve took about 20 - 30 mins to perform, the total perfusion time was about 45 mins. At the end of the functional measurements, the hearts were usually frozen with the aid of Wollenberger clamps precooled in liquid nitrogen, labelled and stored at -80° C for further analysis.

III. Preparation of microsomes enriched in Sarcoplasmic Reticulum

This preparation was made by a modification of the method of Sumida et al. (1978). Hearts were excised and placed in cold 10mM Tris
maleate buffer (pH6.8). The ventricles were separated, weighed and homogenized in about 15mL of 10mM Tris maleate with a teflon pestle for 15 sec at 1500 rpm. The resulting homogenate was placed in a Korex™ tube and centrifuged at 4,000 times g for 10 min. The supernatant was passed through four layers of cheese cloth and centrifuged at 9,000 times g for 20 min. The supernatant was passed through cheese cloth again and centrifuged at 40,000 times g for 100 min. The resulting pellet was suspended in a 10mM Tris maleate buffer containing 0.6M KCl and centrifuged at 40,000 times g for 100 min. The supernatant was discarded and the pellet washed gently with 10 mM Tris maleate buffer. It was then resuspended in 10 mM Tris maleate containing 40% sucrose, quick frozen in acetone on dry ice and stored at -70° C until further use.

IV. Measurement of ATP dependent Ca\(^{2+}\) uptake in SR

ATP-dependent Ca\(^{2+}\) uptake in microsomes enriched in SR was measured by the method of Tada et al., (1974) with a few modifications. The incubation medium used contained 40 mM histidine hydrochloride (pH 6.8), 5 mM MgCl\(_2\), 110 mM KCl, 5 mM Tris-ATP, 2.5 mM Tris-oxalate and varying concentrations of CaCl\(_2\) containing \({^{45}}\)CaCl\(_2\). The desired concentration of free Ca\(^{2+}\) was obtained by the addition of ethylene glycol bis(β-aminoethyl ether)-N,N' - tetraacetate (EGTA), and the free calcium concentration was determined by the equations of Katz et al., (1970). Each incubation tube contained about 20-50 μg of microsomal sarcoplasmic reticular protein. Samples were preincubated for 11 min at 30°C and the reaction was started by the addition of CaCl\(_2\). The incubation was carried out for 5 min after which period the reaction was terminated by filtering an aliquot of the incubation mixture through a
millipore filter (pore size 0.45 μ). The filter was then washed with 20 mL of 40 mM Tris-Cl (pH 7.2), dried and counted for radioactivity in Aquasol® using standard scintillation counting techniques.

ASSAY PROCEDURES

V. Determination of long chain acylcarnitine levels

For estimation of long chain acylcarnitines (LCAC) in the SR, an aliquot of the microsomal suspension containing about 0.5 mg of protein was diluted to 4 mL with 10 mM Tris maleate and centrifuged at 40,000 times g for 45 min. The pellet was resuspended in 0.5 mL of cold 6% perchloric acid. The suspension was centrifuged at 12,000 times g for 10 min. 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, 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 0.4 N KOH at 70° C for 1 hour. Following incubation, 0.575 N HCl (200 μL) was added to neutralize the base.

The actual determination of carnitine was done according to a radioisotopic method developed by McGarry and Foster (1976). A medium containing 120 μM Tris chloride (7.3), 2 μM sodium tetrathionate and 25nmoles (0.025 μCi) of 1-14 C acetyl coenzyme A was prepared and 1.05 mL added to each of the supernatant 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 acetyl transferase. The reaction mixture was incubated at room temperature for 30 min. To this was added 0.3 mL of Dowex 1 X 8-400 anion exchange resin (200-400 mesh in chloride form) suspension (containing 0.08 mL of the resin in 0.22 mL of water) and the sample was placed on ice. The sample
was vortexed twice at 10 min intervals and centrifuged at 3000 times g for 5 min. To a 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 LCAC were expressed as nmoles/mg SR protein.

VI. Protein assay

To a 20 μL aliquot of the microsomal sample, 1 mL distilled water was added. Ten μL of 2% deoxycholate solution were added to the suspension and after 10 min of incubation at room temperature, 0.5 mL 2% trichloroacetic acid were added to precipitate the proteins. The tubes were centrifuged at 3000 times g for 30 min and the precipitated protein was assayed using Lowry's method (Lowry et al., 1951). Bovine serum albumin was used as a standard.

VII. Insulin assay

Plasma immunoreactive insulin was determined by the radioimmunoassay method of Herbert et al., (1965). Reagents for the assay were obtained from Amersham. The insulin assay reagents consisted of human insulin standards and [¹²⁵I] 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.

VIII. Glucose, triglycerides, cholesterol and total lipids

These were estimated using the respective kits. All analyses were done enzymatically followed by a colorimetric determination.

IX. T₃

Thyroid status of the rats was determined by measuring the T₃B index of the serum using a Triobead® - 125 kit. The assay is based on
the principle of radioactivity partitioning between serum proteins which are capable of binding T₃ and charcoal beads which are provided as part of the kit. When the levels of serum T₃ are high, the saturation of serum proteins is high and thus the binding of labeled T₃ to these proteins is low. As a result, the amount of label available for binding to the beads is high. On the other hand, when serum T₃ is low, label bound to the beads is low. The assay procedure involves incubation of beads with the serum and labeled T₃, separation of the beads from the serum and finally estimation of the label bound to the beads by counting in a gamma counter. As mentioned above, this procedure yields a percent of the T₃ uptake value for a reference control provided with the kit and not the actual T₃ content. The assay kits were obtained from Abbott Laboratories.

X. T₄

T₄ was determined by a radioimmunoassay procedure. The antibody to T₄ was coated on glass beads. The procedure consisted of incubating the beads with serum and labeled T₄, separating the beads and estimating the radioactivity of the beads. The kits, marketed as Tetrabead® - 125 was obtained from Abbott laboratories.

XI. Phosphorylase a assay

Phosphorylase a activity was measured in the direction of glycogen synthesis according to a modification of the method of Cori and Cori (1940) as described by McNeill and Brody (1966). The assay was done in the absence of 5' - AMP and involved an enzymatic reaction catalyzed by phosphorylase in which glucose-1- phosphate was converted to glycogen with the release of inorganic phosphate. The inorganic phosphate was determined by the method of Fiske and Subbarow (1928). Phosphorylase a
activity was expressed as a percent of total phosphorylase activity. The latter was estimated in the presence of 5' - AMP and was expressed as umoles of inorganic phosphate released /mg tissue/min.

XII. 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 or two way analysis of variance (ANOVA) followed by Newman-Keuls test were used for comparing results depending on the number of comparisons. A probability of less than 0.05 (p<0.05) was used as the level of significance.
RESULTS

GENERAL FEATURES OF ANIMALS USED IN THE STUDIES

Rats treated with streptozotocin (STZ) exhibited signs and symptoms that are characteristic of the diabetic state. They appeared emaciated 6 weeks after induction of diabetes and this was reflected in their lower body weights as compared to age-matched controls that had been injected with the buffer alone. Although not quantitatively measured, their food and water intake was greater than controls as was their faecal and urine output. These features were not apparent in rats 3 days after they had been injected with STZ. However, unlike control rats, these rats did not gain any weight following the injection of STZ.

Urine glucose, which was qualitatively assessed using Tes Tape® on a scale of 1+ (0.1%) to 4+(2.0%), was between 3+ and 4+ for most of the rats 3 days after they had been injected with STZ. The rats that showed 1+ glycosuria were not used for the study. The rats that showed 2+ were used in the study, but their experimental values were included only if their blood glucose was greater than 350 mg%. The plasma glucose levels of diabetic animals was always elevated 3-4 fold as compared with controls. This was seen in both 6 week as well as 3 day diabetic rats. On the other hand, plasma insulin levels in diabetic animals were found to be about 50% of those of control rats. The overall mortality in all the studies was low. While no mortality occurred in the control groups (treated or untreated) at any time, some mortality did occur in the
diabetic groups. The number of deaths is presented in the respective sections below.

Table 2 summarizes the general features of animals used in the insulin perfusion study. These rats were used about 72 hours after they were injected with STZ. As mentioned above, their body weights were not significantly lower than those of the control animals, but they did not appear to gain any weight in contrast to the control rats. The blood glucose levels were significantly elevated and the plasma insulin levels were significantly lower than those in the control groups.

Animals sacrificed in the 6 week study exhibited the characteristics of diabetes to a greater extent. Their body weights at the time of sacrifice were significantly depressed as compared with controls. At the time of sacrifice, plasma glucose levels were significantly elevated and the insulin levels significantly depressed. Out of a total of 41 diabetic rats used in the study, only 3 rats died, the deaths occurring about 4 weeks after treatment with STZ. These results are summarized in Table 3.

In the next study, (Table 4), control and diabetic rats were treated with methyl palmoxirate. Again, the untreated diabetic rats weighed significantly less than rats in the control groups. While methyl palmoxirate treatment did not affect the body weights of control rats, diabetic rats treated with the drug weighed slightly but not significantly more than untreated diabetics. When the hyperglycemic state of the rats was monitored 3 days after the injection with STZ, all the rats were glycosuric (between 3+ and 4+ on the Tes Tape®). Treatment was then started with methyl palmoxirate and the glycosuria was abolished for the first 3 days with daily treatment. However, the effect was not seen after that time. At the time of sacrifice, these animals exhibited severe
hyperglycemia and, although the plasma glucose levels in the treated group were slightly lower than those of the untreated diabetic rats, they were not significantly different. Plasma glucose levels in control rats were not affected by methyl palmitoate treatment. The insulin levels in both the diabetic groups were decreased to about 35-40% those of control rats. Plasma triglycerides were elevated in diabetic animals and the diabetic treated rats exhibited only slightly lower plasma triglycerides. Similarly, the total lipids were elevated in the diabetic animals and were not affected by the treatment.

During the course of treatment we made two observations that deserve mention. First, of the 8 diabetic and 7 control animals being treated with methyl palmitoate, 2 in each group developed necrosis on the scruff of the neck which started about 2 weeks after the initiation of treatment and spontaneously disappeared by about 5 weeks. Second, on sacrifice, various tissues of the animals treated with the drug (control and diabetic) exhibited a yellow discoloration. The tissues included the kidney, liver and intestine.

Results similar to those described above were also seen in the next study where animals were treated with both methyl palmitoate and T₃. Diabetic rats (untreated and treated) weighed significantly less than animals in the control groups. The diabetic animals treated with T₃ weighed slightly less than untreated diabetics or those treated with methyl palmitoate. Plasma glucose levels were elevated and the insulin levels significantly lower in all diabetic groups as compared with controls. Plasma lipids were elevated in diabetics and remained elevated despite the treatments. The T₃B index of diabetic animals was depressed in untreated diabetic rats. This depression was normalised in diabetics
treated with T3. Controls treated with T3, on the other hand, had higher T3B indices than untreated controls. Untreated diabetics also exhibited lower plasma T4 levels than controls while diabetics treated with methyl palmoxirate had normal T4 levels. All the rats treated with T3 had significantly lower T4 levels than those which did not receive T3. Of the 8 diabetic rats receiving the combination treatment, 2 developed necrosis and 2 died during the course of the study. The general features of animals used in this study are shown in Table 5.

Finally, in the last study carried out, controls and diabetics were treated with T3 and carnitine. The general features of these animals are described in Table 6. In this set of experiments, as in the previous experiments, the untreated and treated diabetic rats weighed significantly less than the control groups. Rats in both the diabetic groups were hyperglycemic and hypoinsulinemic. While the untreated diabetic rats were hypothyroid, the T3 treated diabetics were euthyroid and the treated controls had T3 indices which were greater than those of controls.

**EFFECT OF INSULIN PERFUSION ON PERFORMANCE OF 3 DAY DIABETIC RAT HEARTS**

Cardiac performance was studied by determining the changes in left ventricular developed pressure and the rates of pressure development and decline in response to the changes in left atrial filling pressures. When the maximum LVDP of hearts from 3 day diabetic rats was compared with that in control rat hearts in the presence of 5 mM glucose no significant depression was seen (Fig. 5). Similarly, the maximum positive dP/dt and maximum negative dP/dt were also not depressed at any filling pressure (Figs. 6, 7). When insulin (5 mU/mL regular insulin) was included in the perfusion medium, control hearts developed greater ventricular pressures
as compared to control hearts perfused without insulin (Fig. 5). This increase was statistically significant at filling pressures greater than 15cm H₂O. Similarly, the rates of pressure development and decline were also greater (Figs. 6, 7). On the other hand, diabetic rat hearts did not appear to be affected by perfusion with insulin (Figs. 5, 6, 7).

When the glucose concentration was increased to 10 mM, no enhancement of function was seen in either control or diabetic hearts (Fig. 8). Again, as in the study with 5 mM glucose, insulin (5 mU/mL) enhanced the function of control hearts at higher left atrial filling pressures while that of diabetic rat hearts was unaffected (Figures 9, 10, 11).

**EFFECT OF INSULIN PERFUSION ON CARDIAC FUNCTION OF 6 WEEK DIABETIC RATS**

Having observed a loss of sensitivity to insulin in vitro in diabetic rat hearts three days after they were injected with STZ, we studied the effects of insulin perfusion and increasing glucose concentrations on 6 week diabetic rat heart function. Increasing the concentration of glucose from 5mM to 20mM did not cause any significant change in the cardiac performance of either control (Fig. 12) or diabetic (Fig. 13) rat hearts. When the function of control rat hearts was compared with that of diabetic rat hearts at a glucose concentration of 5mM, it was noted that the diabetic rat hearts did not develop ventricular pressures of the same magnitude as the control rat hearts especially at the higher filling pressures (Fig. 14). Similarly, both positive and negative dP/dt were also lower in hearts from diabetic animals at the higher filling pressures (Figs. 15, 16). When perfused with regular insulin (5 mU/mL), all three parameters of function of control rat hearts were enhanced significantly at the higher filling pressures (Figs. 14, 15, 16). Diabetic
rat hearts, on the other hand, did not respond to insulin (Figs. 14, 15, 16). These experiments were carried out using 5 mM glucose. When the experiments were repeated in the presence of 10 mM glucose in an effort to increase the availability of substrate, identical results were obtained (Figs. 17, 18, 19). Phosphorylase a levels which were determined in myocardial tissues of both control and diabetic animals are shown in Figure 20. The levels of phosphorylase a were elevated in diabetic myocardium as compared with those of control. Insulin perfusion did not seem to affect the levels in either group of animals.

As regular insulin may be contaminated with glucagon due to its source, the study was repeated with human insulin (Humulin®) (5 mU/mL) which is glucagon-free. These experiments were carried out using 10 mM glucose as substrate and the results are shown in Figures 21, 22 and 23. The results obtained were similar to those found using commercial animal insulin in that the insulin stimulated the contractility of only control rat hearts and not that of diabetic rat hearts.

Thus, there appears to be a loss of sensitivity in diabetic hearts to insulin whose onset seems to be as early as 3 days after the induction of diabetes and which persists through the more chronic stages of the disease. Furthermore, the results suggest that the effects seen are due to insulin itself and not to glucagon which may contaminate commercially available animal insulin.

EFFECTS OF METHYL PALMOXIRATE TREATMENT ON CARDIAC FUNCTION AND BIOCHEMISTRY

The left ventricular developed pressure (LVDP) at various filling pressures in hearts from treated and untreated control and diabetic rats is shown in Fig. 24. As reported previously (Tahiliani et al., 1983), hearts from diabetic rats developed lower pressures as compared with
control hearts at filling pressures greater than 17.5 cm H₂O. Also, pressure development was lower in diabetic hearts at the lower filling pressures as compared with controls, but the difference was not statistically significant. Similarly, when the rate of contraction (+dP/dt) and rate of relaxation (-dP/dt) in diabetic rat hearts were compared with those in hearts from control animals, a depression was observed in diabetics (Figs. 25, 26). Treatment of either control or diabetic rats with methyl palmoxirate did not appear to have any significant effect on heart function in the respective groups (Figs. 24, 25, 26).

When calcium transport in the sarcoplasmic reticulum was studied, a depression in the ability of SR from diabetic rat hearts to take up calcium was seen at all the calcium concentrations used. However, statistical significance was observed at concentrations above 0.7 μM free calcium (Fig. 27). While methyl palmoxirate treatment did not seem to have any significant effect on calcium uptake in control rats, diabetic rats treated with the drug did not exhibit any depression as compared with controls suggesting a prevention of the depression of calcium uptake in the SR of diabetic rats (Fig. 27).

Levels of various carnitines (total-, acid soluble or free- and long chain acyl-carnitines) were also determined in the SR of treated and untreated control and diabetic rat hearts. No significant change in the levels of total or free carnitines resulted from the diabetic state. However, as reported in earlier studies (Lopaschuk et al., 1983), the levels of long chain acyl carnitines were significantly elevated in the SR of diabetic rat hearts (Fig 28). No such elevation was seen in methyl palmoxirate-treated diabetic rats. Levels of either free or total carnitines in SR were not affected by the treatment. Carnitine levels
(total, free or long chain) in control rats were not affected by the drug (Fig. 28).

EFFECTS OF METHYL PALMOXIRATE AND T₃ TREATMENT ON CARDIAC FUNCTION

As mentioned above, diabetic rats treated with either T₃ alone or in combination with methyl palmoxirate exhibited normal levels of T₃ and T₄ (Table 5). When the function of hearts from these animals was studied, T₃ treatment alone did not affect the function in either control or diabetic rats. Figure 29 shows the LVDP in these hearts. Positive and negative dP/dt, which were depressed in the diabetic group, were not affected by T₃ alone (Figs. 30, 31). Results similar to those seen previously were obtained when function of hearts from rats treated with methyl palmoxirate alone was studied (Figs. 32, 33, 34). However, the maximum ventricular pressure developed in hearts from diabetic rats treated with both methyl palmoxirate and T₃ was not significantly different from that developed in either control or untreated diabetic rat hearts at the higher filling pressures (Fig. 35) suggesting that the treatment was at least partially successful in preventing the diabetes-induced functional alterations in the myocardium. This prevention was more prominent with the combination when either the positive or the negative dP/dt were studied (Figs. 36, 37).

EFFECTS OF CARNITINE AND T₃ TREATMENT ON CARDIAC FUNCTION

In the last set of experiments, control and diabetic rats were treated with a combination of carnitine and T₃. Myocardial performance of the diabetic rats was depressed at left atrial filling pressures greater than 7.5cm H₂O as reflected by either the LVDP (Fig. 38),
positive dP/dt (fig. 39) or the negative dP/dt (fig. 40). The combination treatment did not appear to affect contractile function at the higher filling pressures. However, at the lower filling pressures (less than about 15 cm H₂O), hearts from diabetic rats treated with the combination performed significantly better than untreated diabetics. These results are depicted in figures 38, 39 and 40.
TABLE 2

GENERAL FEATURES OF ANIMALS USED IN THE 3 DAY STUDY

<table>
<thead>
<tr>
<th></th>
<th>Body Weight before injection of STZ or buffer (g)</th>
<th>Body Weight at sacrifice (g)</th>
<th>Blood Glucose (mg %)</th>
<th>Plasma Immunoreactive Insulin (μU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>171.9±1.5</td>
<td>174.8±1.9</td>
<td>103.8±8.0</td>
<td>22.1±1.5</td>
</tr>
<tr>
<td>(36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIABETIC</td>
<td>173.5±1.5</td>
<td>165.6±1.7</td>
<td>408.1±12.1*</td>
<td>10.6±2.1*</td>
</tr>
<tr>
<td>(31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* : Significantly different from control animals (p<0.05)
Figure 5: Effect of various left atrial filling pressures on the left ventricular developed pressure (LVDP) in hearts from control and 3 day diabetic rats using 5mM glucose in the buffer.

Results are expressed as mean ± S.E.M. (* denotes significantly different from all other groups at p<0.05).
(x)—(x) control (n=9)
(x)—(x) insulin perfused control (n=12)
(■)—(■) diabetic (n=6)
(■)—(■) insulin perfused diabetic (n=8)
Figure 6: Effect of various left atrial filling pressures on the positive dP/dt in hearts from control and 3 day diabetic rats in the presence of 5 mM perfusate glucose perfused with (CON + INS; DIA + INS) or without (CON; DIA) insulin.

Results are expressed as mean ± S.E.M. (* denotes significantly different from all other groups at p<0.05).
Figure 7: Effect of various left atrial filling pressures on the negative dP/dt in hearts from control and 3 day diabetic rats with 5mM glucose either in the presence (CON + INS; DIA + INS) or absence (CON; DIA) of insulin.

Results are expressed as mean ± S.E.M. (* denotes significantly different from all other groups at p<0.05).
Figure 8: Effects of various glucose concentrations on myocardial function of control and 3 day diabetic rat hearts.

Results are expressed as mean ± S.E.M.
(○) Control (5mM Glucose) (N=9)
(□) Control (10mM Glucose) (N=8)
(△) Diabetic (5mM Glucose) (N=6)
(▼) Diabetic (10mM Glucose) (N=8)
Figure 9: Effect of various left atrial filling pressures on the left ventricular developed pressure (LVDP) in hearts from control and 3 day diabetic rats using 10mM glucose in the buffer.

Results are expressed as mean ± S.E.M. (* denotes significantly different from all other groups at p<0.05).
(x)—(x) control (n=8)
(x)—(x) insulin perfused control (n=7)
(*)—(*) diabetic (n=8)
(*)—(*) insulin perfused diabetic (n=8)
Figure 10: Effect of various left atrial filling pressures on the positive dP/dt in hearts from control and 3 day diabetic rats in the presence of 10mM perfusate glucose with (CON + INS; DIA + INS) or without (CON; DIA) insulin.

Results are expressed as mean ± S.E.M. (* denotes significantly different from all other groups at p<0.05).
Figure 11: Effect of various left atrial filling pressures on the negative dP/dt in hearts from control and 3 day diabetic rats with 10mM glucose either in the presence (CON + INS; DIA + INS) or absence (CON; DIA) of insulin.

Results are expressed as mean ± S.E.M. (* denotes significantly different from all other groups at p<0.05).
<table>
<thead>
<tr>
<th></th>
<th>Body Weight before injection of STZ or buffer (n)</th>
<th>Body Weight at sacrifice (g)</th>
<th>Blood Glucose (mg %)</th>
<th>Plasma Immunoreactive Insulin (μU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>(52) 158.2±3.8</td>
<td>224.0±4.2</td>
<td>91.1±2.6</td>
<td>15.8±1.6</td>
</tr>
<tr>
<td>DIABETIC</td>
<td>(41) 163.8±2.2</td>
<td>169.8±8.0*</td>
<td>320.9±9.0*</td>
<td>6.8±0.4*</td>
</tr>
</tbody>
</table>

* Denotes significantly differently from solvent controls at p<0.05.
Figure 12: Performance of control rat hearts perfused in vitro with various (5mM to 20mM glucose) concentrations of glucose in the buffer. The only statistical difference seen was between 5mM and 20mM at the higher filling pressures.

(* denotes significantly different from 5mM).
---(x) 5 mM glucose (n=9)
---(•) 10 mM glucose (n=10)
---(o) 20 mM glucose (n=8)
Figure 13: Performance of 6 week diabetic rat hearts perfused in vitro with various (5mM to 20mM glucose) concentrations of glucose in the buffer. No statistical difference was seen between any of the groups.
— (x) 5 mM glucose (n=8)
--- (o) 10 mM glucose (n=7)
--- (●) 20 mM glucose (n=7)
Figure 14: Function of hearts from control and 6 week diabetic rats perfused with 5mM glucose in the presence or absence of insulin (5mU/ml). Results are expressed as LVDP at various filling pressures.

Each point represents the mean ± S.E.M. (* denotes significantly different from diabetic groups; ** denotes significantly different from all other groups).
(x)—(x) control (n=9)
(x)—(x) insulin perfused control (n=10)
(*)—(*) diabetic (n=8)
(*)—(*) insulin perfused diabetic (n=7)
Figure 15: Function of hearts from control and 6 week diabetic rats perfused with 5mM glucose in the presence (CON + INS; DIA + INS) or absence (CON; DIA) of insulin (5mU/ml). Results are expressed as +dP/dt at various filling pressures.

Each point represents the mean ± S.E.M. (* denotes significantly different from diabetic groups; ** denotes significantly different from all other groups).
Figure 16: Function of hearts from control and 6 week diabetic rats perfused with 5mM glucose in the presence (CON + INS; DIA + INS) or absence (CON; DIA) of insulin (5mU/ml). Results are expressed as \(-dP/dt\) at various filling pressures.

Each point represents the mean ± S.E.M. (* denotes significantly different from diabetic groups; ** denotes significantly different from all other groups).
Figure 17: Cardiac performance of control and 6 week diabetic rats. The hearts were perfused with 10mM glucose either in the presence or absence of 5mU/ml regular insulin.

Each value represents the LVDP at that left atrial filling pressure and is expressed as the mean ± S.E.M. (*) denotes statistical difference from diabetic groups; ** denotes statistical difference from all other groups.)
(x)—(x) control (n=10)
(x)—(x) insulin perfused control (n=10)
(■)—(■) diabetic (n=7)
(■)—(■) insulin perfused diabetic (n=6)
Figure 18: Cardiac performance of control and 6 week diabetic rats. The hearts were perfused with 10mM glucose either in the presence (CON + INS; DIA + INS) or absence (CON; DIA) of 5mU/ml regular insulin.

Each value represents the $+dP/dt$ at that left atrial filling pressure and is expressed as the mean ± S.E.M. (* denotes statistical difference from diabetic groups; ** denotes statistical difference from all other groups).
Positive \( \frac{dP}{dt} \) (mm Hg/sec)

Filling Pressure (Cm H2O)

CON + INS (10)

CON (10)

DIA (7)

DIA + INS (8)
Figure 19: Cardiac performance of control and 6 week diabetic rats. The hearts were perfused with 10mM glucose either in the presence (CON + INS; DIA + INS) or absence (CON; DIA) of 5mU/ml regular insulin.

Each value represents the negative dP/dt at that left atrial filling pressure and is expressed as the mean ± S.E.M. (* denotes statistical difference from diabetic groups; ** denotes statistical difference from all other groups).
Filling Pressure (Cm H2O)

Negative dP/dt (mm Hg/sec)

CON + INS (10)

CON (10)

DIA + INS (6)

DIA (7)
Figure 20: Effect of insulin (5mU/ml of perfusate) on the basal levels of phosphorylase in hearts from control and 6 week diabetic rats.

Each bar represents the mean ± S.E.M. of 4 hearts. (* denotes significantly different from control groups; ** denotes statistical significance from all other groups).
Figure 21: Effect of various left atrial filling pressures on the left ventricular developed pressure (LVDP) in hearts from control and 6 week diabetic rats using 10mM glucose in the buffer. The hearts were perfused either in the presence or absence of human insulin (Humulin®).

Results are expressed as mean ± S.E.M. (* Denotes significantly different from diabetic groups; ** Denotes significantly different from all other groups).
LVEDP (mm Hg)

FILLING PRESSURE (cm H₂O)
Figure 22: Effect of various left atrial filling pressures in the rate of pressure development in hearts from control and 6 week diabetic rats using 10mM glucose in the buffer. The hearts were perfused either in the presence (CON + INS; DIA + INS) or absence (CON; DIA) of human insulin (Humulin®).

Results are expressed as mean ± S.E.M. (* Denotes significantly different from diabetic groups; ** Denotes significantly different from all other groups).
Figure 23: Effect of various left atrial filling pressures on the rate of pressure decline in hearts from control and 6 week diabetic rats using 10mM glucose in the buffer. The hearts were perfused either in the presence (CON + INS; DIA + INS) or absence (CON; DIA) of human insulin (Humulin®).

Results are expressed as mean ± S.E.M. (* Denotes significantly different from diabetic groups; ** Denotes significantly different from all other groups).
### TABLE 4

**GENERAL FEATURES OF ANIMALS USED IN THE METHYL PALMOXIRATE STUDY**

<table>
<thead>
<tr>
<th></th>
<th>Body Weight (g)</th>
<th>Plasma Glucose (mg %)</th>
<th>Plasma Insulin (μU/mL)</th>
<th>Triglycerides (mg %)</th>
<th>Total lipids (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONTROL UNTREATED</strong></td>
<td>227±4</td>
<td>112±6</td>
<td>34±3</td>
<td>95±7</td>
<td>326±29</td>
</tr>
<tr>
<td><strong>CONTROL + METHYL PALMOXIRATE</strong></td>
<td>224±5</td>
<td>118±6</td>
<td>36±5</td>
<td>72±5</td>
<td>336±21</td>
</tr>
<tr>
<td><strong>DIABETIC UNTREATED</strong></td>
<td>173±7*</td>
<td>657±43*</td>
<td>12±3*</td>
<td>529±40*</td>
<td>2260±119*</td>
</tr>
<tr>
<td><strong>DIABETIC + METHYL PALMOXIRATE</strong></td>
<td>197±5*</td>
<td>579±36*</td>
<td>15±5*</td>
<td>403±45*</td>
<td>2127±117*</td>
</tr>
</tbody>
</table>

* : significantly different from all control groups
Figure 24: Left ventricular pressure development in hearts from control and 6 week diabetic rats. These rats were treated with methyl palmoxirate (25mg/kg/day as a suspension in 1.5% tragacanth) for 6 weeks, the treatment being initiated 3 days after the induction of STZ diabetes.

Results are expressed as mean ± S.E.M. (* denotes significantly different from both diabetic groups).
FILLING PRESSURE (cm H₂O)

LVDP (mm Hg)

(○) control (n=5)
(△) control-treated (n=4)
(●) diabetic (n=4)
(▼) diabetic-treated (n=5)
Figure 25: Rate of left ventricular pressure development in hearts from control and 6 week diabetic rats. These rats were treated with either methyl palmoxirate (25mg/kg/day as a suspension in 1.5% tragacanth) or the vehicle alone for 6 weeks, the treatment being initiated 3 days after the induction of STZ diabetes.

Results are expressed as mean ± S.E.M. (* denotes significantly different from both diabetic groups).
Figure 26: Rate of left ventricular pressure decline in hearts from control and 6 week diabetic rats. These rats were treated with either methyl palmoxirate (25mg/kg/day as a suspension in 1.5% tragacanth) or the vehicle alone for 6 weeks, the treatment being initiated 3 days after the induction of STZ diabetes.

Results are expressed as mean ± S.E.M. (* denotes significantly different from both diabetic groups).
Figure 27: Calcium uptake activity of sarcoplasmic reticulum prepared from hearts of control and diabetic rats. These rats were treated with either methyl paloxirate (25mg/kg/day as a suspension in 1.5% tragacanth) or the vehicle alone for 6 weeks, the treatment being initiated 3 days after the induction of STZ diabetes. Calcium uptake was ATP-dependent and carried out in the presence of oxalate.

Results are expressed as mean ± S.E.M. (* denotes statistical significance from all other groups).
Ca UPTAKE (nmol/mg protein/min)

FREE Ca (log conc) µM

- ○ control (n=4)
- △ control-treated (n=4)
- ● diabetic (n=4)
- ▼ diabetic-treated (n=4)
Figure 28: Levels of total, free and long chain acyl carnitines in the SR prepared from methyl-palmoxirate treated and untreated control and diabetic rats. Treatment protocol used was as described in legend to Figure 27.

The bars represent mean ± S.E.M. of 4 preparations. (* denotes significantly different from all other groups).
nmol CARNITINE/MG SR PROTEIN

CARNITINE LEVELS IN CARDIAC SR

DIABETIC (N=4)
CONTROL (N=4)
CON+M. P. (N=4)
DIAB+M. P. (N=4)
<table>
<thead>
<tr>
<th></th>
<th>Body Weight (g)</th>
<th>Plasma Glucose (mg %)</th>
<th>Plasma Insulin (μU/mL)</th>
<th>Total Lipids (mg %)</th>
<th>T₃B Index (%)</th>
<th>Plasma T₄ (μg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL UNTREATED</td>
<td>226±6</td>
<td>97±2</td>
<td>48±1</td>
<td>674±122</td>
<td>58.3±2.4</td>
<td>4.8±0.7**</td>
</tr>
<tr>
<td>CONTROL + T₃</td>
<td>223±1</td>
<td>109±5</td>
<td>52±6</td>
<td>846±99</td>
<td>65.6±0.6</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>CONTROL + METHYL PALMOXIRATE</td>
<td>233±7</td>
<td>112±6</td>
<td>46±6</td>
<td>575±69</td>
<td>57.9±2.9</td>
<td>3.7±0.5**</td>
</tr>
<tr>
<td>CONTROL + T₃ + METHYL PALMOXIRATE</td>
<td>223±6</td>
<td>119±3</td>
<td>50±4</td>
<td>619±40</td>
<td>61.8±2</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>DIABETIC UNTREATED</td>
<td>187±10*</td>
<td>481±24*</td>
<td>20±2*</td>
<td>2143±254*</td>
<td>45.0±1.3***</td>
<td>2.5±0.3***</td>
</tr>
<tr>
<td>DIABETIC + T₃</td>
<td>163±3*</td>
<td>484±37*</td>
<td>19±4*</td>
<td>2628±194*</td>
<td>53.6±1.5</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>DIABETIC + METHYL PALMOXIRATE</td>
<td>198±11*</td>
<td>513±25*</td>
<td>23±2*</td>
<td>2005±210*</td>
<td>44.3±1.6***</td>
<td>3.2±0.4**</td>
</tr>
<tr>
<td>DIABETIC + T₃ + METHYL PALMOXIRATE</td>
<td>162±8*</td>
<td>428±43*</td>
<td>17±4*</td>
<td>2581±232*</td>
<td>52.4±1.3</td>
<td>1.3±0.3</td>
</tr>
</tbody>
</table>

* : Significantly different from all control groups
** : Significantly different from all T₃ treated groups
*** : Significantly different from all other groups
Figure 29: Effect of $T_3$ (30μg/kg/day s.c.) treatment on cardiac performance in control and diabetic rats. Treatment was initiated 3 days after the rats were made diabetic and carried out for 6 weeks thereafter.

Each value represents mean ± S.E.M. of LVDP (* denotes significantly different from diabetic groups).
Figure 30: Effect of T₃ (30μg/kg/day s.c.) treatment on cardiac performance in control and diabetic rats. Treatment was initiated 3 days after the rats were made diabetic and carried out for 6 weeks thereafter.

Each value represents mean ± S.E.M. of +dP/dt (* denotes significantly different from diabetic groups).
(○) CONTROL (n = 5)
(△) CONTROL + T₃ (n = 4)
(●) DIABETIC (n = 4)
(▼) DIABETIC + T₃ (n = 6)

FILLING PRESSURE (Cm H₂O)

POSITIVE dP/dt (mm Hg/s)
Figure 31: Effect of T₃ (30μg/kg/day s.c.) treatment on cardiac performance in control and diabetic rats. Treatment was initiated 3 days after the rats were made diabetic and carried out for 6 weeks thereafter.

Each value represents mean ± S.E.M. of -dP/dt (* denotes significantly different from diabetic groups).
Figure 32: Effect of methyl palmoxirate (25 mg/kg/day p.o.) treatment on cardiac performance in control and diabetic rats. Treatment was initiated 3 days after the rats were made diabetic and carried out for 6 weeks thereafter. This study was carried out as part of the combination treatment with $T_3$ and methyl palmoxirate.

Each value represents mean ± S.E.M. of LVDP (* denotes significantly different from diabetic groups).
Figure 33: Effect of methyl palmoxirate (25 mg/kg/day p.o.) treatment on cardiac performance in control and diabetic rats. Treatment was initiated 3 days after the rats were made diabetic and carried out for 6 weeks thereafter. This study was carried out as part of the combination treatment with T₃ and methyl palmoxirate.

Each value represents mean ± S.E.M. of +dP/dt (* denotes significantly different from diabetic groups).
Figure 34: Effect of methyl palmoxirate (25 mg/kg/day p.o.) treatment on cardiac performance in control and diabetic rats. Treatment was initiated 3 days after the rats were made diabetic and carried out for 6 weeks thereafter. This study was carried out as part of the combination treatment with T₃ and methyl palmoxirate.

Each value represents mean ± S.E.M. of -dP/dt (* denotes significantly different from diabetic groups).

CON, CON+M.P.: control, methyl palmoxirate-treated control
DIA, DIA+M.P.: diabetic, methyl palmoxirate-treated diabetic
Figure 35: Cardiac function of control and diabetic rats treated with a combination of methyl palmoxirate and T₃ using doses and treatment protocol as in the previous figures.

Each value represents mean ± S.E.M. of LVDP (* denotes significantly different from untreated diabetics; ** denotes significantly different from all other groups).
**Graph:**

- **Y-axis:** LVDP (mm Hg)
- **X-axis:** FILLING PRESSURE (Cm H₂O)

- **Legend:**
  - ○ CONTROL (n = 5)
  - ▽ CONTROL + T₁₃ + MP (n = 4)
  - ● DIABETIC (n = 4)
  - ▼ DIABETIC + T₁₃ + MP (n = 4)
Figure 36: Rate of pressure development in left ventricles from hearts of control and diabetic rats treated with the combination of methyl palmoxirate and T₃.

Each value represents mean ± S.E.M. (* denotes significantly different from untreated diabetics; ** denotes significantly different from all other groups).
Figure 37: Rate of pressure decline in left ventricles from hearts of control and diabetic rats treated with the combination of methyl palmoxirate and T₃.

Each value represents mean ± S.E.M. (* denotes significantly different from untreated diabetics).
T₃ & METHYL PALMOXIRATE STUDY

-\frac{\text{d}P}{\text{d}t} (\text{mm Hg/s})

FILLING PRESSURE (cm H₂O)

(○) CONTROL (n=4)
(▼) CONTROL + T₃ + MP (n=4)
(●) DIABETIC (n=4)
(▼) DIABETIC + T₃ + MP (n=4)
<table>
<thead>
<tr>
<th></th>
<th>Body Weight (g)</th>
<th>Plasma Glucose (mg %)</th>
<th>Plasma Insulin (μU/mL)</th>
<th>T₃B Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL UNTREATED</td>
<td>216±6</td>
<td>122±7</td>
<td>22.5±0.4</td>
<td>58.6±4.2</td>
</tr>
<tr>
<td>CONTROL + T₃ + CARNITINE</td>
<td>209±4</td>
<td>115±6</td>
<td>28.6±1.0</td>
<td>70.5±5.0**</td>
</tr>
<tr>
<td>DIABETIC UNTREATED</td>
<td>168±12*</td>
<td>425±22*</td>
<td>10.8±0.9*</td>
<td>45.4±3.1*</td>
</tr>
<tr>
<td>DIABETIC + T₃ + CARNITINE</td>
<td>149±9*</td>
<td>456±24*</td>
<td>11.2±0.8*</td>
<td>56.9±4.5</td>
</tr>
</tbody>
</table>

* : Significantly different from all control groups
** : Significantly different from all other groups
Figure 38: Left ventricular developed pressure in hearts from control and diabetic rats treated with or without the combination of T₃ (30µg/kg/day s.c.) and carnitine (3g/kg/day in the drinking water).

Data are expressed as means ± S.E.M. (* denotes statistical difference from untreated diabetic groups; + denotes statistical difference from both diabetic groups).
Figure 39: Rate of Left ventricular pressure development in hearts from control and diabetic rats treated with or without the combination of T2 (30μg/kg/day s.c.) and carnitine (3g/kg/day in the drinking water).

Data are expressed as means ± S.E.M. (* denotes statistical difference from untreated diabetic groups; + denotes statistical difference from both diabetic groups).
Figure 40: Rate of Left ventricular pressure decline in hearts from control and diabetic rats treated with or without the combination of T3 (30μg/kg/day s.c.) and carnitine (3g/kg/day in the drinking water).

Data are expressed as means ± S.E.M. (* denotes statistical difference from diabetic groups).
DISCUSSION

Animals treated with STZ exhibited the classical features of diabetes. Quantitative determinations carried out revealed hyperglycemia, hypoinsulinemia, hypothyroidism and hyperlipidemia in the diabetic rats which also weighed less than controls. Qualitative observations confirmed the presence of polyuria, polyphagia and polydipsia. All of these features of diabetes have been reported in a number of previous studies with STZ (Fein et al., 1980; 1981; Vadlamudi et al., 1982).

It cannot be conclusively stated as to the type of human diabetes represented by this model. Pancreatic beta cell damage, which is the basis of diabetes in these rats, suggests that the diabetes is similar to Type I (insulin dependent-type). However, the presence of significant amounts of circulating insulin (although they are reduced by about 50%) and the survival of these rats without the administration of exogenous insulin argues against classifying this diabetes as Type I. On the other hand, if left untreated, these rats develop a number of complications, including those affecting the cardiac (Fein et al., 1980), vascular (Reinila, 1981), renal (Jensen et al., 1981) and the nervous (Bestetti et al., 1981) systems so it would be reasonable to refer to these rats as poorly controlled diabetics. Whatever the type, it is definite that the model has features which are seen clinically in diabetics.

It is also apparent from the study that the effects of STZ are seen within 3 days of its injection. The rats exhibit glycosuria, hyperglycemia and hypoinsulinemia and, simultaneously, their weight gain stops
(Table 2). It is possible that these observations are due to the direct effects of STZ. However, a more likely possibility is that the effects are due to the diabetes induced by STZ since insulin treatment prevents all the detrimental effects of diabetes in rats. (Fein et al., 1981; Tahiliani et al., 1983). Also, symptoms similar to those seen with STZ diabetes are seen in spontaneously diabetic rats (BB rats) when they become diabetic (Chapell and Chapell, 1983). The severity of symptoms is much greater in the latter model but this is probably due to the drastic reduction in insulin levels at the onset of diabetes in these rats (Marliss et al., 1983), as opposed to the situation in STZ-treated rats where the insulin levels usually remain around 50% those of controls (Tables 2-6).

With respect to the effects of experimentally-induced diabetes on cardiac function, various studies have shown a depression of cardiac performance in rats so treated. In one study by Miller (1979), 3 day alloxan-diabetic rats exhibited depression of cardiac function at a 5mM glucose perfusate concentration. The depression could be reversed in vitro by insulin perfusion or by increasing the glucose concentration to 10mM. It was thus suggested that acute experimental diabetes in the rat results in impaired myocardial glucose uptake which can be reversed in vitro. However, using a similar preparation, Vadlamudi et al., (1982) were unable to demonstrate any depression of function 7 days after either alloxan or streptozotocin treatment. The discrepancy between the two studies was suggested to be due to the higher concentration of glucose in the perfusion medium used by the latter group which could have overcome the depression of function. Also, the time points studied by the two groups were different and it was possible that alloxan
diabetes resulted in acute myocardial changes which then normalized (around 7 days and so were missed by Vadlamudi et al) and at a later point in time resulted in chronic changes. In the present investigation, STZ diabetic rats did not exhibit myocardial depression as compared to controls when 5mM glucose was used. It thus seems that the effects seen in the study by Miller (1979) are unique to acute alloxan diabetes in the rat since Downing et al. (1981) did not observe a depression of ventricular function of 2 - 4 day alloxan diabetic lambs.

When we added insulin to the buffer containing 5mM glucose, function of control rat hearts was significantly enhanced. However, when 3 day diabetic rat hearts were perfused with insulin, no such enhancement was seen. A number of studies have examined the effects of insulin on various cardiac preparations. Positive inotropic effects of insulin have been shown by Rieker et al., (1975) in piglets; by Sassine et al., (1975) in isolated rabbit atria and on the isolated rabbit heart by Klinge and Wafin (1971). The effects of insulin on glucose metabolism are well known. In control hearts, an increase in glucose uptake and glucose phosphorylation in response to insulin was shown by Morgan et al., (1961a), the kinetics of which were studied more recently by Cheung et al., (1978). Also, insulin has been shown to increase CO₂ production from glucose in normal working hearts (Chain et al., 1969) and to increase the concentrations of various glucose metabolites (Das and Chain, 1976). Recently, Haworth et al., (1984) demonstrated the ability of insulin to stimulate 3-O-methyl glucose uptake into adult rat heart cells. Thus, the effects of insulin on control hearts seen in our study may be due to increased glucose transport and metabolism in the myocardial cells. The mechanism by which insulin stimulates glucose
transport into cells is not very well understood. Glucose transport into cells is mediated via the glucose transporter. Evidence exists that there are two pools of the glucose transporter units in cells. One is at the surface of cells and it is this pool which is responsible for the actual transport of glucose across the cell membrane while the other pool may be at a distant site in the cell. One hypothesis suggests that insulin enhances the transport of glucose by increasing the translocation of glucose transporters from the intracellular to the surface pool and thus increases the actual number of transporters available (Cushman and Wardzala, 1980; Hissin et al., 1982). Whatever the mechanism, glucose transport is increased by insulin and this increase may contribute to the enhancement of function induced by insulin. However, as discussed later, this may not be the sole mechanism of insulin inotropy.

In diabetic rat hearts, the effects of insulin are not as prominent. Morgan et al., (1961b) have shown that glucose transport is the limiting factor for glucose uptake in the absence of insulin in diabetic hearts and that, in the presence of insulin, the transport of glucose can be normalized but the depressed phosphorylation of glucose cannot be returned to normal. In diabetic rat hearts, however, acceleration of glucose transport by insulin is slower than in normal hearts (Morgan et al., 1961a; b) and insulin was also shown not to be capable of stimulating CO₂ production from glucose to an extent seen in normal rat hearts (Chain et al., 1969). These defects in the handling of glucose seen in diabetic rat hearts may be reflected in our study as an inability of insulin to stimulate myocardial function in diabetic animals. Increasing the glucose concentration from 5mM to 10mM did not alter the responsiveness of diabetic hearts to insulin suggesting that the uptake and
perhaps the metabolism of glucose in diabetic rat hearts is impaired and cannot be corrected by high concentrations of glucose even in the presence of insulin.

Diabetogenic agents induce diabetes by selectively destroying pancreatic beta cells (Veleminsky, 1970). Alloxan has been shown to result in an acute transient hypoglycemic phase which could be due to release of insulin caused by the degranulation of beta cells of the islets in the pancreas (Lundquist and Rerup, 1967). A decrease in the number of functioning cell surface insulin receptors is known to result from exposure to insulin in various tissues (Krupp and Lane, 1981; Marshall and Ofelsky, 1980). Thus, the mechanism of the lack of insulin response in diabetic rat hearts could be that streptozotocin caused a release of insulin from the pancreas which resulted in internalization of the insulin receptors. It is possible that three days after injection with the agent, the cardiac insulin receptors may still be low, and so a loss of sensitivity to insulin may be seen. However, this hypothesis still does not explain the results obtained by Miller (1979) in which insulin was found to elicit a response in diabetic rat hearts. A possibility is that the release of insulin caused by alloxan may be more acute than that caused by STZ, and three days after treatment, the insulin receptor population is at least partially restored (and hence responsiveness to insulin) in alloxan-treated rats.

If the hypothesis that the loss of insulin sensitivity in 3 day diabetic rat hearts is due to a decrease in the insulin receptors in the myocardium were true, then if we allowed a sufficient period for the receptors to regenerate, a response to insulin would be expected in diabetic rat hearts. This was one of the reasons why the second part of
our study was undertaken. The other reason for undertaking this study was to determine whether insulin perfusion could reverse the depression of myocardial function. In this study, the experiments were repeated six weeks after the induction of diabetes. Six week diabetic rat hearts exhibited a depression of cardiac function which is in agreement with previous studies from our laboratory.

Various biochemical factors have been suggested to be involved with such a depression. These include a depressed calcium uptake in the SR (Penpargkul et al., 1981; Lopaschuk et al., 1983); depressed myosin ATPase activity of the myocardium (Dillmann, 1980). Results from more recent studies seem to suggest that the elevated levels of circulating lipids may be important towards the development of cardiac problems in diabetes (Rodrigues et al., 1985; McNeill and Rodrigues, 1985). These data are discussed in the following sections.

Cardiac contractility was not significantly affected by increasing the glucose concentration in either control or diabetic rat hearts. This suggests that the availability of glucose or energy available for contraction by way of glucose metabolism could not be enhanced any further in the absence of insulin. The uptake of glucose and its phosphorylation are known to increase as substrate concentration is increased in control and in diabetic rat hearts, though at a slower rate in diabetics as compared to controls (Morgan et al., 1961a; b). The finding that the function of diabetic hearts is depressed despite the elevated levels of glucose is not surprising as diabetes is known to induce depressed myocardial performance in vivo in various species including dog (Regan et al., 1974) and man (Shapiro et al., 1980a; b). Also, it is evident from our results that acutely elevated glucose levels and thus the resul-
tant increase in osmolality are not detrimental to performance in control hearts.

As in the previous experiments, insulin perfusion significantly enhanced myocardial function in control animals. In diabetic animals, however, the function was unaffected by insulin. These results would suggest that the receptors were down-regulated 3 days after STZ injection and did not recover up to six weeks later. As we have not determined receptor populations, it is not possible to draw any such conclusions as yet. However, this seems to be a likely possibility in view of findings from various studies in which responsiveness to insulin disappears in diabetes but reappears when insulin treatment is instituted. Also, responsiveness to insulin is known to increase during chronic hyperinsulinism in experimental diabetes and this has been referred to as the property of insulin to maintain sensitivity of various organs (and cells) towards itself. Whether or not the insulin receptor is directly responsible for the positive inotropic effect of insulin is not known. The finding that the positive inotropic effects of insulin are seen even in the absence of glucose one would suggest that the pathways by which insulin increases glucose uptake and that by which it produces positive inotropy are different and may not be related to each other. On the other hand, if it is assumed that insulin receptors are altered by acute diabetes and normalized in the chronic phases of diabetes, then our studies would suggest that the positive inotropic effects of insulin are not indirectly (through increased glucose transport) receptor-mediated. This latter view is supported by findings in which the positive inotropic effects of insulin are seen even in the absence of glucose (see Christensen et al., 1983).
However, if the insulin inotropy is not receptor-mediated and thus not dependent on glucose, then the question regarding the mechanism of insulin action arises. Catecholamine release has been suggested, but the involvement appears to be unlikely (for review, see Christensen et al., 1983). In an attempt to elucidate the mechanism of action, we studied the effect of insulin on the cyclic AMP system, as stimulation of this system results in positive inotrophic effects. An increase in the levels of phosphorylase a in diabetic rat hearts was observed. This is in agreement with previous data from our laboratory (Vadlamudi and McNeill, 1984). However, insulin did not affect the levels of phosphorylase a in either control or diabetic rat hearts suggesting that the cyclic AMP system was not involved in the positive inotropic effect of insulin. This finding also seems to rule out the possibility that the effects of insulin may be mediated via a release of noradrenaline which is consistent with data from previous studies (Christensen et al., 1983). In brush border cells, glucose transport is accompanied by an influx in Na⁺. If such a system existed in the myocardium, an increase in Na⁺ influx could lead to an increase in Ca²⁺ via the Na-Ca exchanger system which could then explain the positive inotropy of insulin. However there are two drawbacks to this hypothesis. First, no such system has been demonstrated in the myocardium and secondly, even if such a system did exist, it would still not explain the insulin inotropy as the latter apparently does not require the presence of glucose in the perfusion medium. The other possibility, which is still to be tested, is that insulin could increase the influx of calcium directly into myocardial cells and thus produce inotropic effects.

Finally, commercially available insulin has been suggested to be
contaminated with glucagon (see Farah and Alousi, 1981). In other studies, inhibitors of insulin action have been found in commercial insulin preparations (Klinge and Wafin, 1971; Bloom et al., 1978). Contamination with glucagon is probably due to the source of manufacture of commercial insulin. In order to eliminate the possibility that the effect of insulin we were getting in the control hearts was due to glucagon, we repeated the last part of our study using glucagon-free insulin. The results were identical to those obtained using regular insulin, suggesting that the observed effects were probably due to insulin itself.

In conclusion, the study brings out a number of features with respect to the myocardial effects of insulin. First, the hormone produces a positive inotropic effect in the control rat heart which is not the result of any contaminants in the insulin preparation. The effect does not change as the perfusate concentration of glucose is increased suggesting that the maximal effect occurs at 5 mM glucose. The inotropy may be the result of increased glucose transport and metabolism but this is unlikely in view of previous findings. Other mechanisms such as increased calcium influx may be involved but data are still not available to substantiate this hypothesis. Secondly, inotropic responses to insulin are not seen in diabetic rat hearts and the loss of sensitivity is seen 3 days and 6 weeks after STZ injection. The mechanism for this is not known. Finally, the study shows that the beneficial effects of in vivo insulin treatment are not due to direct inotropic effects of insulin in diabetic hearts.

EFFECTS OF VARIOUS TREATMENTS ON DIABETES-INDUCED MYOCARDIAL ALTERATIONS
Diabetes is known to result in a number of alterations, the most prominent ones being those of abnormal carbohydrate and fat metabolism and the resultant elevation of these substances in the blood of diabetics. The extent of the contribution of either of these towards diabetes-induced myocardial abnormalities is not known definitely. Elevated levels of glucose may alter enzyme activities by inducing glycosylation of the enzymes eventually leading to or contributing towards functional alterations. Elevated lipids, on the other hand, may alter the cardiac function by a variety of modes. Among other changes, increased myocardial lipid metabolism, occurring as a result of decreased glucose transport and elevated free fatty acid levels causes elevation of metabolic intermediates such as long chain acyl carnitines (LCAC) and long chain acyl CoA in the heart. LCAC have been shown to inhibit various enzymes in the heart including Na\(^+\), K\(^+\) ATPase (Wood et al., 1977; Pitts and Okhuysen, 1984), and calcium transport in the SR (Adam et al., 1978; Pitts et al., 1978). The latter changes are also known to occur as a result of diabetes (Ku and Sellers, 1982; Lopaschuk et al., 1983). It is thus possible that diabetes-induced alterations of lipid metabolism may be at least partially responsible for the cardiac functional alterations associated with the disease and LCAC may be contributing factors.

In order to test this hypothesis, we studied the effectiveness of methyl palmoxirate (McN-3716; methyl tetradecylglycidate) on diabetes-induced myocardial alterations. The drug is a fatty acid analog which blocks oxidation of free fatty acids in the heart by inhibiting carnitine palmitoyl transferase and thus inhibiting the transport of fatty acyl CoA into the mitochondria (Tutwiler and Ryzlak, 1980). By virtue of this
action, methyl palmoxirate is thought to increase glucose metabolism and thus decrease the levels of glucose in the blood of either diabetic or starved animals i.e. in animals dependent on fats as their source for metabolism (Tutwiler et al., 1978). In the present study, however, we were unable to demonstrate any significant decrease in blood glucose levels in the diabetic animals by methyl palmoxirate treatment. We did observe a decrease in the severity of glycosuria during the initial stages of treatment (during the first two days) from 4+ (>2.0%) to 1+ (0.1%) using Lilly Tes Tape® when the animals were tested 24 hours after administration of the drug. This decrease was not seen in the latter stages. In the study by Tutwiler et al. (1978), the effect of methyl palmoxirate was most prominent in fasted, severely diabetic rats rather than in fed diabetics in which only a moderate lowering of glucose levels is seen. This may explain the ineffectiveness of the drug in our study where the animals had free access to food at all times. We did observe slightly lower blood glucose levels as well as slightly elevated body weights in diabetic rats treated with methyl palmoxirate. It may be argued that the rats in the treated group were not as severely diabetic before treatment was initiated, but this seems unlikely as insulin levels were similar in both these groups. This finding suggests that the drug probably did increase glucose uptake into cells to a small extent and it may help to explain some of our later results. Another observation we made was a yellow discoloration in the liver, kidney and intestine which is suggestive of fatty infiltration. Similar observations have been reported by Bachmann et al., (1984).

Analysis of cardiac function revealed that six weeks after induction of diabetes, depression of LVDP, +dP/dt and -dP/dt were present in the
STZ-diabetic rats. These results agree with those reported previously (Tahiliani et al., 1983; Tahiliani and McNeill, 1984a). The finding that methyl palmoxirate did not prevent functional alterations from occurring suggested that the drug was either not having any effects or that the inhibition of fatty acid metabolism was not sufficient to prevent the myocardial abnormalities. The latter possibility cannot be eliminated as we have not studied the extent of inhibition of fat metabolism in the hearts of fed diabetic rats. Alternatively, it suggested that fatty acid metabolism was not causally related to myocardial abnormalities in the heart. In view of the detrimental effects elevated levels of fatty acids can have on myocardial function directly (Myos, 1971; Henderson et al., 1970), as well as the effects that may be exerted by fatty acid metabolites such as acyl carnitines and acyl CoA (see Corr et al., 1984), it seems unlikely that elevated lipids are not causally related to the myocardial dysfunction in diabetic animals. Furthermore, recent data from our laboratory have shown that myocardial dysfunction can be reversed by hydralazine treatment of diabetic rats and this reversal is accompanied by a reversal of elevated levels of circulating lipids to normal (McNeill and Rodrigues, 1985). Also, in a different study, it was demonstrated that, in the WKY strain of rats, STZ induced hyperglycemia but did not cause hyperlipidemia. Interestingly, these WKY rats did not demonstrate a depression of cardiac function (Rodrigues et al., 1985). Although preliminary, these studies further support the hypothesis that elevated levels of circulating lipids are detrimental to cardiac function in diabetic rats.

When we determined the levels of free, total and long chain acyl (LCAC) carnitines in the SR, only the levels of LCAC were significantly
elevated in untreated diabetic rat hearts. This has been reported in previous studies (Feuvray et al., 1979; Lopaschuk et al., 1983; Tahiliani et al., 1983) and is probably due to availability of greater amounts of fatty acids for metabolism in diabetic rat hearts. Further, this elevation could be prevented by methyl paloxirate treatment. Also, we found that the depression of cardiac SR calcium uptake in diabetic animals was not present in those animals treated with the drug. These results suggest that the drug is indeed inhibiting fatty acid oxidation to some extent. As fatty acid metabolism is being affected while function is not, it would seem that the former is not the only responsible factor in diabetes-induced myocardial dysfunction and that other factors may be involved. On the other hand, it may also suggest that the inhibition is not severe enough for myocardial function to be affected. Another possibility is that the treatment could be having effects which may be detrimental and might nullify the beneficial effects of decreased fatty acid metabolism. A possibility of the latter could be that by inhibiting the metabolism of free fatty acids, methyl paloxirate could elevate circulating free fatty acids in the diabetics. We did observe a slight but insignificant increase in the levels of plasma fatty acids of diabetic rats. However, this seems to be an unlikely possibility since no depression of myocardial function was seen in control animals that received the drug treatment. However, it should be pointed out that the fatty acid levels were not elevated in the plasma of control animals. The finding that methyl paloxirate-treated controls exhibited normal function is interesting in view of the findings of Bachmann et al. (1984), who reported that the drug caused a progressive uncoupling of oxidative phosphorylation of heart mitochondria in control rats. This was
found to be due to inhibition of the formation of high energy bonds and their transfer to the cytosol. Probably, the process was not sufficiently impaired for functional damage to occur. The finding that oxidative phosphorylation was depressed in methyl palmoxirate-treated rat hearts may be another side effect of methyl palmoxirate which could neutralise any beneficial effects it may have had on myocardial contractility.

The study also demonstrates that myocardial functional depression occurs in the hearts of diabetic animals despite normal calcium uptake in the SR. The finding confirms previous studies in our laboratory in which carnitine treatment normalised the SR calcium uptake but failed to affect cardiac function in diabetic rat hearts (Lopaschuk et al., 1984). The explanation forwarded was that diabetes-induced myocardial alterations were not solely the result of depressed cardiac SR calcium uptake and other factors such as a depressed myosin ATPase activity which were probably not affected by carnitine treatment were equally important. However, in the case of methyl palmoxirate, a recent study has demonstrated the effectiveness of the drug in preventing the depression of myosin ATPase in diabetic rat hearts (Barrieux and Dillamnn, 1985). This finding is interesting as hypothyroidism (which was originally thought to induce the depression of myosin ATPase in rats) was not corrected by methyl palmoxirate in our studies. Thus it would seem that diabetes involves factors besides depressed myosin ATPase and SR calcium uptake which are detrimental towards function and are not corrected by methyl palmoxirate treatment.

An interesting question arises from this study with regard to the source of metabolic energy in hearts treated with methyl palmoxirate. In the case of diabetic rat hearts, the ability to take up glucose is
impaired by the lower levels of circulating insulin and in case of diabetic rats treated with methyl palmoxirate the ability to metabolise fatty acids is now impaired by the drug. In a recent study by Kiorpes et al. (1984), it was found that methyl palmoxirate (1X10^{-7} M) produced an 80% inhibition of hepatic carnitine palmityl transferase. However, it is not possible to translate these data to numbers that would be relevant for determining the extent of inhibition of cardiac carnitine palmityl transferase as these studies were carried out in the liver. In addition, the pharmacokinetics of methyl palmoxirate have not been investigated and the plasma concentration following a 25 mg/kg dose in rats is not known. Since glucose uptake was probably stimulated to at least some extent by methyl palmoxirate treatment (as mentioned in the preceding sections) and besides, since the diabetic rats are not totally devoid of insulin, some energy must be derived from glucose. The remainder would have to come from fatty acids. This is supported by our finding that normal levels of long chain acyl carnitines were found in the hearts of diabetic rats treated with methyl palmoxirate. This suggests that cardiac carnitine palmityl transferase is not completely inhibited by methyl palmoxirate in the dosage schedule used.

In the other part of our study, we used a combination of T_3 and methyl palmoxirate in an attempt to prevent the cardiac dysfunction from occurring. T_3 treatment in diabetic rats has been shown to prevent the depression of myosin ATPase in diabetic rat hearts (Dillmann, 1982) suggesting that diabetes - induced hypothyroidism is responsible for the myosin ATPase depression in diabetic rats. Since our hypothesis was that methyl palmoxirate affected only the SR but not myosin ATPase in hearts from diabetic rats (which was why we did not get a normalisation of
cardiac function), we treated rats with methyl palmoxirate as well as T₃. T₃ alone was found to be ineffective in preventing the functional alterations seen in diabetes (Tahiliani et al., 1984). Garber and Neely (1983) have also reported that physiological doses of T₃ were ineffective in preventing the myocardial dysfunctional abnormalities induced by diabetes. However, they reported that the function could be normalised with large doses of T₃. This effect was probably a direct toxicity of the hyperthyroidism so induced. In agreement with our previous study, Barbee and Burns, (1985) were unable to prevent the diabetes induced alterations in function with T₃ treatment. These studies suggested that other factors besides myosin ATPase are involved in the functional depression.

In the present study, we found that while methyl palmoxirate alone did not affect T₃ or T₄ levels, the combination of T₃ and methyl palmoxirate was effective in preventing diabetes-induced hypothyroidism as reflected by the T₃B index. However, the T₄ levels which were depressed in diabetic rats were further depressed by T₃ treatment. T₃ is formed in the liver by the process of monodeiodination from T₄ and the lower levels of T₃ in diabetic rats are due to the depression of monodeiodinating enzymes in the liver (Pittman et al., 1979; Balsam et al., 1978). The production of T₃ is controlled by a feedback loop and thus when the levels of T₃ are elevated, its production from T₄ as well as the production of T₄ is shut off. This explains the lower levels of T₄ in diabetic rats treated with T₃.

The other important feature of this study was the success of the combination in preventing cardiac functional alterations seen in diabetic rats. It should be pointed out that while function of treated diabetic
rat hearts at the lower filling pressures was similar to that in untreated diabetics no depression was seen at the higher filling pressures. Although the prevention was statistically significant, in our opinion, it was not as impressive as that produced by insulin in the same preparation (Tahiliani et al., 1983). This view is based on the observation that in the present study, prevention of LVDP depression in untreated diabetic hearts was seen only at the highest filling pressure (22.5 cm H₂O) while that in insulin-treated diabetic rat hearts was seen at left atrial filling pressures above 15 cm H₂O. Also, while insulin-treated diabetic rat hearts performed slightly (although not significantly) better than control rat hearts, no such observation was made in the present study. It thus seems that the hearts from diabetic rats treated with T₃ and methyl palmitoxirate have a normal reserve. At the higher filling pressures, energy consumption increases which is met by increased energy production through accelerated metabolism (Crass et al., 1971). In diabetic hearts, due to excessive availability of circulating free fatty acids and restriction of glucose transport, fat metabolism increases. This increase in fatty acid metabolism increases the oxygen demand to a greater extent than that in control hearts where the increased energy demand is met by increased fat and carbohydrate metabolism since the ratio of energy produced to oxygen consumed is greater for glucose than that for fats. Cardiac dysfunction seen in diabetic rats may in part reflect the imbalance between supply and demand of oxygen. Our results suggest that this may be true since inhibition of fatty acid oxidation by the drug, which has been shown to increase carbohydrate utilisation, may in fact increase cardiac glucose metabolism and thus reduce the oxygen demand. Further, as mentioned in preceding sections, treatment with
methyl palmoxirate did decrease the blood glucose levels slightly suggesting that the uptake of glucose into cells was increased by the drug and this finding may be taken as support for the above suggestion. However, this mechanism does not account for the dysfunction entirely since methyl palmoxirate alone was unable to normalise the function.

It would appear that $T_3$ treatment has certain effects which in combination with those exerted by methyl palmoxirate are beneficial to the diabetic hearts. This is probably not merely a reversal of the myosin ATPase to normal (as hypothesized previously) as methyl palmoxirate itself can produce such a normalisation (Barrieux and Dillmann, 1985). $T_3$ may be doing something to compensate for the side effects induced by methyl palmoxirate (which as suggested earlier may mask its beneficial effects). On the other hand, the hypothyroidism induced by diabetes is not corrected by methyl palmoxirate alone. Thus hypothyroidism may be an additional factor (besides the depressed calcium uptake and depressed myosin ATPase) which is of major significance in diabetes-induced myocardial alterations which is corrected by $T_3$ treatment. This correction, in combination with the beneficial effects of methyl palmoxirate, may explain the effectiveness of the combination. The mechanism by which hypothyroidism exerts its influence in diabetes obviously extends beyond the depression of myosin ATPase.

In summary, the combination of $T_3$ and methyl palmoxirate seems effective as it normalises myosin ATPase and calcium uptake in the SR. Other possibilities such as decreased oxygen demand and the effects of $T_3$ on metabolism may also contribute to the prevention of cardiac dysfunction.

In the last study, we investigated the effects of a combination of
and carnitine on the diabetes-induced myocardial alterations. In previous studies, carnitine was shown to lower the levels of LCAC in cardiac SR of diabetic rats and also to normalise the depression of SR calcium uptake in these animals (Lopaschuk et al., 1984). The mechanism by which carnitine produces a lowering of LCAC in the myocardium is only speculative. As suggested by Liedtke et al. (1981), carnitine may inhibit free fatty acid uptake at the plasma membrane. Thus the amount of fatty acids available for conversion to LCAC may be lower than normal and thus the lowering of LCAC. Other mechanisms by which carnitine may lower intracellular LCAC that have been suggested include a carnitine-LCAC translocase system in the sarcolemma, similar to that found in the mitochondrial membrane (Lopaschuk, 1984). However, it did not produce any significant effects on the depressed myocardial function in diabetic animals. Our study revealed that the combination did have some effects on the myocardial function of diabetic rats. At the lower filling pressures, the functional parameters developed in the treated diabetic rats were similar to those in normal rats but at the higher filling pressures, these parameters were depressed. This is in contrast to the previous study with the combination of methyl palmoxirate and T₃ where a normalisation of function was obtained at the higher filling pressures as well.

Although the reasons for these differences are not clear, it is obvious that the two combinations have effects which are different from each other. We had originally assumed that carnitine or methyl palmoxirate treatments affected only the calcium uptake in SR while T₃ treatment affected only the cardiac myosin ATPase. While this assumption did not hold with regard to methyl palmoxirate, this cannot be said about the
effect of carnitine on myosin ATPase as this has not been determined. Also, we thought that myosin ATPase and SR calcium uptake were the two major factors in producing the cardiac functional derangements. This also does not appear to be true as methyl palmoxirate alone, which affects both these parameters did not affect function. Thus the situation of diabetes-induced myocardial dysfunction is more complex than originally thought and must involve various other factors.

One such factor could be the accumulation of collagen in the hearts from diabetics which would result in increased stiffness of the ventricular wall. Regan et al. (1981) have reported an increase in the concentrations of collagen in hearts from 12 month alloxan-diabetic dogs. However, this increase was not affected by the insulin therapy which they used. Similarly, in a postmortem study of human subjects, Regan et al. (1977) found histologic evidence of increased amounts of collagen and mucopolysaccharides in the interstitium without evidence of large or small vessel coronary atherosclerosis. Since these observations were made in parallel with abnormalities of cardiac function, it was suggested that the accumulation of myocardial collagen may indeed be a significant problem in diabetics. However, in a study on collagen metabolism on STZ-diabetic rats 18 and 26 weeks after they were made diabetic, no increase in the concentrations of myocardial collagen was seen (Modrak, 1980). It may be argued that changes in collagen metabolism may occur in the later stages of diabetes. However, keeping in view the results of Modrak (1980), it is unlikely that collagen deposition was a significant factor in our studies which were relatively short term.

Another factor that may also contribute to a significant extent towards the diabetes-induced myocardial abnormalities is an altered
sarcolemma. A depression of the cardiac Na\(^+\), K\(^+\) ATPase activity has been shown (Ku and Sellers, 1982; Pierce and Dhalla, 1983). Cardiac adenylate cyclase activity is also depressed in diabetic rat hearts (Smith et al., 1984). In another study, the calcium binding capability was shown to be altered in diabetes. In the same study, the content of sarcolemmal calcium was also found to be depressed in chronically diabetic rats (Pierce et al., 1984). In a recent study, Ganguly et al. (1984) have identified a defect in the sarcolemmal phosphatidylinositol-ethanolamine N-methylation. Such a defect may alter the lipid microenvironment and thus alter the biophysical properties of the cardiac membranes and could lead to altered ion fluxes through the sarcolemma and alter mechanics of the heart. Thus, the sarcolemma certainly seems to be altered in diabetes and may well be a significant causative factor in the production of diabetes-induced myocardial alterations.
SUMMARY AND CONCLUSIONS

1. Streptozotocin-induced diabetes in rats did not affect myocardial function 3 days after the induction of the disease. However, a significant depression of cardiac function was seen in 6 week diabetic rats.

2. Increasing the buffer concentration of glucose from 5 mM to 20mM did not affect the function of either control, 3 day or 6 week diabetic rat hearts. Inclusion of insulin (5 mU/ml) in the perfusion medium (containing 5 mM glucose) enhanced the function of control rat hearts to a significant extent. However, 3 day or 6 week diabetic rat heart function was not affected by the insulin perfusion. Similar results were obtained in the presence of 10 mM glucose.

3. Results similar to those obtained with regular insulin were obtained when glucagon free insulin (Humulin®) was used instead of regular insulin.

4. In vitro insulin perfusion did not affect the basal levels of phosphorylase a in hearts from either control or diabetic rats.

5. Six weeks of methyl palmoxoritate treatment did not affect the levels of plasma glucose, lipids or insulin in either control or STZ-treated rats. Also, the depression of myocardial function in diabetic rats 6 weeks after the induction of diabetes was not affected by the treatment. However, the depression of the cardiac sarcoplasmic reticulum calcium
uptake which was depressed in diabetic rats was normalised by methyl palmoxirate treatment. Also, levels of long chain acyl carnitines, which were elevated in SR from diabetic rat hearts were normalised by the drug.

6. Treatment of rats with either T<sub>3</sub> alone or in combination with methyl palmoxirate corrected the diabetes-induced hypothyroidism in rats. While T<sub>3</sub> or methyl palmoxirate alone did not affect the diabetes-induced depression of myocardial function, combination treatment with both the agents was effective in preventing the depression.

7. Unlike the effectiveness of the T<sub>3</sub> and methyl palmoxirate combination, treating the diabetic rats with a combination of T<sub>3</sub> and carnitine did not affect the myocardial depression in diabetic rats.

In conclusion, insulin produces an enhancement of function in control rat hearts while hearts from STZ-diabetic rats do not respond to insulin. The mechanism for the inotropy in control rat hearts may involve increased glucose transport and perhaps metabolism, but the finding that inotropic effects of insulin are seen even in the absence of glucose argue against the involvement of glucose. Involvement of the adenylate cyclase-cAMP system or the possibility that the effects of insulin are mediated by a release phenomenon seem unlikely since the levels of phosphorylase a were not affected by insulin. The mechanism by which diabetes induces resistance to insulin is not clear. The study also suggests that the effectiveness of insulin treatment in preventing and reversing diabetes-induced myocardial alterations is not due to the direct effects of insulin but is the result of the effects of insulin on metabolism in vivo.
The other part of this study shows that the diabetes-induced myocardial functional alterations cannot be accounted for by the combination of the depression of myosin ATPase and the depression of calcium uptake in the SR. Hypothyroidism may be an important factor as methyl palmoxirate alone, which has been shown to correct both the above mentioned parameters did not affect the function, but used in combination with T₃ prevented the functional depression. However, the combination of carnitine and T₃ which would also be expected to normalise the depression of cardiac myosin ATPase and SR calcium uptake did not normalise the functional depression. These findings suggest that the combination of T₃ and methyl palmoxirate does something in addition to normalising myosin ATPase and SR calcium uptake.
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ABSTRACTS


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