DIABETES-INDUCED ALTERATIONS IN
ISOLATED RAT HEART PERFORMANCE

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in

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

We accept this thesis as conforming
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THE UNIVERSITY OF BRITISH COLUMBIA
March 1983
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Date May 20th, 1983
ABSTRACT

Chronic diabetic patients have a higher incidence of and mortality from cardiac disease. A wide spectrum of cardiac problems plague the chronic diabetic including coronary artery disease, congestive heart failure and diabetic cardiomyopathy. Cardiac disease in the diabetic is not simply due to accelerated atherosclerosis alone, but is also due to a combination of microangiopathy, autonomic neuropathy, and various other factors which produce biochemical, functional and structural alterations in the heart. Recently, cardiac function was studied in animals with experimentally-induced diabetes and cardiac dysfunction was reported in acute as well as chronic phases of experimental diabetes. Since cardiac disease is a consequence of long-standing diabetes in diabetic patients, investigation of myocardial function at various time points after induction of experimental diabetes would yield information regarding the development and progression of cardiac dysfunction in diabetes.

We therefore investigated cardiac function and pharmacology in isolated perfused working hearts obtained from 7, 30, 100, 180, 240 and 360-day alloxan and streptozotocin (STZ) diabetic and age-matched control hearts. Diabetes was induced in the rat by injecting either alloxan (65 or 40 mg/kg) or STZ (50 or 60 mg/kg) into the tail vein. Diabetic and age-matched control rats were sacrificed at various time points after the induction of diabetes and hearts were isolated and perfused on a working heart apparatus. Cardiac function was studied at various left atrial filling pressures and was expressed in terms of left ventricular developed pressure (LVDP), rate of rise of left
ventricular pressure (positive dP/dt) and rate of decline of left ventricular pressure (negative dP/dt). Dose-response curves to carbachol and isoproterenol were also performed. Blood samples were collected at the time of sacrifice, serum was separated and analyzed for insulin and glucose content.

Both alloxan and STZ produced diabetes in the rat as shown by fasting hypoinsulinemia and hyperglycemia. Cardiac function was not altered in 7-day alloxan and STZ diabetic rats. Depressed function at various left atrial filling pressures was seen in hearts isolated from 30-day alloxan diabetic rats but not in 30-day STZ diabetic rats. Hearts isolated from 100-day alloxan and STZ diabetic rats, 180- and 360-day STZ diabetic rats and 240-day alloxan diabetic rats, all exhibited cardiac functional abnormalities. Cardiac functional abnormalities observed in diabetic rats were depressed LVDP and positive and negative dP/dt at high left atrial filling pressures. Diabetic rat hearts exhibited no change in either sensitivity or responsiveness to the negative inotropic effect of carbachol at 7 and 30 days after induction of the disease. A sub-sensitivity to carbachol was observed in diabetic rat hearts at 100 days after induction of diabetes as compared to age-matched control rat hearts. However, 180- and 240-day diabetic rat hearts exhibited supersensitivity to the negative inotropic effect of carbachol. Isoproterenol produced an identical positive inotropic effect in control as well as diabetic rat hearts at all of the time points studied. However, the maximum changes produced by isoproterenol in negative dP/dt of diabetic rat hearts were depressed at various time points as compared to those in age-matched control rat hearts.
We also studied the effect of isoproterenol on the cyclic AMP content and phosphorylase activity in hearts obtained from 3 and 100 to 120 day control and diabetic rats. Basal cyclic AMP content and phosphorylase activity were not altered in acute and chronic diabetic and age-matched control rat hearts. Isoproterenol produced similar time- and dose-dependent changes in cyclic AMP content and positive and negative dP/dt in isolated perfused working hearts obtained from 3 and 100 to 120 day control and diabetic rats. However, isoproterenol caused a significantly greater activation of phosphorylase enzyme in hearts isolated from 3 and 100 to 120 day diabetic rats as compared to age-matched controls. Diabetic rat hearts had a significantly higher total phosphorylase activity at 100 to 120 days as compared to age-matched controls. Prostaglandin E₁, a drug which increases cyclic AMP content without altering phosphorylase activity in perfused rat hearts, increased phosphorylase activity in acute as well as chronic diabetic rat hearts but not in control rat hearts.

Cholinergic muscarinic receptors in the ventricles obtained from 180-day control and STZ diabetic rats were studied by performing radioligand binding studies. [³H]NMS was used as a radioligand to stereospecifically label all of the muscarinic receptor binding sites present in the ventricular membrane preparation. There was no change in either the receptor density or in the binding constants for antagonists and agonists at the muscarinic receptor site in 180-day diabetic rat hearts as compared to control. Ventricular noradrenaline content was estimated using an HPLC method, in 180-day alloxan and STZ diabetic and age-matched control rat hearts. There was no significant change in the
noradrenaline content of diabetic rat hearts.

Results obtained in the above studies demonstrate that various functional, pharmacological and biochemical alterations occur in the heart in experimental diabetes. Depressed cardiac performance was observed in isolated perfused diabetic rat hearts at various time points after the induction of diabetes and may represent the preclinical ventricular dysfunction phase of a developing diabetic cardiomyopathy. Changes noticed in the sensitivity of the diabetic myocardium towards the negative inotropic effect of carbachol may represent various stages of a parasympathetic autonomic neuropathy of the heart in diabetes. The unaltered positive inotropic effect of isoproterenol and unchanged noradrenaline content in diabetic rat hearts indicate the absence of a sympathetic autonomic neuropathy. The depressed cardiac relaxant effect (maximum changes produced in negative dP/dt) of isoproterenol in diabetic rat hearts suggest defects in cardiac muscle relaxation, Ca$^{2+}$ handling by the sarcoplasmic reticulum and perhaps ATP production and utilization. The enhanced sensitivity of the phosphorylase enzyme to agonists in diabetic rat hearts may be an outcome of alterations in Ca$^{2+}$ homeostasis and other acute metabolic derangements in the heart caused by diabetes. All these changes could contribute to the pathogenesis of a diabetic cardiomyopathy.

John H. McNeill, Ph.D.
Thesis Supervisor
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>xv</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CLASSIFICATION AND ETIOLOGY OF DIABETES</td>
<td>1</td>
</tr>
<tr>
<td>PATHOPHYSIOLOGY OF DIABETES MELLITUS</td>
<td>5</td>
</tr>
<tr>
<td>THE HEART AND DIABETES</td>
<td>19</td>
</tr>
<tr>
<td>PRODUCTION OF EXPERIMENTAL DIABETES, ALLOXAN AND STREPTOZOTOCIN</td>
<td>29</td>
</tr>
<tr>
<td>METHODS</td>
<td>37</td>
</tr>
<tr>
<td>ANIMAL MODEL</td>
<td>37</td>
</tr>
<tr>
<td>WORKING HEART APPARATUS</td>
<td>38</td>
</tr>
<tr>
<td>HEART PERFUSION</td>
<td>42</td>
</tr>
<tr>
<td>CARDIAC FUNCTION CURVES</td>
<td>44</td>
</tr>
<tr>
<td>DOSE-RESPONSE CURVES TO CARBACHOL</td>
<td>47</td>
</tr>
<tr>
<td>DOSE-RESPONSE CURVES TO ISOPROTERENOL</td>
<td>48</td>
</tr>
<tr>
<td>CYCLIC AMP AND PHOSPHORYLASE ENZYME STUDIES</td>
<td>49</td>
</tr>
<tr>
<td>CYCLIC AMP DETERMINATION</td>
<td>51</td>
</tr>
<tr>
<td>PHOSPHORYLASE (_a) ASSAY</td>
<td>51</td>
</tr>
<tr>
<td>MUSCARINIC RECEPTOR BINDING EXPERIMENTS</td>
<td>52</td>
</tr>
<tr>
<td>ESTIMATION OF NORADRENALINE CONTENT IN VENTRICLES</td>
<td>54</td>
</tr>
<tr>
<td>SERUM ANALYSES</td>
<td>56</td>
</tr>
<tr>
<td>SERUM IMMUNOREACTIVE INSULIN DETERMINATION,</td>
<td>56</td>
</tr>
<tr>
<td>SERUM GLUCOSE DETERMINATION</td>
<td>57</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Schematic representation of the working heart apparatus</td>
<td>41</td>
</tr>
<tr>
<td>2. Calculation of positive and negative dP/dt from left ventricular pressure trace</td>
<td>46</td>
</tr>
<tr>
<td>3. Effect of changing atrial filling pressure on left ventricular pressure development in 7 day control and diabetic rat hearts</td>
<td>80</td>
</tr>
<tr>
<td>4. Effect of changing atrial filling pressure on positive dP/dt in 7 day control and diabetic rat hearts</td>
<td>82</td>
</tr>
<tr>
<td>5. Effect of changing atrial filling pressure on negative dP/dt in 7 day control and diabetic rat hearts</td>
<td>84</td>
</tr>
<tr>
<td>6. Effect of changing atrial filling pressure on left ventricular pressure in 30 day control and diabetic rat hearts</td>
<td>86</td>
</tr>
<tr>
<td>7. Effect of changing atrial filling pressure on positive dP/dt in 30 day control and diabetic rat hearts</td>
<td>88</td>
</tr>
<tr>
<td>8. Effect of changing atrial filling pressure on negative dP/dt in 30 day control and diabetic rat hearts</td>
<td>90</td>
</tr>
<tr>
<td>9. Effect of changing atrial filling pressure on left ventricular pressure in 100 day control and diabetic rat hearts</td>
<td>92</td>
</tr>
<tr>
<td>10. Effect of changing atrial pressure on positive dP/dt in 100 day control and diabetic rat hearts</td>
<td>94</td>
</tr>
<tr>
<td>11. Effect of changing atrial filling pressure on negative dP/dt in 100 day control and diabetic rat hearts</td>
<td>96</td>
</tr>
<tr>
<td>12. Effect of changing atrial filling pressure on left ventricular pressure in 180 day control and diabetic rat hearts</td>
<td>98</td>
</tr>
</tbody>
</table>
13. Effect of changing atrial filling pressure on positive dP/dt in 180 day control and diabetic rat hearts

14. Effect of changing atrial filling pressure on negative dP/dt in 180 day control and diabetic rat hearts

15. Effect of changing atrial filling pressure on left ventricular pressure in 240 day control and diabetic rat heart

16. Effect of changing atrial filling pressure on positive dP/dt in 240 day control and diabetic rat hearts

17. Effect of changing atrial filling pressure on negative dP/dt in 240 day control and diabetic rat hearts

18. Effect of changing atrial filling pressure on left ventricular pressure in 360 day control and diabetic rat hearts

19. Effect of changing atrial filling pressure on positive dP/dt in 360 day control and diabetic rat hearts

20. Effect of changing atrial filling pressure on negative dP/dt in 360 day control and diabetic rat hearts

21. Effect of the duration of diabetes on positive dP/dt at 15 cm H₂O filling pressure in control and diabetic rat hearts

22. Effect of the duration of diabetes on negative dP/dt at 15 cm H₂O filling pressure in control and diabetic rat hearts

23. Dose-response curve of carbachol on basal positive dP/dt in 7 day control and diabetic rat hearts
FIGURE

24. Dose-response curve of carbachol on basal positive $dP/dt$ in 30 day control and diabetic rat hearts 122
25. Dose-response curve of carbachol on basal positive $dP/dt$ in 100 day control and diabetic rat hearts 124
26. Dose-response curve of carbachol on basal positive $dP/dt$ in 180 day control and diabetic rat hearts 126
27. Dose-response curve of carbachol on basal positive $dP/dt$ in 240 day control and diabetic rat hearts 128
28. Dose-response curve of carbachol on basal positive $dP/dt$ in 360 day control and diabetic rat hearts 130
29. Dose-response curve of isoproterenol on positive $dP/dt$ in 7 day control and diabetic rat hearts 133
30. Dose-response curve of isoproterenol on positive $dP/dt$ in 30 day control and diabetic rat hearts 135
31. Dose-response curve of isoproterenol on positive $dP/dt$ in 100 day control and diabetic rat hearts 137
32. Dose-response curve of isoproterenol on positive $dP/dt$ in 180 day control and diabetic rat hearts 139
33. Dose-response curve of isoproterenol on positive $dP/dt$ in 240 day control and diabetic rat hearts 141
34. Dose-response curve of isoproterenol on positive $dP/dt$ in 360 day control and diabetic rat hearts 143
35. Dose-response curve of isoproterenol on negative $dP/dt$ in 7 day control and diabetic rat hearts 146
36. Dose-response curve of isoproterenol on negative $dP/dt$ in 30 day control and diabetic rat hearts 148
FIGURE

37. Dose-response curve of isoproterenol on negative dP/dt in 100 day control and diabetic rat hearts 150
38. Dose-response curve of isoproterenol on negative dP/dt in 180 day control and diabetic rat hearts 152
39. Dose-response curve of isoproterenol on negative dP/dt in 240 day control and diabetic rat hearts 154
40. Dose-response curve of isoproterenol on negative dP/dt in 360 day control and diabetic rat hearts 156
41. Time course of the effect of isoproterenol on cardiac cyclic AMP content in 3 day control and diabetic rats 159
42. Time course of the effect of isoproterenol on cardiac cyclic AMP content in 100-120 day control and diabetic rats 161
43. Time course of the effect of isoproterenol on cardiac phosphorylase a activity in 3 day control and diabetic rats 163
44. Time course of the effect of isoproterenol on cardiac phosphorylase a activity in 100-120 day control and diabetic rats 165
45. Total phosphorylase activity in 3 and 100-120 day control and diabetic rat hearts 167
46. Time course of the effect of isoproterenol on the rate of left ventricular pressure rise in 3 day control and diabetic rat hearts 169
<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>47. Time course of the effect of isoproterenol on the rate of left ventricular pressure rise in 100-120 day control and diabetic rat hearts</td>
<td>171</td>
</tr>
<tr>
<td>48. Time course of the effect of isoproterenol on the rate of left ventricular pressure decline in 3 day control and diabetic rat hearts</td>
<td>173</td>
</tr>
<tr>
<td>49. Time course of the effect of isoproterenol on the rate of left ventricular pressure decline in 100-120 day control and diabetic rat hearts</td>
<td>175</td>
</tr>
<tr>
<td>50. Dose-response curve of isoproterenol on cardiac cyclic AMP content in 100-120 day control and diabetic rats</td>
<td>177</td>
</tr>
<tr>
<td>51. Dose-response curve of isoproterenol on cardiac phosphorylase a activity in 100-120 day control and diabetic rats</td>
<td>129</td>
</tr>
<tr>
<td>52. Dose-response curve of isoproterenol on the rate of left ventricular pressure development in 100-120 day control and diabetic rat hearts</td>
<td>181</td>
</tr>
<tr>
<td>53. Dose-response curve of isoproterenol on the rate of left ventricular pressure decline in 100-120 day control and diabetic rat hearts</td>
<td>183</td>
</tr>
<tr>
<td>TABLE</td>
<td>PAGE</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>I</td>
<td>76</td>
</tr>
<tr>
<td>II</td>
<td>77</td>
</tr>
<tr>
<td>III</td>
<td>78</td>
</tr>
<tr>
<td>IV</td>
<td>131</td>
</tr>
<tr>
<td>V</td>
<td>144</td>
</tr>
<tr>
<td>VI</td>
<td>157</td>
</tr>
<tr>
<td>VII</td>
<td>184</td>
</tr>
<tr>
<td>VIII</td>
<td>185</td>
</tr>
<tr>
<td>IX</td>
<td>186</td>
</tr>
<tr>
<td>X</td>
<td>187</td>
</tr>
</tbody>
</table>

I Certain features of control and diabetic rats
II Certain features of control and diabetic rats used in the time course study
III Features of control and diabetic rats
IV Geometric mean ED$_{30}$ values of carbachol
V Sensitivity of the rat myocardium to the positive inotropic effect of isoproterenol (pD$_2$ values)
VI Cardiac relaxant effect of isoproterenol
VII Effect of PGE$_1$ on cardiac cyclic AMP and phosphorylase a activity in control and diabetic rats
VIII Effect of 10$^{-6}$M indomethacin on isoproterenol-induced cardiac phosphorylase activity in 100-120 day control and diabetic rats
IX $[^3]$H] NMS binding in 180 day control and diabetic rat hearts
X Left ventricular noradrenaline content in 180 day control and diabetic rats
ACKNOWLEDGEMENTS

I am deeply grateful to Dr. John H. McNeill for his constant guidance and patience throughout the present study.

I would like to thank Dr. J.W. Wells for allowing me to carry out cholinergic receptor binding studies in his laboratory at the University of Toronto. I would also like to thank Dr. Vladimir Palaty for carrying out the HPLC determination of noradrenaline.

I wish to thank my research committee members for their genuine interest and valuable suggestions. I would also like to express my sincere thanks to Dr. Gary Lopaschuk, Dr. Kath MacLeod, Mr. David Harris, Ms. Danielle Wenkstern and to all my colleagues for their valuable help, encouragement and friendship.

My special thanks are due to Ms. Judy Wyne for her expert typing skills. The financial support of the Canadian Heart Foundation is gratefully acknowledged.
INTRODUCTION

CLASSIFICATION AND ETIOLOGY OF DIABETES:

In recent years, the disease diabetes has been recognized as a heterogeneous group of metabolic disorders characterized by inappropriate hyperglycemia and in more severe cases with accelerated lipolysis and excessive ketogenesis (Lefebvre and Luyckx, 1979). Most of the various forms of diabetes have a genetic background; however, the genetic mechanisms involved appear to vary (Salans, 1982). Recently, an international workgroup sponsored by the National Diabetes Data group (1979) has developed a new classification for diabetes and other categories of glucose intolerance. According to this classification, the heterogeneous syndrome diabetes has been divided into three major clinical classes and two statistical risk classes (Craig, 1980). The new classification is as follows:

Clinical classes:
1. Diabetes mellitus (DM)
2. Gestational diabetes (GDM)
and 3. Impaired glucose tolerance (IGT)

Statistical risk classes:
1. Previous abnormality of glucose tolerance (Pre AGT)
and 2. Potential abnormality of glucose tolerance (Pot AGT)

Diabetes mellitus (DM):

DM has been further categorized into three groups, which are:
Type I: insulin-dependent (IDDM)
Type II: non-insulin-dependent (NIDDM)
and other types of diabetes associated with certain conditions and genetic syndromes.

Type I: insulin-dependent diabetes mellitus (IDDM):

IDDM represents about 20% of all the clinically encountered cases of diabetes. The usual age of onset is childhood or early adulthood and it was therefore known previously as juvenile-onset diabetes. IDDM is usually associated with no detectable insulin activity in the blood and insulin administration is an absolute necessity for IDDM patients to prevent the development of ketosis. Total destruction of pancreatic β-cells is a common finding in IDDM. Islet cell destruction may result from autoimmunity or from a viral infection (Irvine, 1977). In most cases, a combination of autoimmunity and viral infection appear to be implicated in the development of IDDM. A viral invasion of pancreatic islet cells could result in damage of the islets which, in turn, would trigger antibody production and cytotoxicity directed against islet cells (Craig, 1980). In most of the cases of newly diagnosed IDDM, islet cell cytoplasmic antibodies were found and these antibodies disappeared with increasing duration of the disease (Lernmark and Baekkeskov, 1981). Pancreatic islet cell susceptibility to viral infection is thought to be genetically predetermined. IDDM has been shown to be frequently associated with certain histocompatibility antigen (HLA) linked genes such as HLA-B8, BW15 and B18 (Cudworth and Woodrow, 1976). However, studies with identical twins (Gottlieb and Root, 1968; Pyke, 1979) show only about a 50% concordance for IDDM between twins, suggesting a role for nongenetic factors in the expression of this disease. For example,
environmental factors such as viruses can precipitate diabetes in genetically susceptible individuals (Salans, 1982).

Type II: non-insulin-dependent diabetes (NIDDM).

The majority of the clinically encountered cases of diabetes are of the non-insulin-dependent type. NIDDM was formerly referred to as maturity-onset diabetes owing to the age at onset, which usually is in later adulthood. NIDDM is associated with either normal or low levels of circulating insulin and sometimes even with high levels. Patients with NIDDM do not usually require insulin therapy for the prevention of ketoacidosis as they are not ketosis-prone. Two types of abnormalities in insulin action are demonstrable in NIDDM. Firstly, lack of the first-phase (acute release of insulin) insulin response to an intravenous glucose challenge has been reported in NIDDM patients (Brunzell et al. 1976). It appears that glucose is a poor stimulator of insulin release in these patients. Defects in the hypothetical 'gluoreceptor' on the β-cell have been proposed to be responsible for the inability of glucose to release insulin (Robertson and Metz, 1979). Secondly, insensitivity of the target tissues (liver, adipose tissue and muscle) to the action of insulin has been shown to be a common feature of NIDDM (Reaven, 1980). The insensitivity to the action of insulin is referred to as insulin resistance and is a common feature of obesity. Most of the NIDDM patients are obese (Craig, 1980). Insulin resistance usually results in a compensatory rise in circulating insulin levels in patients with normally functioning β-cells, thereby producing hyperinsulinemia (Flier et al. 1979).
Almost all cases of NIDDM appear to have a genetic cause (Craig, 1980). Studies of diabetes in monozygotic twins show almost a 100% concordance for NIDDM between twins (Gottlieb and Root, 1968; Pyke, 1979). However, the genetic factors that contribute to the development of NIDDM appear to be different from those which determine IDDM, as no association was found between HLA and NIDDM (Ganda and Soeldner, 1977). Environmental factors also influence the expression of NIDDM. One of the important environmental factors for NIDDM is obesity (Salans, 1982).

Other types of diabetes:

Various types of diabetes that develop secondary to pancreatic disease, certain genetic syndromes, endocrine disorders or due to treatment with some hormones and drugs are grouped in this class (National Diabetes Data Group, 1979). These types of diabetes represent a very small proportion of clinically encountered diabetes cases.

Gestational diabetes (GDM):

Diabetes mellitus that transiently develops in a previously non-diabetic woman during pregnancy owing to the complex metabolic changes occurring in pregnancy is termed GDM. Usually GDM disappears after delivery but these patients face an increased risk of becoming overt diabetics in the future (Craig, 1980).

Impaired glucose tolerance (IGT):

This class is comprised of cases which are in the borderline
region between diabetes and normal glucose tolerance. Formerly this class was referred to as subclinical diabetes, borderline diabetes or latent diabetes. Individuals in this class have a higher risk of becoming diabetic. On the other hand they may remain in the intermediate stage or become completely normal. However, an increased prevalence of atherosclerotic disease has been reported to be associated with this class, suggesting the pathogenic nature of IGT (Keen, 1970).

Statistical risk classes:

Previous abnormality of glucose tolerance (Pre-AGT):

Individuals with normal glucose tolerance, but with a history of impaired glucose tolerance such as GDM belong in this class. These individuals however, are at a higher risk for overt diabetes.

Potential abnormality of glucose tolerance (Pot-AGT):

Individuals categorized in this group have never shown impaired glucose tolerance, but have a statistical risk of developing diabetes. Examples of such persons include monozygotic twins of either an insulin-dependent or a non-insulin dependent diabetic, first-degree relative of a non-insulin-dependent diabetic and persons with islet cell antibodies or HLA linked genes.

PATHOPHYSIOLOGY OF DIABETES MELLITUS:

In diabetes mellitus, regardless of its etiology, the major defect is insufficient insulin action on target tissues. Since insulin regulates carbohydrate, fat and amino acid metabolism in target tissues, in the event of inadequate insulin action a number of abnormalities can
be predicted to occur. Insulin exerts both short and long-term effects on the metabolism, in responsive target tissues (Denton et al. 1981). The short-term effects of insulin are on carbohydrate and lipid metabolism in liver, muscle and adipose tissue, whereas the long-term effects are mainly on protein metabolism and growth. The short-term effects of insulin include enhancement of glucose uptake into muscle and adipose tissue, stimulation of glycogen synthesis in liver and muscle, and synthesis of fatty acids and triglycerides in liver and adipose tissue. Insulin decreases glycogenolysis and gluconeogenesis in liver and lipolysis in adipose tissue. Insulin also exerts a few short-term effects on protein metabolism such as increasing the amino acid uptake into muscle and adipose tissue. The long-term effects of insulin include increasing the synthesis of both general and specific proteins, RNA and DNA, promotion of growth and inhibition of protein breakdown.

During an episode of acute insulin lack, all of the above documented short-term insulin dependent processes are inhibited and a number of metabolic abnormalities take place. Glucose uptake into muscle and adipose tissue is reduced, resulting in elevated blood glucose levels. Glycogenesis is impaired in liver and muscle, at the same time glycogenolysis and gluconeogenesis increase in the liver and large quantities of glucose are released into the blood stream, causing dramatic increases in blood glucose levels. When the blood glucose levels exceed the renal threshold for glucose, glucose starts to spill into the urine causing glucosuria. Increased amounts of glucose in the urine cause osmotic diuresis resulting in a loss of water, electrolytes and calories. This situation gives rise to the characteristic clinical
symptoms such as polyuria, polydipsia and polyphagia. In the absence of insulin, synthesis of fatty acids and triglycerides is reduced in the liver and lipolysis becomes uninhibited in adipose tissue. Large quantities of fatty acids are released into blood causing hyperlipidemia. Protein synthesis is impaired and protein breakdown is increased during prolonged insulin lack. Excessive lipolysis and protein breakdown result in weight loss, another characteristic symptom of IDDM.

Complications of diabetes mellitus:

Diabetes mellitus is associated with several secondary complications. Clinical and biochemical manifestations of diabetes include acute as well as chronic complications (Fajans and Freinkel, 1976). Acute complications result from either a sudden and severe insulin deficiency or loss of metabolic control due to severe stress or acute illness or certain additional drug therapies, while chronic complications of diabetes appear slowly and are consequences of various biochemical, structural and functional alterations produced by chronic hyperglycemia and other metabolic errors.

Acute complications of diabetes:

Acute metabolic complications often occur in diabetics and if not treated in time, can lead to coma and death. There are two types of acute complications.

1. Diabetic ketoacidosis with or without dehydration
2. Hyperosmolar non-ketotic diabetic coma.
Diabetic ketoacidosis or diabetic coma results whenever there is a profound insulin deficiency associated with changes in other hormones such as increased levels of growth hormone and glucagon (Foster, 1976). The following metabolic events and their interrelationships are considered to be involved in the development of ketoacidosis. Unimpeded gluconeogenesis brought about by the above mentioned hormonal changes, results in marked hyperglycemia followed by osmotic diuresis and dehydration. Excessive lipolysis in adipose tissue elevates free fatty acid levels in the blood. Free fatty acids are taken up by liver and oxidized to ketone bodies, which are released into the blood. Blood ketone levels gradually increase and cause metabolic acidosis leading to coma. Death may result from peripheral vascular collapse and renal failure. Hyperosmolar, non-ketotic diabetic coma is another life-threatening situation which most commonly occurs in elderly diabetic patients (Field, 1976). Very severe hyperglycemia is considered to be the main cause for the hyperosmolarity. However, azotemia and hypernatremia also contribute to the hyperosmolarity. This syndrome differs from diabetic coma in certain aspects. It is more insidious in development than diabetic coma and ketogenesis is completely absent due to either an impaired release of free fatty acids from adipose tissue or a decreased hepatic synthesis of ketone bodies (Podolsky, 1981). Plasma glucagon levels in hyperosmolar coma are higher than those in diabetic coma (Lindsey et al., 1974). Hyperosmolar non-ketotic coma is frequently associated with a number of neurological signs ranging from confusion to convulsions.
Chronic complications of diabetes:

With the discovery of insulin in 1921 (Banting et al. 1922) and the commercial availability of various forms of pure insulin in recent years, management of the acute metabolic complications of diabetes ceased to be a major problem for the clinician. However, the clinician has now been presented with an array of debilitating and life-threatening complications which characteristically appear in long-term diabetics (Kannel, 1978). In general three major groups of abnormalities have been recognized in chronic diabetics (Renold et al. 1978). These are: 1. A disease of the small blood vessels, which is also known as microangiopathy and is thought to be the underlying defect in diabetic retinopathy, nephropathy and perhaps cardiomyopathy. There appears to be a direct correlation between microangiopathy and the degree and duration of hyperglycemia. 2. A disease of medium and large size blood vessels, known as macroangiopathy, which is probably responsible for accelerated atherogenesis and a number of cardiovascular diseases such as coronary artery disease and myocardial infarction, cerebrovascular diseases and hypertension. Macroangiopathy is definitely more severe in patients with marked hyperglycemia, but has not been identified as a significantly prevalent problem in diabetics when compared to the non-diabetic population (Renold et al. 1978), and 3. A mixture of micro and macroangiopathies and certain other metabolic abnormalities giving rise to diabetic neuropathies, gestational problems and diabetic senile cataracts. Identification of the specific biochemical and pathophysiological processes underlying these secondary complications of long-term diabetes is very important in order to prevent or treat
them (Winegrad and Clements, 1976). Secondary complications have been found in both insulin-dependent and non-insulin-dependent diabetics and a feature common to these two types of diabetes is abnormal glucose tolerance. Therefore it is reasonable to assume that long-standing hyperglycemia and other metabolic abnormalities associated with it, play a role in the pathogenesis of the late complications of the diabetic. The following are some of the pathophysiological processes that occur in diabetes and probably are related to long-standing hyperglycemia.

The polyol pathway:

The polyol or sorbitol pathway is a minor pathway for glucose by which non-phosphorylated glucose is converted to fructose through the formation of an intermediate compound sorbitol (Hers, 1956). The reaction sequence is as follows:

1. \[ \text{D-glucose} + \text{NADPH} + H^+ \xrightarrow{\text{aldose reductase}} \text{sorbitol} + \text{NADP}^+ \]
2. \[ \text{sorbitol} + \text{NAD}^+ \xrightarrow{\text{sorbitol dehydrogenase}} \text{D-fructose} + \text{NAD} + H^+ \]

The enzymes involved in this pathway have been identified in many tissues such as the aorta, brain, kidney, lens, nerves, placenta and seminal vesicles (Gabbay, 1975). Most of these tissues do not require insulin for glucose uptake and during hyperglycemia the intracellular glucose concentration approaches that of blood. The large intracellular pool of glucose in these tissues is converted to sorbitol. Since sorbitol is unable to permeate through the cell membrane, large quantities of sorbitol accumulate intracellularly. Sorbitol can be converted to fructose, but the net result is accumulation of sorbitol...
and fructose as fructose is metabolized rather poorly in some of these tissues. Accumulation of sorbitol results in hypertonicity and osmotic swelling of these cells (Gabbay, 1973). It has been demonstrated that among various other tissues, lens, nerve and renal papilla accumulate sufficiently large quantities of sorbitol in diabetes (Gabbay, 1975). Accumulation of sorbitol in the lens has been shown to be accompanied with loss of osmotic integrity, electrolyte imbalance and various other changes finally resulting in lenticular opacity (Kinoshita et al., 1962). In diabetes, the activity of the sorbitol pathway has been demonstrated to be markedly elevated leading to accumulation of sorbitol and fructose in the nerves (Ward et al., 1972), particularly in the Schwann cells (Thomas and Lascelles, 1965). Schwann cells are responsible for the formation and regulation of myelin in the peripheral nervous system, and accumulation of polyols in Schwann cells somehow results in suppression of myelin production (Gabbay and O'Sullivan, 1968). These findings suggest a role for the polyol pathway in the pathogenesis of diabetic neuropathy (Gabbay, 1975; Giachetti, 1981). Sorbitol accumulation in the kidney cells is high enough in diabetes to cause osmotic disturbances and this pathway might contribute to the development of a diabetic nephropathy (Gabbay, 1975). Increased activity of the sorbitol pathway has also been implicated in producing vascular injury and microangiopathy (Williamson and Kilo, 1980).

Glycosylation of proteins:

Prolonged hyperglycemia has been shown to be associated with increased levels of glycosylated hemoglobin (Bunn et al., 1978) and
other serum proteins (McFarland et al., 1979). Glycosylation of hemoglobin is a non-enzymatic, nearly irreversible reaction and its rate is dependent on blood glucose concentration (Koenig and Cerami, 1980; Jovanovic and Peterson, 1981). This reaction involves addition of a molecule of glucose to the N-terminal valine of the \( \beta \)-chains of hemoglobin. In general various body proteins such as serum albumin, plasma proteins, insulin and lens crystalline protein undergo non-enzymatic glycosylation of N-terminal lysine groups in a manner analogous to that of hemoglobin (Rahbar, 1981). Glycosylation of hemoglobin increases with an increase in the blood glucose level and once synthesized remains in circulation until the end of 120-day life span of the erythrocytes. Therefore determination of glycosylated hemoglobin should give an estimation of blood glucose levels over a period of previous eight to twelve weeks (Koenig and Cerami, 1980). Measurement of glycosylated hemoglobin levels is presently used as an accepted method for assessing the control of diabetes in patients (Rahbar, 1981). Increased glycosylation of hemoglobin and other body proteins has been hypothesized to play a role in the pathogenesis of the late complications of diabetes (Renold et al., 1978). Glycosylation of hemoglobin has been shown to cause an increase in the oxygen affinity and a reduction in the sensitivity to 2,3-diphosphoglycerate (Bunn and Briehl, 1970), a regulator of hemoglobin oxygenation (Benesch and Benesch, 1969). Increased levels of glycosylated hemoglobin thus may cause tissue hypoxia and other vascular responses leading to the development of microangiopathy (Williamson and Kilo, 1980). Glycosylation of intracellular proteins might alter their function by bringing about changes in their solubility, activity and antigenicity (Koenig
and Cerami, 1980). Widespread glycosylation of various proteins has been implicated in the pathogenesis of diabetic cataracts, thickening of glomerular basement membrane and nephropathy, changes in myelin content and nerve metabolism leading to neuropathy, various microangiopathies and marked increases in carbohydrate-containing connective tissue in the heart resulting in a cardiomyopathy (Renold et al., 1978; Koenig and Cerami, 1980; Rahbar, 1981).

Circulating factors and circulatory changes:

Diabetic microangiopathy, a disease of small blood vessels, produces alterations in the microcirculation of various organs and is probably involved in the development of nephropathy, retinopathy and cardiomyopathy. Diabetes has been shown to cause changes in the circulating factors and hemorrheology and these changes might play a role in the pathogenesis of microangiopathy (McMillan, 1976). Viscosity of plasma and serum is elevated in diabetes and has been shown to be associated with an increase in red-blood cell aggregability (McMillan, 1976; Schmid-Schonbein and Volger, 1976). Red blood cells also exhibit decreased deformability and these changes in red cells cause alterations in blood-rheology in diabetics (Schmid-Schonbein and Volger, 1976). Elevated blood viscosity can be correlated with plasma protein changes. Acute-phase proteins such as α1-acid glycoprotein, complement proteins and fibrinogen are elevated in diabetes with a concurrent decrease in serum albumin. These changes in plasma proteins cause alterations in hemorrheology and plasma viscosity. Viscous blood creates resistance to flow and as a result pressure in the microcirculation increases (McMillan, 1976). Increased aggregability of platelets
has been demonstrated in diabetics (Colwell et al. 1976). Changes have also been shown to occur in various properties of polymorphonuclear leukocytes and lymphocytes in diabetes (Jones and Peterson, 1981).

Capillary wall permeability to small and large molecules has been shown to increase in diabetes (Parving, 1976; Bollinger et al. 1982). Due to the increased permeability of the microvasculature, plasma proteins leak out and during this process get deposited in the vessel wall, thereby providing the morphological basis for the development of a microangiopathy (Parving, 1976). The increased permeability of microvasculature has been postulated to result either from an increase of hydrostatic pressure in the microcirculation (Parving, 1976) or from the thickening of capillary basement-membrane (Williamson and Kilo, 1976).

Basement membrane changes:

One of the characteristic features of diabetic microangiopathy is the thickening of basement membrane (lamina densa) of capillaries that supply the glomerulus, retina, skin and skeletal muscle (Kefalides, 1981). Hyperglycemia, insulin deficiency and elevated levels of plasma growth hormone are some of the possible factors that might contribute to the pathogenesis of basement membrane thickening (Spiro, 1976; Kefalides, 1981). There appears to be a good correlation between thickening of capillary basement membrane and the duration of diabetes (Østerby, 1975). Thickening of capillary basement membrane could result from any one of or combinations of the following mechanisms,
which include a) increased synthesis, b) decreased degradation, c) deposition of serum proteins in basement membrane or d) increased turnover of basement membrane synthesizing cells (Kefalides, 1977).

Basement membrane is composed of collagenous glycoprotein material with hydroxyllysine-linked carbohydrate units (Spiro, 1967). In diabetes, the basement membranes are found to have abnormally high hydroxyllysine and hydroxyllysine-linked glycoside content suggesting an increase in collagen-like subunits. Concomitant increases in the activities of the enzymes protocollagen lysyl-hydroxylase, lysyl and prolyl hydroxylases and glycosyl transferases have also been found (Spiro, 1976; Kefalides, 1981). The pathophysiological significance of capillary basement membrane thickening in diabetic microangiopathy is not very clear. Williamson and Kilo (1977) have suggested that greatly thickened capillary basement membrane might contribute to defects in glomerular function and diabetic nephropathy, increased vascular permeability leading to defects in microcirculation and probably diabetic retinopathy. Capillary basement membrane thickening, however, can be used as a useful index for detection of the deleterious changes produced by diabetes on the vascular system.

Other hormonal changes in diabetes:

Although diabetes is considered as a disorder primarily characterized by an absolute or relative deficiency of insulin it is also associated with changes in various other hormones such as glucagon, growth hormone, somatostatin, thyroid hormones, etc.,
which may contribute either directly or indirectly to the pathophysiology of the disease. Glucagon, a polypeptide hormone secreted by the α-cells of pancreatic islets, is a potent hyperglycemic factor and its plasma levels are generally elevated in diabetes mellitus (Pek, 1977; Lefebvre and Luyckx, 1979). Since disturbances in glucagon secretion usually accompany insulin deficiency, diabetes has recently been referred to as a bihormonal disorder (Unger and Orci, 1975). It has been shown that in diabetes, the inhibitory effect of hyperglycemia on glucagon secretion is lost and in fact, in certain cases, elevated blood glucose levels may even increase glucagon concentrations. Another characteristic finding in diabetes is that a protein meal or arginine infusion produces an exaggerated glucagon response (Felig et al., 1976; Unger, 1978). Since glucagon has been shown to produce hyperglycemia by stimulation of glycogenolysis and gluconeogenesis in the liver, hyperglucagonemia observed occasionally in diabetes is expected to contribute to the hyperglycemia (Pek, 1977). Markedly elevated levels of glucagon have been found in diabetic ketoacidosis (Miller et al., 1973; Foster, 1976) and hyperosmolar non-ketotic coma (Lindsey et al., 1974), and may be involved in the pathogenesis of these acute complications.

Growth hormone is secreted by the anterior pituitary gland and it generally exhibits antagonistic effects to insulin on glucose tolerance. Growth hormone produces a reduction in the uptake and utilization of glucose by various tissues and thus cause a diabetogenic effect. Abnormal growth hormone secretion occurs in diabetes and insulin-dependent diabetics have been shown to have elevated levels
of growth hormone (Johansen and Hansen, 1969). Increased serum growth hormone levels in diabetes have been hypothesized to be involved in the pathogenesis of diabetic microangiopathy (Lundbaek, 1976). Growth hormone has also been proposed to be one of the causal factors which cause capillary basement-membrane thickening (Kefalides, 1981). Hypophysectomy or suppression of growth hormone has been shown to produce an improvement of retinopathy in diabetic patients (Wright et al. 1969), thus suggesting a role for growth hormone in the development of a retinopathy.

Somatostatin, a hormone secreted by both the hypothalamus and the D-cells of the islets of Langerhans, is able to inhibit release of glucagon, growth hormone and insulin. Studies with somatostatin have helped in the understanding of the role of glucagon in diabetes, and somatostatin has even been tried therapeutically in order to suppress glucagon and growth hormone in diabetic patients but without much success (Tzagournis, 1982). However, the exact physiologic role of somatostatin in diabetes is unclear. Elevated somatostatin levels and an increase in the density of somatostatin containing D-cells have been demonstrated in diabetics as well as in experimentally diabetic rats (Orci et al. 1976; Patel et al. 1980).

Hypothyroidism is a frequent finding in diabetic patients (Cooppan, 1982). The thyroid hormones thyroxine (T4) and triiodothyronine (T3) are produced by the thyroid gland and the thyroid gland secretes about 8 to 10 times more T4, compared to T3. In the liver and kidney, T4 is monodeiodinated to form T3 and reverse T3, a biologically inactive compound. Thyroid hormone metabolism appears to be altered in
diabetes mellitus resulting in a decreased production of T₃. Diabetic ketoacidosis has always been found to be associated with low T₃ levels (Cooppan, 1982). Recently a number of reports have demonstrated that in diabetes, most of the monodeiodination reactions are impaired resulting in low serum levels of T₃ (Madsbad et al., 1981; Pittman, et al., 1981). However, the contribution of the prolonged low serum T₃ syndrome to various pathological manifestations of diabetes mellitus is not known at the present time (Pittman et al., 1979).

Cellular abnormality:

All of the pathological manifestations of diabetes mellitus have been proposed to result from an underlying somatic cell defect which may be acquired genetically. The defect in somatic cells is characterized by enhanced cell death and replenishment, thereby causing an accelerated somatic cell turnover. The increase in somatic cell turnover rate probably reflects a high susceptibility of cells to injurious events in diabetes (Vracko and Benditt, 1974). Most of the cellular lesions such as basement-membrane thickening, atherosclerotic lesions in large vessels, behavioural alterations in fibroblasts etc. that occur quite early in diabetics, have also been shown to occur in non-diabetics, but only with increasing age. Studies involving estimation of collagen content and cross-linking have shown that diabetic collagen has undergone accelerated aging (Hamlin et al., 1975). It has also been proposed that premature senescence of β-cells is responsible for the development of NIDDM (Craig, 1980). From the above documented evidence, Renolds et al. (1978) have suggested that diabetes is a form of premature cellular senescence.
THE HEART AND DIABETES:

Chronic diabetics in general have a higher incidence of and mortality from cardiac disease (Kannel, 1978). A wide spectrum of cardiac problems plague the chronic diabetic and include coronary artery disease, congestive heart failure and the newly recognized clinical entity, diabetic cardiomyopathy (Smith, 1982). Coronary artery disease has been recognized as one of the most common causes of death in middle-aged, non-insulin-dependent diabetics (Knowles, 1978 and 1982) and is estimated to be responsible for at least 40-50% of diabetic deaths (Bennett, 1982). Autopsy studies comparing the frequency of coronary artery disease in diabetics and non-diabetics showed a prevalence rate ranging from 18 to 75% in diabetic patients (Knowles, 1978; Smith, 1982). A number of epidemiologic studies reported on the prevalence and incidence of coronary artery disease in living diabetic populations during the follow-up periods. The University Group Diabetic Programme (UGDP) study indicated a prevalence rate of 9.5% and an incidence rate of 12% for coronary artery disease in diabetics over a period of 6.5 years (Knowles, 1978). In the Framingham study, a prevalence rate of 1.6% and an incidence rate of 17% have been reported for coronary artery disease in the diabetic population (Kannel et al., 1961; Garcia et al., 1974). Similar data were obtained from various other population studies and led to the conclusion that the frequency of coronary artery disease in diabetics was about two and one-half times more than that in non-diabetics (Knowles, 1982). These population studies have also shown that diabetes increases the risk for coronary artery disease in the female more than in the male.
Coronary artery disease is clinically manifested in the diabetic as angina pectoris, myocardial infarction, sudden cardiac death and occasionally as congestive heart failure (Palumbo et al. 1982). Angina pectoris and acute myocardial infarction are more common in the diabetic population than in the general population. Myocardial infarction is also more severe in diabetics and results in a higher rate of in-hospital mortality, which again in the case of female diabetics is considerably higher compared to male diabetics (Partamian and Bradley, 1965). Recovery from acute myocardial infarction is also poor in diabetic patients (Smith, 1982). Occasionally acute myocardial infarction in diabetics is not associated with any chest pain and offers a diagnostic problem to the physician (Bradley and Schonfeld, 1962). This type of painless myocardial infarction is considered to be secondary to a diabetic neuropathy affecting the autonomic nerve control of the heart (Fearman et al. 1977). Myocardial infarction is known to produce a state similar to diabetes in a number of patients owing to the increased production of cortisol, catecholamines, glucagon and growth hormone (Opie, et al. 1979b). The beneficial effect of promoting glucose metabolism in the place of free fatty acid metabolism on ischemic and infarcted myocardium has been emphasized by Opie (1975). In diabetes, myocardial metabolism has been subjected to various alterations which result in a decrease in glucose metabolism and an increase in free fatty acid metabolism in the heart. These changes would decrease the resistance of the diabetic myocardium to anoxia and ischemia and thus increase the severity of myocardial infarction.
Coronary heart disease of the diabetic has been reported to be due to severe atherosclerosis which occurs at an earlier age (Rubler, 1977). Disease of large blood vessels, also known as macroangiopathy was considered to be responsible for the high prevalence of atherosclerotic heart disease in diabetics. However, many investigators have failed to differentiate morphologically macroangiopathy in diabetics from atherosclerotic changes that occur in non-diabetics, except that the atherosclerotic lesion in diabetics is much more frequent (Berkman and Zucker, 1978; Stout, 1979). The increased frequency of atherosclerosis in diabetics can be attributed to risk factors such as hypertension, obesity, hypercholesterolemia, hypertriglyceridemia and hyperlipoproteinemia which are more common to diabetics as compared to non-diabetics (Rubler, 1977; Berkman and Zucker, 1978).

Diabetics also have a higher incidence of congestive heart failure compared to non-diabetics (Kannel, 1978). The increased incidence of congestive heart failure is not always a consequence of co-existing coronary heart disease. Epidemiological data suggested that diabetics devoid of atherosclerosis or hypertension have also faced a five-fold increased risk for congestive heart failure. The increased risk for congestive heart failure has also been shown to be confined more to insulin-treated diabetics (Kannel, 1974; Jarrett, 1977). In the absence of atherosclerosis, it is believed that congestive heart failure is a consequence of myocardial small vessel disease, since a number of reports have pointed to a possible relationship between the extent of clinically detectable microangiopathy and the
degree of left ventricular dysfunction (Shapiro, 1982). Evidence for the existence of microangiopathy in the diabetic myocardium has been provided by recent reports showing thickening of myocardial capillary basement-membrane (Fischer et al. 1979) and capillary microaneurysms in the hearts of diabetic patients (Factor et al. 1980). Finally, it has also been noted that chronic diabetics, particularly the insulin-dependent ones, have been found to have obscure cardiomyopathies, which might arise from either a small vessel disease, impaired myocardial metabolism or some other pathological processes peculiar to diabetes (Kannel, 1978).

A number of studies have demonstrated the existence of myocardial dysfunction in diabetic patients in the absence of significant coronary heart disease (Regan et al. 1977; D'elia et al. 1979). Recently Ledet et al. (1979) have correctly pointed out that the cardiac disease of the diabetic is not simply due to atherosclerosis alone, but is also due to other factors such as microangiopathy and autonomic neuropathy which give rise to a specific type of cardiomyopathy. Evidence for the existence of a specific cardiomyopathy in chronic diabetics has been provided by a number of clinical studies. Rubler et al. (1972) have demonstrated a cardiomyopathy characterized by left ventricular hypertrophy and diffuse myocardial fibrosis in chronic diabetic patients. These patients did not show any major coronary artery disease, but had proven glomerulosclerosis. A significantly high prevalence of idiopathic cardiomyopathy has been demonstrated in chronic diabetic patients by Hamby et al. (1974). Functional abnormalities in myocardial microcirculation and capillary transport owing to the development of
microangiopathy have been proposed as the underlying cause for the cardiomyopathic condition. Recently, a number of clinical studies have demonstrated preclinical left ventricular dysfunction in diabetics. Left ventricular function was studied using the non-invasive systolic time interval technique. Diabetics have been shown to have a prolonged pre-ejection period (PEP), a shortened left ventricular ejection time (LVET) and an abnormally high PEP to LVET ratio (Ahmed et al. 1975; Seneviratne, 1977). These abnormalities suggest a defect in the rate of myocardial contraction. The abnormalities in left ventricular function were attributed to pathological alterations in the interstitium of cardiac muscle (Ahmed et al. 1975) and to microangiopathy (Seneviratne, 1977). More recently Shapiro et al. (1981a) have studied left ventricular function in a large group of diabetic patients using non-invasive techniques and found left ventricular dysfunction in the majority of cases. These abnormalities were mainly in diastole and included a significantly prolonged isovolumic relaxation time and a delay in mitral valve opening relative to minimal dimension or aortic valve closure. Abnormal systolic time intervals have also been noted frequently in these patients. Preclinical left ventricular dysfunction has been observed in both insulin-dependent and non-insulin dependent diabetics; however, it was more common in insulin-dependent diabetics. In another study Shapiro et al. (1981b) demonstrated a close relationship between abnormal left ventricular function and the severity of microvascular complications. These authors have also proposed that myocardial dysfunction in diabetes, which initially is seen as an impairment in diastolic relaxation, may occur fairly early during the course of the
disease, and when more extensive, is found both in relaxation and contraction. Hemodynamic abnormalities such as elevated end-diastolic pressure and decreased end-diastolic and stroke volumes have been found in diabetics without heart failure (Ahmed and Regan, 1982). Histopathological changes that are found to accompany a cardiomyopathic condition in diabetics include, cardiac dilatation and hypertrophy of muscle fibres, endothelial proliferation, perivascular and interstitial fibrosis, increased accumulation of periodic acid-Schiff (PAS) positive or glycoprotein material and focal necrosis (Badeer and Zoneraich, 1978; Shapiro et al. 1981b; Ahmed and Regan, 1982). All the above histopathological changes occur independently of coronary atherosclerosis.

Autonomic neuropathy is one of the major complications of chronic diabetes and is responsible for disturbances in cardiovascular, gastrointestinal, urogenital, endocrine, respiratory and thermo-regulatory systems (Clarke et al. 1979). Autonomic neuropathy of the heart, although not frequent in occurrence, is associated with a high mortality rate (Clarke et al. 1979; Feldman, 1981). Cardiovascular abnormalities owing to the loss of parasympathetic activity are more frequent and have been reported to occur quite early during the development of a diabetic autonomic neuropathy (Wheeler and Watkins, 1973; Bennett et al. 1975). The early detectable features of defective parasympathetic control of the heart are persistent resting tachycardia (Rundles, 1945; Wheeler and Watkins, 1973) and loss of beat-to-beat variation or sinus arrhythmia during deep breathing (Lloyd-Mostyn and Watkins, 1975; Watkins and MacKay, 1980; Feldman, 1981). The defect in parasympathetic innervation of the diabetic heart has been shown occasionally to
progress to the extent of total vagal denervation (Lloyd-Mostyn and Watkins, 1976). In contrast to parasympathetic neuropathy, defects in the sympathetic innervation of the heart occur very late and are often associated with other systemic complications such as diarrhea and impotence (Hosking, et al. 1978; Clarke et al. 1979). Orthostatic hypotension is one of the most significant consequences of a sympathetic autonomic neuropathy of the cardiovascular system (Hosking et al. 1978; Clarke et al. 1979). Painless myocardial infarction, which occurs frequently in chronic diabetics, is yet another consequence of an autonomic neuropathy of the heart (Clarke et al. 1979; Feldman, 1981). Post-mortem histopathological studies of the autonomic nerves in diabetics with neuropathy have revealed severe loss of myelinated axons, collagen deposits and distended giant ganglia with vacuoles in the vagus nerve and other sympathetic nerves (Duchen et al. 1980).

Recently a number of studies have been published in which cardiac function was investigated in animals with experimentally-induced diabetes. Cardiac functional alterations independent of vascular abnormalities have been demonstrated to occur in dogs with chronic alloxan-induced diabetes (Regan et al. 1974). Diabetic dog hearts exhibited altered diastolic pressure-volume relationships, which were attributed to increased ventricular wall stiffness owing to accumulation of PAS positive glycoprotein-like material. More recently, Regan et al. (1981) have also demonstrated that the above functional and structural alterations were not prevented by insulin-replacement. Cardiac function, metabolism and biochemistry have been studied quite
extensively in isolated perfused working hearts obtained from rats with chemical-induced diabetes. Acute alloxan diabetic rat hearts have been reported to exhibit a reduced tolerance to severe ischemia (Feuvray et al., 1978) or prolonged anoxia (Ingebretsen et al. 1980). Isolated perfused working hearts from acute alloxan-diabetic rats have also been shown to have reduced abilities to develop normal systolic (aortic) pressure (Miller, 1979), basal left ventricular pressure and rate of rise of left ventricular pressure (Ingebretsen et al. 1980) in response to increased cardiac work. Reductions in cardiac output, stroke work, peak left ventricular pressure and maximal rate of decline in left ventricular pressure were observed in isolated perfused working hearts obtained from chronic streptozotocin diabetic rats, in response to high atrial filling pressures (Penpargkul et al. 1980). Furthermore, left ventricular papillary muscles isolated from chronic streptozotocin diabetic rats have been reported to exhibit depressions in the rate of relaxation and velocity of shortening at various loads (Fein et al. 1980).

A number of changes have been reported to occur in the metabolism and various biochemical events in the hearts of experimentally diabetic animals. Multiple abnormalities occur in carbohydrate and lipid metabolism in the heart in diabetes. Glucose uptake into cardiac cells is decreased and so is glucose phosphorylation and glycolysis. As a result free glucose accumulated within the cell and cardiac glycogen content increased in spite of decreased glycogen synthase activity (Opie et al. 1979a). Lipid metabolism is increased in diabetic hearts owing mainly to the increased availability of free fatty acids in the
blood. A marked increase in the triglyceride content of the diabetic myocardium has been reported (Paulson and Crass, 1980). Feuvray et al. (1979) have demonstrated increased tissue levels of total CoA, long-chain acyl CoA and long-chain acylcarnitine esters in isolated working hearts from alloxan-diabetic rats. Increased levels of these intermediates of fatty acid metabolism have been proposed to be involved in the development of cardiac functional disturbances (Farah and Alousi, 1981). Acute experimental-diabetes has also been found to result in reduced levels and production of ATP (Allison et al., 1976) decreased protein synthesis (Williams et al., 1980) and changes in myosin components (Dillmann, 1980) in the heart.

Various biochemical processes have been found to be altered in experimentally-induced diabetes and these alterations may contribute to the cardiac dysfunction observed in diabetic animals. Depressions in the activity of myofibrillar basal and Ca$^{2+}$ stimulated ATPases, acto-myosin ATPase and Ca$^{2+}$ ATPase of pure myosin have been demonstrated in streptozotocin diabetic rats (Dillmann, 1980; Malhotra et al., 1981; Pierce and Dhalla, 1981). Cardiac sarcoplasmic reticulum from diabetic rats has been shown to exhibit a decreased ability to take up calcium and a depressed activity of the membrane-bound Ca$^{2+}$ ATPase (Penpargkul et al., 1981; Lopaschuk et al., 1983). It has been postulated that the elevated levels of long-chain acylcarnitines in diabetic hearts inhibit Ca$^{2+}$ uptake and Ca$^{2+}$ ATPase activity of sarcoplasmic reticulum (Lopaschuk et al., 1983). The ability of acylcarnitine esters to inhibit Ca$^{2+}$ ATPase activity of cardiac sarcoplasmic reticulum (Pitts et al., 1978) and cardiac Na$^+$, K$^+$, ATPase activity (Wood et al., 1977)
Very few reports have been published thus far in which the effects of exogenously administered cholinergic and adrenergic drugs have been studied on the heart in experimentally-induced diabetes. Foy and Lucas (1976 and 1978) have reported reductions in the inotropic effects to exogenous adrenergic amines and the depressor response to acetylcholine in both pithed diabetic rats and in isolated diabetic rat atria. These authors also reported that the chronotropic responses to isoproterenol were reduced in pithed diabetic rats whereas they were increased in isolated rat atrial preparations. Ingebretsen et al. (1981) have reported no change in the dose-dependent inotropic effect of isoproterenol in isolated working hearts from acute alloxan-diabetic rats. The above documented studies were performed with acute (7 to 14 day) diabetic rats. The only study in which chronic diabetic rats have been used to study the effects of cardioactive drugs is by Tomlinson and Yusof (1981). These investigators, using isolated atria obtained from 7 to 8 month alloxan-diabetic rats, have demonstrated no change in the responses of diabetic atria to noradrenaline and a supersensitivity to the negative inotropic effect of acetylcholine. Autonomic neuropathy has been demonstrated in chronic streptozotocin-diabetic rats by Schmidt et al. (1981) and Schmidt and Schapp (1982), using ultrastructural, histochemical and biochemical methods. Swollen degenerating unmyelinated terminal axons were frequently found in the colon and mesenteric nerves of chronic diabetic rats. Histochemical studies showed an overall decrease in the intensity of catecholamine fluorescence and cholinesterase staining in chronic diabetic rats. Decreased activities
of the enzymes dopamine β-hydroxylase and choline acetyltransferase have also been found.

Recently a number of studies have been published in which the effects of experimental-diabetes on cardiac cyclic AMP and phosphorylase were investigated. Chaudhury and Shipp (1973) have demonstrated that basal cardiac cyclic AMP levels were four-fold higher in acute alloxan-diabetic rats, whereas several other reports showed no changes in either basal adenylate cyclase activity or basal cyclic AMP content in hearts isolated from acute diabetic rats (Das, 1973; Menahan et al. 1977; Ingebretsen et al. 1981; Miller et al. 1981). However, Ingebretsen et al. (1981) reported a 50 percent reduction in isoproterenol-induced increases in cardiac cyclic AMP content in acute alloxan-diabetic rats, but Miller et al. (1981) failed to show such a reduction in the effect of epinephrine on cardiac cyclic AMP in a similar diabetic rat model. Basal cardiac phosphorylase a activity has been shown to be unaltered in acute diabetic rats (Das, 1973; Ingebretsen et al. 1981; Miller et al. 1981). However Miller et al. (1981) have reported that epinephrine caused a significantly enhanced activation of phosphorylase a in acute diabetic rat hearts, and this observation has been partially confirmed by Ingebretsen et al. (1981), as these authors showed only a slight, but not significant, enhancement in the activation of phosphorylase by isoproterenol in acute diabetic rat hearts.

PRODUCTION OF EXPERIMENTAL DIABETES, ALLOXAN AND STREPTOZOTOCIN:

Diabetes mellitus can be produced in laboratory animals with chemical agents which cause immediate damage to the β-cells of the islets of Langerhans. These chemical substances have been referred to as
β-cytotoxic agents or β-cytotoxins (Rerup, 1970). The most important of these compounds are alloxan and streptozotocin (STZ). Alloxan was first reported to have diabetogenic activity by Dunn and McLetchie in 1943 and since then has been widely used to induce experimental diabetes in laboratory animals. The diabetogenic effect of STZ was first discovered by Rakieten et al. in 1963.

Alloxan monohydrate:

Alloxan (2,4,5,6-tetraoxohexahydropyrimidine) is usually employed in its monohydrate form. It is freely soluble in water and its stability is pH and temperature dependent. Alloxan is quite stable in acidic solution, but is highly unstable in neutral solution at room temperature. Its half-life in biological fluids such as blood is less than 1 minute. Alloxan has been reported to produce diabetes in almost all of the laboratory animal species tested except the guinea-pig (Rerup, 1970). Alloxan has been shown to be effective by all routes of administration; nevertheless, the intravenous route is most preferred since it is highly effective and less toxic (Lazarow and Palay, 1946). The diabetogenic dose of alloxan in rats varies between 30 to 70 mg/kg and is subject to variations in mean sensitivity within strains and between laboratories (Rerup, 1970). Alloxan administration has been shown to produce a characteristic triphasic response in blood glucose levels (Lundquist and Rerup 1967). These phases are:

1. An initial hyperglycemia of short duration, probably due to increased glycogenolysis in the liver and insulin deficiency;
2. A transient hypoglycemia which lasts up to 48 hours and often results in convulsions and death. This phase could be due to either a surplus
of insulin or a complete lack of glucose production in the liver and 3. A permanent hyperglycemia representing alloxan diabetes. The mechanism of action of the \( \beta \)-cytotoxic effect of alloxan is not very clear. Recently several hypotheses have been put forward to explain the highly specific \( \beta \)-cytotoxic effect of alloxan. According to one of these hypotheses (Boquist, 1980) alloxan is supposed to pass the \( \beta \)-cell membrane, probably through a glucose transport system. Inside the \( \beta \)-cell, alloxan appears to produce an increased concentration of inorganic phosphate (Pi) in the cytosol by inhibiting a sulfhydryl-dependent transport system for phosphate in the mitochondria. The increased concentration of Pi in the cytosol is believed to produce a fall in intracellular pH and a change in intracellular \( \text{Ca}^{2+} \). A fall in the cytosolic pH not only increases the stability of alloxan thereby prolonging its action but also inhibits the synthesis and glucose-induced release of insulin. Blockade of mitochondrial phosphate transport may result in the inhibition of NAD-dependent oxidative reactions including oxidative phosphorylations. In the absence of energy production, necrosis of the \( \beta \)-cell occurs resulting in the development of diabetes. More recently a new and alternative hypothesis has been put forward by Malaisse (1982) for the highly selective \( \beta \)-cytotoxic effect of alloxan. This hypothesis deals with two distinct features of alloxan which may be responsible for the \( \beta \)-cytotoxic effect. The first is the rapid rate at which alloxan is taken up by the pancreatic islet cells and the second is the ability of alloxan to undergo reduction to dialuric acid in the presence of reducing agents. Alloxan and dialuric acid then form a reduction-oxidation cycle resulting in
the generation of highly reactive oxygen-containing free radicals such as $O_2^\cdot$, $H_2O_2$ and $OH^\cdot$. Islet cells are highly sensitive to the toxic effects of these free radicals. This hypothesis partly explains why guinea pigs are the least sensitive species to the diabetogenic effect of alloxan since guinea pig islet cells have been shown to be at least 5 times less sensitive than rat islets to the toxic effects of tert-butyl hydroperoxide, a compound often used as an exogenous peroxide (Malaisse-Lagae et al. 1981).

Streptozotocin (STZ):

STZ (2-deoxy-2-(3-methyl-3-nitrosourea)1-d-glucopyranose) is a broad-spectrum antibiotic isolated from Streptomyces achromogenes (Herr et al. 1960). STZ has been reported to possess carcinogenic, antitumor and diabetogenic effects in addition to its antibiotic properties (Srivastava et al. 1982). STZ is an unstable compound at either room or refrigerator temperatures and must be stored below -20°C. It is freely soluble in water, but is very unstable in solution. STZ, however, is quite stable in solution at pH 4.0 and low temperatures (Rerup, 1970). STZ produces diabetes in most of the laboratory animal species tested. In the rat a single intravenous injection of 50 mg/kg was reported to produce 100% diabetes (Rakieten et al. 1963). Due to the unstable nature of STZ in solution, a rapid intravenous injection is the most preferred route of administration. STZ has been shown to produce a characteristic triphasic response in blood sugar levels which was similar to that observed after administration of alloxan (Junod et al. 1969). The diabetogenic effect of STZ has been shown to be dose-dependent (Junod et al. 1969) and inversely
related to the age of the animals (Masiello et al. 1979). The mechanism of the diabetogenic action of STZ is not known, and any of the following effects of STZ might be involved in its β-cytotoxicity. Pancreatic islet cells exhibit a marked specificity and capacity to take up STZ. STZ is believed to be taken-up through a specific transport system (Srivastava et al. 1982) and the glucose moiety of STZ is necessary for the transport process (Agarwal, 1980). STZ has been shown to cause a marked depletion of liver and islet NAD and NADH levels, within 24 hours after administration and this effect might contribute to the diabetogenic action (Rerup 1970; Agarwal, 1980) as pretreatment with nicotinamide has been shown to protect against STZ-induced diabetes (Schein et al. 1967). Histopathological changes observed after the administration of STZ include: hypertrophy of β-cell Golgi apparatus, nuclear pyknosis and cytoplasmic vacuolization resulting in degeneration of β-cell (Agarwal, 1980; Srivastava et al. 1982).

Comparison of alloxan- and STZ-induced diabetes:

Diabetes mellitus induced by either alloxan or STZ exhibited the classical symptoms of type-I diabetes (IDDM) such as polydipsia, polyphagia, polyuria, glycosuria and weight loss (Rerup, 1970). However, certain differences have been reported in the metabolic abnormalities caused by alloxan or streptozotocin. Mansford and Opie (1968) have demonstrated that alloxan diabetes is characterized by hyperglycemia, elevated free fatty acid levels in the blood and ketosis, whereas STZ diabetes only exhibited hyperglycemia without any changes in blood free fatty acid and
ketone levels. On the contrary, Junod et al. (1969) showed that the severity of STZ-diabetes is dose-dependent and larger doses of STZ produced diabetes with ketonuria. Recently, Opie et al. (1979a) have reported that acute STZ-induced diabetes is associated with elevated blood levels of free fatty acids and ketone bodies and also that Mansford and Opie (1968) have incorrectly contrasted acute alloxan-diabetes with chronic streptozotocin-diabetes in their study. Furthermore Hofteizer and Carpenter (1973) have reported that the clinical courses of alloxan- and streptozotocin-induced diabetes were almost similar. From the above discussion it is evident that both alloxan and STZ produce diabetes in laboratory animals in a dose-dependent manner and the diabetic state produced by either drug very closely resembles the insulin-dependent type of diabetes.

THE PURPOSE OF THE INVESTIGATION:

From the information documented thus far in this section, it is evident that cardiac function, metabolism, biochemistry and to some extent pharmacology have all been studied in experimental diabetic animal models. In most cases acute diabetic animal models were used in these studies. Nevertheless, there have been a few studies in which chronic diabetic animal models were used. In general, cardiac complications of diabetic patients are a consequence of long-standing diabetes and rarely appear in the early phases of clinical diabetes. In a recent review, Farah and Alousi (1981) have pointed out a need for the re-evaluation of the morphological, functional and biochemical conditions in hearts obtained from chronic diabetic animals, instead
of those obtained from animals with acute diabetes. We therefore investigated cardiac function, pharmacology and certain biochemical events in isolated perfused hearts obtained from diabetic rats at various time points after induction of the disease. We examined three important phases in the course of the experimental disease, namely acute, moderately chronic and chronic phases, in order to obtain information regarding when cardiac functional alterations appeared and whether these alterations progressed with the disease state.

We chose the rat as a model since it has been known for some time that atherosclerotic complications do not readily develop in this model. In fact in a recent report Chobanian et al. (1982) have pointed out that macrovascular disease was not common in rats made diabetic with either alloxan or streptozotocin. By using such a model, one would be able to investigate cardiac functional alterations that might be occurring independently of coronary artery disease and such functional alterations may represent the development of a specific heart muscle disorder also known as diabetic cardiomyopathy.

We used both alloxan and streptozotocin to induce diabetes in the rat. Most of the acute studies that have been published thus far were done on alloxan-diabetic rats and the chronic studies were done predominantly on STZ-diabetic rats. By employing both of these diabetogenic drugs to induce diabetes, we would be able to compare our results with almost all of the previously published information on acute as well as chronic diabetic animals regardless of which diabetogenic drug was employed. At the same time we could also compare these
two models with regard to the onset and progression of cardiac complications. We used the isolated perfused working heart preparation to study cardiac performance and the effects of cardioactive drugs. The working heart preparation has advantages over the conventional Langendorff preparation since the cardiac performance of the working hearts more nearly approximates physiological conditions and left ventricular function can be estimated with more accuracy. Finally, we used age-matched rats as controls for the diabetic rats in order to nullify the effects of aging on cardiac function.

SPECIFIC AIMS:

1. To study cardiac function in alloxan and STZ diabetic and age-matched control rats at various time-points after the induction of diabetes.

2. To study the negative inotropic effect of carbachol on control and diabetic rat hearts at various time-points.

3. To study the positive inotropic and cardiac relaxant effects of isoproterenol on control and diabetic rat hearts.

4. To study the time-course of the effect of isoproterenol on cyclic AMP and the phosphorylase system in acute as well as chronic diabetic and control rat hearts.
ANIMAL MODEL:

Female Wistar rats weighing 175-200g, corresponding to 9-12 weeks of age, were used throughout the study. The rats were allowed to recover from stress of transportation for a week before they were given any treatment. The rats were randomly divided into 3 groups, the first two groups were made diabetic and the third group was used as age-matched controls. Diabetes was induced by single intravenous injection of either alloxan monohydrate or STZ dissolved in 0.1 M citrate buffer, pH 4.5. Both alloxan and streptozotocin are unstable in solution but are relatively more stable in acidic solution compared to either basic or neutral solution. Solutions of alloxan and STZ were freshly made before injecting and were kept on ice. Initially diabetes was induced by using either 45 mg/kg alloxan or 60 mg/kg STZ. In the latter part of this study, these doses produced a high rate of mortality in rats within the first week of injection owing to the production of a very severe state of diabetes. Seasonal variations in the mean sensitivity of Wistar rats towards these diabetogenic drugs could have resulted in such an effect. Dose response curves were performed to determine a safer but effective dose for these drugs and the new doses used were: 40 mg/kg alloxan and 50 mg/kg STZ.

Solutions of alloxan containing either 65 or 40 mg/ml and of STZ containing either 60 or 50 mg/ml were prepared. Each rat received a fraction of a ml, in the range of 0.17 to 0.2 ml, depending on the body weight. Such small volumes helped in achieving rapid injections
without causing major changes in blood pH. Intravenous administration of the drugs was made into the rat tail vein. Rats were anesthetized with ether prior to injection. Tail veins were dilated by applying warm water to the tail, followed by the application of 95% ethyl alcohol. Diabetogenic drugs were rapidly injected into the dilated tail vein using a 1 ml tuberculin syringe fitted with a 25 1/2 gauge hypodermic needle. Rats in the control group received appropriate volumes of citrate buffer alone. Rats recovered from ether anesthesia within 5 minutes after injection. The three groups of rats were randomly divided into various sets and were used at various times after injection. Control and treated rats were housed separately and were given food (Purina Rat Chow) and water ad libitum. Production of a diabetic state was diagnosed 48 hours after injection by a positive test for glycosuria with Lilly Test-tape. The diabetic state was monitored by periodical tests for the presence of glucosuria thereafter. Blood samples were collected at the time of sacrifice, allowed to clot, centrifuged and the clear serum was analyzed for serum glucose, immunoreactive insulin (IRI), thyroxine (T4) and triiodothyronine (T3) levels.

WORKING HEART APPARATUS:

The working heart perfusion apparatus used in this study is a non-recirculating type and is a modified version of Neely's original working heart apparatus (Neely et al. 1967) as described previously by Rodgers et al. (1981).
The working heart apparatus as shown in Figure 1, contained two jacketed primary and two jacketed filling reservoirs. One of the primary reservoirs is connected through a 3-way stop cock to a 15 gauge steel aortic cannula or to one of the filling reservoirs. The other primary reservoir is connected to the second filling reservoir. The primary reservoir connected to the aortic cannula is situated 45 cms above the aortic cannula. Both the filling reservoirs are connected to a 16 gauge steel perfusion cannula and the reservoirs are fixed such that they can be moved up and down in steps to provide various filling pressures. Buffer level in the jacketed reservoirs is maintained at a constant level with the help of small glass float valves. The aortic cannula is also connected to a series of 1 ml pipettes, through a second 3-way stop cock. The 1 ml pipettes are arranged to give various combinations of pipettes in parallel and series and served as an external peripheral resistance. The perfusate in the primary and filling reservoirs is maintained at 37°C by means of a Haake constant-temperature water recirculating pump. The perfusate in the reservoirs is also constantly aerated with a mixture of 95% oxygen and 5% carbon dioxide. The perfusate used is the one described previously by Chenoweth and Koelle (1946). The Chenoweth-Koelle (C-K) solution contained the following in millimolar quantities: NaCl, 120; KCl, 5.6; CaCl$_2$, 2.18; MgCl$_2$, 2.1; NaHCO$_3$, 19; glucose, 9.9; and EDTA, .03. When aerated with 95% O$_2$-5% CO$_2$ mixture at 37°C, this solution gave a pH of 7.4.
FIGURE 1:

Schematic representation of the working heart apparatus.

The working heart apparatus contained two jacketed primary (1) and two jacketed filling reservoirs (2). One of the primary reservoirs is connected through 3-way stop cocks (7) to a 15-gauge steel aortic cannula (3) or to one of the filling reservoirs. The other primary reservoir is connected to the second filling reservoir. Both the filling reservoirs are connected to a 16 gauge steel left atrial cannula (4). The primary reservoir connected to the aortic cannula is situated 45 cm above the cannula. The filling reservoirs can be moved up or down in steps to provide various filling pressures between 5 and 22.5 cm H₂O. The aortic cannula is also connected to a series of 1 ml pipettes, which serve as an external peripheral resistance (8). The perfusate in the reservoirs is constantly aerated through the built-in oxygenators (10) and is maintained at 37°C by means of a constant temperature water recirculating pump (not shown in the figure).

The isolated rat heart (12) is attached to the aortic cannula through the aorta and perfused initially in the retrograde manner at 45 cm H₂O filling pressure. The left ventricular chamber is connected to a pressure transducer (5) as described in the methods section. The left atrium is cannulated with the atrial cannula and the perfusion is switched to the working mode. In the working mode, perfusate from filling reservoirs, entered the left ventricular chamber, pumped out through the aorta into the peripheral resistance (as indicated by the arrows). Intra aortic pressure is recorded through the second pressure transducer (6) and the hearts are paced at 300 beats/min by placing a bipolar platinum electrode (11) on the left atrium.

Aortic output is determined by measuring the volume of perfusate flowing out of the aortic out-flow system (9).
HEART PERFUSION:

Animals were sacrificed by stunning them with a blow to the head followed by decapitation using a guillotine. Hearts were quickly removed from the animals and were immediately placed in aerated C-K solution. The aortic stump was located, separated from the surrounding tissue and attached to the 15 gauge aortic perfusion cannula. Perfusion was initiated in the retrograde manner through the aorta at 45 cm H₂O (30 mm Hg) aortic filling pressure. Remnants of the pulmonary vein were located and a small piece (3 cm in length) of PE 90 tubing was inserted into the left ventricle through the pulmonary vein and pulled out through the apex of the heart such that one end of the tube remained inside the left ventricular chamber. Later on in this study, a 20 gauge hypodermic needle was attached to the PE 90 tubing and the needle was then inserted into the left ventricle through the apex, instead of inserting the PE 90 tube into the left ventricle through the pulmonary vein. The second method was chosen to prevent any damage that may occur to the mitral valve while passing the PE 90 tube through it. The left atrium was then attached to the 16 gauge cannula through the pulmonary vein. Cardiac work was initiated by switching the perfusion system from the retrograde mode to the working heart mode. In the working mode the perfusate entered the left ventricle through the left atrium and was pumped out into the aortic stump. The aortic outflow was redirected into the external peripheral resistance, which provided a resistance of 340 PRU (peripheral resistance units) or 45.32 x 10⁴ dyne sec/cm⁵. Intra-ventricular pressure development
was measured by attaching a Statham P23AA pressure transducer to the PE 90 tube. Intraaortic pressure was measured with a second pressure transducer attached to the aortic outflow system through a side-arm. Aortic and left ventricular pressure development and the first derivative of left ventricular pressure development were recorded on a Grass 79D polygraph. The first derivative of left ventricular pressure was obtained through a Grass 7P20 differentiator.

Hearts were electrically stimulated at 300 beats/min by means of a bipolar platinum electrode placed on the left atrium with pulses of 5 m sec duration and twice threshold voltage (1.4 V) from a Grass model SD9D stimulator. Each heart was equilibrated for 15 min at 15 cm H₂O left atrial filling pressure. At 15 cm H₂O left atrial filling pressure, rat hearts developed peak left ventricular pressures in the range of 90-110 mm Hg which resulted in mean aortic pressures in the range of 70-80 mm Hg. Under these conditions adequate coronary perfusion was achieved. Coronary flow was measured during the initial 5 min period of retrograde perfusion at 45 cm H₂O perfusion pressure. Coronary flow was not measured during the working heart perfusion mode since artifactually high flow rates were obtained owing to leakages.

Cardiac performance was expressed in terms of left ventricular developed pressure (LVDP), rate of rise of left ventricular pressure (+dP/dt) and rate of decline of left ventricular pressure (-dP/dt).

DP/dt was calculated by hand by drawing a tangent line to the pressure trace at the point of maximum slope. The slope of this
line is normally calculated by constructing a right angle triangle by dropping a vertical and drawing a horizontal back to the tangent line. However, as the recording system used is curvilinear, this method was modified slightly. It was assumed that the maximum positive slope of the pressure curve occurred at approximately 25 mm Hg and the negative slope at 75 mm Hg. At these deflections the recording pen is about 6° and 2° respectively from the horizontal. Therefore to correct for this the constructed right angled triangles were also tilted at 6° or 2° as shown in Figure 2. In the latter part of this study, dP/dt was obtained using a microcomputer as previously described by Harris et al. (1982). Briefly, a Mountain Hardware A/D and D/A converter was used to digitize the left ventricular pressure pulses from the Grass polygraph and the resulting digitized data was stored to disk by an Apple II microcomputer. The pressure transducer signal from the polygraph was sampled at 667 Hz over 1.5 seconds. This resulted in data being collected for six complete cardiac pulses. Three of these were analyzed using various curve fitting techniques to determine pulse height, positive and negative dP/dt, start and finish.

CARDIAC FUNCTION CURVES:

Cardiac function was estimated in isolated perfused working hearts obtained from 7, 30, 100, 180, 240 and 360 day alloxan- and STZ-diabetic and age-matched control rats. Following the 15 min equilibration period at 15 cm H₂O left atrial filling pressure, left ventricular function was estimated by varying the left atrial filling
FIGURE 2.

Calculation of positive and negative dP/dt from left ventricular pressure trace

Tangent lines (2 and 3) are drawn to the left ventricular pressure trace (1) at the points of maximum slope, which are approximately at 25 mm Hg for the positive slope and 75 mm Hg for the negative slope of the pressure curve. Slopes of these tangents are calculated by constructing right angled triangles by dropping verticals (4 and 5) and drawing horizontals back to tangent lines. Since the recording pen is about 6° at 25 mm Hg and 2° at 75 mm Hg from the horizontal, the right angled triangles A and B are tilted by 6° and 2° respectively. The slope is calculated from the formula:

\[
\text{Slope of the hypotenuse} = \frac{\text{opposite side}}{\text{adjacent side}} \quad \text{or} \quad \frac{\text{vertical}}{\text{horizontal}}
\]

Vertical can be converted to pressure (mm Hg) from chart calibration and horizontal to time (sec) from chart speed.

Then slope = \[\frac{\text{mm Hg}}{\text{sec}}\]
pressures, which in turn influenced the left ventricular preload. During this manoeuvre, conditions which influence left ventricular afterload, such as the external peripheral resistance were held constant. Left atrial filling pressures were varied by changing the height of the filling reservoirs from 5.0 to 22.5 cm $H_2O$ in 2.5 cm steps. The function curve was performed by initial stepwise reduction of filling pressure from 15 to 5 cm $H_2O$, followed by stepwise increments up to 22.5 cm $H_2O$ and back to 15 cm $H_2O$. At each filling pressure hearts were allowed to perfuse until stable pressure development was obtained before changing to next filling pressure. In general stable pressure development was achieved within 2 min after changing the atrial filling pressure and a complete function curve was usually performed in about 20 to 30 min. As hearts were perfused at 15 cm $H_2O$ filling pressure during the equilibration period, filling pressures which were lower than 15 cm $H_2O$ were referred to as low work loads and filling pressures higher than 15 cm $H_2O$ were referred to as high work loads.

DOSE-RESPONSE CURVES TO CARBACHOL:

Dose-response curves to carbamylcholine (carbachol) were performed in isolated perfused working hearts obtained from alloxan- and STZ-diabetic and control rats at various time points after induction of the disease state. Isolated perfused working hearts were paced at 300 beats/min by placing two bipolar platinum electrodes, one on the left atrium and the other on the apex of the heart. Each pair of electrodes was connected to a Grass model SD9D stimulator. The stimulators were connected such that each atrial pulse triggered
a separate ventricular pulse 6 msec later. The pulses were of 5 msec duration and were four times threshold voltage (2.8 V). This method of pacing was used to overcome the A-V block that may occur after the administration of carbachol. Cumulative dose-response curves to carbachol were performed by adding increasing concentrations of carbachol to the perfusion medium. The concentration of carbachol which produced a 65 to 70 % reduction in the basal rate of rise in left ventricular pressure or +dP/dt was considered as the maximum dose of carbachol and doses greater than this were not tested. The maximal concentration of carbachol was normally 1 x 10^{-5} M but in a few cases the maximal concentration was 3 x 10^{-5} M. Sensitivities of hearts obtained from control and diabetic rats towards the negative inotropic effect of carbachol were compared by comparing the ED_{30} values for carbachol on these hearts. The ED_{30} value is expressed as the geometric mean (with 95% confidence intervals) of the dose of carbachol required to produce a 30% reduction in the basal +dP/dt and was calculated as described by Fleming et al (1972). After the completion of dose-response curve to carbachol, each heart was perfused with normal perfusion buffer to completely wash out any residual carbachol. Hearts which reached stable left ventricular pressure development after washing out carbachol were used for isoproterenol dose-response curves.

DOSE-RESPONSE CURVE TO ISOPROTERENOL:

Dose-response curves to isoproterenol were performed in isolated perfused working hearts from diabetic and control rats at various time
points after induction of diabetes. For this study, hearts were paced at 300 beats/min at twice threshold voltage (1.4 V) with platinum electrodes placed only on the left atrium. Cumulative dose-response curves to isoproterenol were performed by adding increasing concentrations of isoproterenol (from $1 \times 10^{-10}$ to $1 \times 10^{-7}$ M) to the perfusion medium. Changes produced by various concentrations of isoproterenol in positive and negative left ventricular $dP/dt$ were monitored. The positive inotropic effect of isoproterenol was expressed as changes produced by isoproterenol in the rate of rise of left ventricular pressure and the cardiac relaxant effect of isoproterenol was expressed as changes produced in the rate of decline of left ventricular pressure. Sensitivity of the diabetic and control myocardium towards the positive inotropic effect of isoproterenol were compared by determining $pD_2$ values. The $pD_2$ value was defined as the negative log concentration of the drug required to produce 50% of the maximum change produced in $+dP/dt$.

CYCLIC AMP AND PHOSPHORYLASE ENZYME STUDIES:

Isolated perfused working hearts obtained from 3 and 100-120 day diabetic and control rats were used to study the time-course of the changes produced by an $ED_{50}$ concentration ($5 \times 10^{-9}$M) of isoproterenol on cardiac cyclic AMP levels, phosphorylase activity and inotropy. Hearts were isolated and perfused according to the working heart technique as described under the section: Heart perfusion. Following the 15 min equilibration period at 15 cm H$_2$O left atrial filling pressure, sets of control and diabetic hearts were quick
frozen before and at various time points (5, 10, 20, and 40 sec) after the administration of $5 \times 10^{-9}$ M isoproterenol, by the application of tongs precooled in liquid nitrogen. Isoproterenol was administered by adding a solution of isoproterenol to the perfusion medium to make a final concentration of $5 \times 10^{-9}$ M.

Dose-response of the effect of isoproterenol on cardiac cyclic AMP levels, phosphorylase activity and inotropy was studied in isolated perfused working hearts obtained from 100-120 day control and STZ-diabetic rats. Hearts were quick frozen either before or 20 sec after administration of an ED$_{20}$ dose ($2 \times 10^{-9}$ M) or an ED$_{80}$ dose ($1 \times 10^{-8}$ M) of isoproterenol.

The effect of prostaglandin E$_1$ (PGE$_1$) on cardiac phosphorylase activity in control and diabetic rats was also studied. Isolated perfused working hearts from 3 and 100-120 day diabetic and control rats were quick frozen 30 sec. after the administration of $10^{-6}$ M PGE$_1$. A stock solution of PGE$_1$ was prepared by dissolving an appropriate quantity of PGE$_1$ in absolute alcohol to make a $10^{-2}$ M solution. Serial dilution of the stock solution to obtain $10^{-6}$ M solution was made in Chenoweth-Koelle buffer.

The effect of indomethacin pretreatment on isoproterenol-induced phosphorylase activation was studied in isolated working hearts from 100-120 STZ-diabetic and control rats. Hearts were perfused with buffer containing $10^{-6}$ M indomethacin for 20 minutes before administering $5 \times 10^{-9}$ M isoproterenol. Hearts were quick frozen 20 sec after the administration of isoproterenol. A stock solution of indomethacin was made in absolute alcohol and was serially diluted with Chenoweth-Koelle
buffer to obtain the required 10^{-6} M concentration. All the frozen hearts were stored at -80°C until they were analyzed for cyclic AMP content and phosphorylase activity.

CYCLIC AMP DETERMINATION:

Cyclic AMP content of the myocardial tissue was determined by a radioimmunoassay method according to Steiner et al. (1972), using Becton-Dickinson radioimmunoassay reagents. The assay involved a competitive binding reaction between [^{125}I]-labelled cyclic AMP and non-radioactive cyclic AMP in the standard or sample, for the binding sites present on the cyclic AMP-antibody. Radioactive cyclic AMP that was bound to the antibody was separated by precipitation with 60% saturated ammonium sulfate solution and was counted in a gamma counter. The cyclic AMP content of the myocardium was expressed as femtomoles/mg tissue wet weight.

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) which was previously described by McNeill and Brody (1966). The assay involved an enzymatic reaction catalyzed by phosphorylase, whereby glucose-1-phosphate was converted to glycogen with the release of inorganic phosphate. The inorganic phosphate was estimated photometrically according to the method of Fiske and Subbarow (1928). Phosphorylase a activity was expressed as a percent of total phosphorylase activity. Total phosphorylase activity was estimated
in the presence of 5'-AMP and was expressed as micromoles of inorganic phosphate released per mg tissue per min. Phosphorylase a activity was estimated in the absence of 5'-AMP. Most results are expressed as percent phosphorylase a which is:

\[
\frac{\text{phosphorylase a activity}}{\text{total phosphorylase activity}} \times 100
\]

MUSCARINIC RECEPTOR BINDING EXPERIMENTS:

Muscarinic receptor radioligand binding experiments were carried out on ventricular tissue obtained from 180-day control and STZ diabetic rats. Isolated perfused working hearts from control and diabetic rats were quick frozen following the 15 min equilibration period and were stored at -80°C until used for binding experiments.

Frozen hearts were suspended in cold 0.32 M sucrose solution, allowed to thaw and ventricles were separated from atria and other extraneous tissue. Each ventricle was suspended in 10 ml of cold 0.32 M sucrose and was homogenized using a Polytron, setting 7 for 20 sec. Homogenate from 6 control or 6 diabetic rat ventricles was combined and further homogenized in a glass homogenization tube using a teflon pestle at 200 rpm, with 8 down strokes and 8 upstrokes. The homogenate was then distributed into plastic centrifuge tubes, loaded into a swinging bucket rotor of type SW28 #903 and centrifuged at 25,000 rpm for 30 min. The supernatant liquid was discarded and the crude membrane pellets used for the binding experiments.
The crude membrane pellet was suspended in 10 ml of Krebs-Henseleit solution containing in mM: NaCl, 118; KCl, 4.7; NaHCO₃, 25; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2 and dextrose, 11.1 and was homogenized using a teflon pestle. The homogenate was filtered through cheese cloth to remove connective tissue. The protein content of the homogenate was determined using the Lowry's protein assay. The homogenate was diluted with Krebs-Henseleit buffer to adjust the protein concentration to approximately 0.7 mg/ml.

The following binding experiments were performed on the diluted membrane preparation:

a. Direct binding or determination of the saturation binding curve for the radioligand.

b. Competition by non-radioactive antagonist for radioligand labelled binding sites.

c. Competition by non-radioactive agonist for radioligand labelled binding sites and the effect of guanine nucleotides.

Tritiated N-methylscopolamine ([³H]NMS), a muscarinic receptor antagonist, was used as a radioligand to stereospecifically and reversibly label muscarinic receptor sites in the membrane preparation. For competition experiments, non-radioactive N-methylscopolamine was used as an antagonist and carbachol was used as an agonist. Guanyl-5′-yl-imido-diphosphate (GMPPNP), a non-hydrolysable derivative of GTP was used as a guanyl nucleotide.

All the points in each binding experiment were carried out in quadruplicate. In the direct binding experiments, total binding was
determined with varying concentrations of \([^3H]NMS (10^{-11} - 10^{-8} \text{ M})\) and non-specific binding was determined in the presence of a high concentration of non-radioactive NMS \((10^{-5} \text{ M})\). In the competition experiments a single concentration of \([^3H]NMS (1 x 10^{-7} \text{ M})\) was used in the presence of varying concentrations of either a non-radioactive antagonist \((10^{-11} - 10^{-7} \text{ M NMS})\) or a non-radioactive agonist \((10^{-10} - 10^{-2} \text{ M Carbachol})\). When a guanyl nucleotide was included \(10^{-4} \text{ M GMPPNP}\) was added. All the drugs were added to 0.5 ml Eppendorf tubes in 5 µl quantities. The binding reaction was initiated by the addition of 490 µl of the membrane preparation. The reaction mixture was incubated at 30°C for 45 minutes. The reaction was stopped by rapid centrifugation in a Beckmann model bench top microcentrifuge for 5 minutes. The supernatant was decanted and the membrane pellet was washed three times with 0.1 M NaCl solution. The pellets were solubilized in 0.5 ml of Soluene, a tissue solubilizer by keeping at 40°C for 12 hours. The dissolved pellets were mixed with 5 ml of Beckmann Ready Solve NA and counted using standard scintillation counting techniques.

ESTIMATION OF NORADRENALINE CONTENT IN VENTRICLES:

Noradrenaline content of the hearts obtained from 180 day alloxan- and STZ-diabetic and control rats was determined using a slight modification of the HPLC method described by Starke et al. (1981). Isolated perfused working hearts from diabetic and control rats were quick frozen following a 30 min perfusion and the frozen hearts were stored at -80°C until they were assayed for noradrenaline content.
Approximately 100 mg of the frozen ventricular tissue was weighed and homogenized in 4 ml of 0.4 M perchloric and \((\text{HClO}_4)\) containing 0.2 ml of 100 mM \(\text{Na}_2\text{EDTA}\), 0.1 ml of 10% \(\text{Na}_2\text{SO}_3\) and 10 \(\mu\)l of 25 \(\mu\)M dihydroxybenzylamine (DBA) as a marker, using a glass homogenization tube and glass pestle at 1,000 RPM for 45 sec. The homogenate was centrifuged at 20,000 \(\times\) g for 30 min and the pellet was discarded. The clear supernatant was mixed with 100 mg of alumina \((\text{Al}_2\text{O}_3, \text{Woelm neutral, grade #1})\). The mixture was stirred continuously with a magnetic stirrer bar and the pH of the mixture was adjusted to 8.3 with 2 M tris buffer. Stirring was continued for approximately 5 min after adjusting the pH to 8.3. The mixture was then transferred to a Spin Sep\(^R\) microfilter unit and the alumina was allowed to settle. The supernatant was discarded and the alumina column was washed 5 times with distilled water. After the 5th wash, water was completely removed from the column by centrifugation. Then the catecholamines that were adsorbed on the column were eluted twice with 0.5 ml of 0.2 M \(\text{HClO}_4\). The two 0.5 ml portions of the eluate were combined and analyzed for noradrenaline content by a reverse phase HPLC method. A fully capped ODS (octadecylsilica) Altex column (5\(\mu\)M particles, 25 cm long) was used for separation of the catecholamines. The mobile phase was a 5% methanolic solution of 0.1 M \(\text{NaH}_2\text{PO}_4\), 0.5 mM \(\text{Na}_2\text{EDTA}\) and 0.5 mM Na-octane sulfonate, pH 3.0 (adjusted with \(\text{H}_3\text{PO}_4\)). The flow rate through the column was adjusted to 1 ml/min using an Altex model 100 A pump. A standard solution containing 25 \(\mu\)M noradrenaline and 25 \(\mu\)M DBA and samples for analysis were injected in 20 \(\mu\)l quantities. Noradrenaline and DBA were detected amperometrically using a BAS
model LC-4A detector equipped with a +0.7 V CP-0 electrode. Recovery from the alumina chromatogram was determined by comparing the DBA (marker) peak to that in the Standard. All values of noradrenaline were corrected for incomplete recovery from columns. Noradrenaline content was expressed as µg per g heart tissue.

SERUM ANALYSES:

The diabetic and control rats were withdrawn from food for 12-18 hrs prior to sacrifice throughout this study. Blood samples were collected at the time of sacrifice. Blood samples were allowed to clot and centrifuged at 1000 rpm for 15 min to separate serum. The separated clear serum was stored below 0° C until analyzed for serum immunoreactive insulin, glucose, thyroxine (T4) and triiodothyronine (T3) levels.

SERUM IMMUNOREACTIVE INSULIN DETERMINATION;

Insulin content of the serum was measured by a radioimmunoassay, using the Becton-Dickinson insulin radioimmunoassay kit. The assay involved a competitive binding reaction between [125I] labelled insulin (porcine insulin) and the non-radioactive insulin in the standard (human insulin) or serum sample (rat insulin). Cross reactivity between the antibody raised against porcine insulin and rat insulin is 90% for the binding sites present on the antibody which were raised against porcine insulin in the guinea pig. Bound and unbound radioactive insulin were separated using dextran-coated charcoal. Dextran-coated charcoal has been reported to readily adsorb free insulin but not antibody-bound insulin (Herbert et al., 1965). At the end of the incubation period, a suspension of dextran-coated
charcoal was added to the incubation mixture, vortexed, centrifuged and the antibody-bound radioactivity in the supernatant was measured using a gamma counter. Serum immunoreactive insulin was expressed in μU/ml.

SERUM GLUCOSE DETERMINATION:

Glucose content of the serum was determined by an enzymatic method using glucose oxidase according to Raabo and Terkildsen (1960). During the initial phase of this study serum glucose was determined with the Ames Blood Analyzer glucose reagent kit (Sunderland and Logan, 1970). Later on the Sigma glucose assay kit was used to determine serum glucose since the Sigma glucose kit is less expensive. The assay involves an enzymatic reaction in which glucose is converted to gluconolactone in the presence of glucose oxidase. Gluconolactone reacts with water forming gluconic acid and hydrogen peroxide. Hydrogen peroxide, in the presence of the enzyme peroxidase, oxidizes the colorless reagent 0-dianisidine to a reddish-brown dye and the color developed was read at 500 mM using a spectrophotometer. A 100 mg/100 ml glucose standard solution was used as a reference standard. Serum glucose levels were expressed in mg/dl (mg %).

SERUM T4 DETERMINATION:

T4 content of the serum was determined by a radioimmunoassay method using the TetrabeadR-125 T4 diagnostic kit. The assay involves competition between the radioactive and non-radioactive T4 in the standard or serum sample for the binding sites present in a limited quantity of the T4 antibody immobilized on beads. Separation of the bound and free radioactive T4 was achieved by removal of the reaction
liquid from the beads. The radioactivity bound to the beads was estimated using a gamma counter. Serum T₄ content was expressed in μg/dl.

SERUM T₃ DETERMINATION:

Serum T₃ content was determined by a charcoal bead uptake method using the Triobead®-125 T₃ uptake kit. The assay involves partition of the radiolabelled T₃ between the primary binding sites (serum proteins such as thyroxine binding protein, albumin etc.) in the serum and secondary binding sites such as the charcoal coated beads. The radioactivity taken up by the beads gives an indication of the thyroid status. In hypothyroidism the primary binding sites are less saturated and they take up more radioactivity and the beads less. Conversely in hyperthyroidism, the primary binding sites are supersaturated and so the beads take up more radioactivity. The radioactive T₃ bound to the charcoal coated beads was separated by removing the reaction liquid from the beads and was counted in a gamma counter. The charcoal bead uptake was expressed as a percent of the T₃ uptake value for the reference control serum provided with the kit. The percent T₃ bead uptake value provides an indication of the thyroid status and can not be directly translated into definite units of T₃.

STATISTICAL ANALYSES:

All the results presented in this thesis are expressed as mean ± standard error of the mean. Comparisons between control and diabetic groups were made by using the one-way analysis of variance. Whenever
a multigroup comparison was made, a posteriori test such as the Newman-Kuels test for multiple comparisons was then performed to determine significant difference between groups. A multifactional two-way analysis of variance was used to determine the effects of treatment, time after treatment and their interaction on the sensitivity of the myocardium towards carbachol. The level of significance was set at \( p<0.05 \).

**MATERIALS**

Alloxan monohydrate, l-amino-2-napthol-4-sulfonic acid reagent, 5'-AMP, carbachol, D-glucose-1-phosphate, glycogen, DL-isoproterenol, indomethacin, prostaglandin \( \text{E}_1 \), streptozotocin and Tris were all purchased from Sigma Chemical Company, St. Louis, Mo. \(^ {3}\text{N}\)N-methyl scopolamine, N-methyl scopolamine and guanyl-5'-imidodiphosphate were generous gifts from Dr. J.W. Wells, Faculty of Pharmacy, University of Toronto. Dihydroxybenzylamine and alumina were generous gifts from Dr. V. Palaty, Department of Anatomy, University of B.C. All of the chemicals used to prepare various buffer solutions were of analytical reagent grade.

Cyclic AMP radioimmunoassay reagents and insulin radioimmunoassay kits were obtained from Beckton Dickinson and Company, Orangeburg, New York, U.S.A. Glucose assay kits were purchased from Ames Company, Elkhart, Indiana, U.S.A. and Sigma Chemical Company, St. Louis, Mo., U.S.A. Tetrabead\(^ R \)-125 \( \text{T}_4 \) diagnostic kits and Triobead\(^ R \)-125 \( \text{T}_3 \) uptake kits were bought from Abbott Laboratories, North Chicago, Il, U.S.A. The Spinsep\(^ R \) microfilter units used in catecholamine determinations were obtained from Mandel Scientific Company, Calgary, Alberta.
RESULTS

FEATURES OF THE DIABETIC-RAT MODEL:

Administration of either alloxan or STZ to rats resulted in a diabetic state characterized by fasting hyperglycemia and hypoinsulinemia throughout in this study. Table I presents fasting serum insulin and glucose levels, body weights, wet heart weights, heart weight to body weight ratios and coronary flow rates of control and alloxan- and STZ-treated rats at the time points studied in the initial part of this study. Serum insulin levels were significantly reduced in rats treated with alloxan and STZ, compared to controls at all of the time points, except at 30 days where the serum insulin levels in the STZ-treated group were not significantly different from controls. Fasting serum glucose levels were significantly elevated in both the treated groups at all of the time points studied, including the 30 day STZ-treated group. Body weight of alloxan and STZ diabetic rats were significantly lower than controls at all time-points except at 7 days after treatment. Wet heart weights of the diabetic animals were also significantly lower than controls at all time points except at 7 days after treatment. The wet heart weight to body weight ratio was unaltered in alloxan and STZ diabetic rats at 7, 30 and 100 days after induction of diabetes whereas the ratio was significantly larger in diabetic animals at 180, 240 and 360 days after treatment. Coronary flow per gram of heart tissue was unaltered in diabetic rats at any time point studied.

There was no mortality in any group either at 7 or 30 days after treatment. At 100 days and thereafter some mortality was encountered.
Specifically, 5 out of 15 rats injected with alloxan died between 100 and 240 days. One control animal out of 12 died before 100 days. There was no mortality in either group at 180 days. Three out of 12 animals died in both the control and STZ diabetic groups between 180 and 360 days.

Table II presents some features of 3 and 100-120 day alloxan-and STZ-treated and control rats used in the time course study of the effect of isoproterenol on cardiac cyclic AMP content, phosphorylase activity and inotropy. In this study serum T₄ and T₃ levels were estimated in order to check the thyroid status of the diabetic rats. Body weights were significantly lower in rats treated with either alloxan or STZ at 3 days and 100-120 days, when compared to control rats at the corresponding time points. Fasting serum glucose levels were significantly elevated in alloxan- and STZ-treated rats at 3 and 100-120 days compared to age-matched control rats. Serum T₃ bead uptake values were significantly depressed in diabetic rats at both the time points suggesting reduced serum T₃ levels. Serum T₄ levels were significantly lower in alloxan and STZ diabetic rats only at 100-120 days after induction of diabetes but not at 3 days. There was no mortality in any group of rats either at 3 days or at 100-120 days.

Table III shows body weights and fasting serum glucose levels of control and diabetic rats at various time points, that were used in the PGE₁ and indomethacin study, muscarinic receptor binding studies or for heart noradrenaline content estimation. Body weights of diabetic rats were significantly lower compared to control at all
of the time points studied in the above mentioned studies. Fasting serum glucose levels were significantly higher in diabetic rats compared to age-matched control rats.

CARDIAC PERFORMANCE OF CONTROL AND DIABETIC RATS:

Cardiac performance was studied at various left atrial filling pressures in isolated perfused working hearts obtained from control and diabetic rats at various time points after inducing diabetes. Cardiac performance is expressed in terms of LVDP, positive dP/dt and negative dP/dt. Figures 3, 4 and 5 illustrate LVDP, positive dP/dt and negative dP/dt at various left atrial filling pressures in isolated perfused working hearts obtained from 7 day alloxan and STZ diabetic and control rats. There were no differences in cardiac performance between control and diabetic rats at this time point. Left ventricular pressure development, and positive and negative dP/dt of isolated perfused working hearts from 30 day control and diabetic rats is presented in Figures 6, 7 and 8 respectively. Control and STZ diabetic rats exhibited similar cardiac performance at various left atrial filling pressure at this time point. However, alloxan diabetic rat hearts exhibited depressed left ventricular pressure development and positive dP/dt, when perfused at filling pressures between 5 to 17.5 cm H$_2$O and depressed negative dP/dt at filling pressures between 10 and 22.5 cm H$_2$O. Isolated perfused working hearts obtained from 100-day alloxan and STZ diabetic rats developed significantly lower left ventricular pressure, and positive and negative dP/dt at filling pressures above 10 cm H$_2$O when compared to age-matched control rats as shown in Figures 9, 10 and 11 respectively. Similar depressions in cardiac performance were seen in isolated
perfused working hearts from 180-day STZ diabetic rats at most of the left atrial filling pressures studied (Figures 12, 13 and 14). Left ventricular pressure development was slightly but not significantly depressed in 240-day alloxan diabetic rat hearts (Figure 15). However positive dP/dt was depressed at all of the filling pressures studied and negative dP/dt was depressed at high filling pressures in these hearts, as illustrated in Figures 16 and 17. In isolated perfused working hearts obtained from 360-day STZ diabetic rats, there were no depressions in LVDP and negative dP/dt at any filling pressure studied (Figures 18 and 20). On the other hand positive dP/dt in 360-day STZ diabetic rat hearts was significantly depressed compared to controls at all of the filling pressures studied except at 10 cm H2O (Figure 19).

Figure 21 demonstrates the changes produced in positive dP/dt with time in control and alloxan and STZ diabetic rat hearts at 15 cm H2O left atrial filling pressure. Both control and diabetic rat hearts exhibited similar positive dP/dt, 7 days after induction of diabetes. Alloxan diabetic rat hearts exhibited a significant decline in the rate of pressure development, starting from 30 days after treatment until the end of the study, whereas STZ diabetic rat hearts exhibited similar positive dP/dt as controls at 30 days after treatment but thereafter showed a significant decline until the end of the study. The control rat hearts showed a slight decline in positive dP/dt with increasing age. Figure 22 illustrates the changes occurring in rate of left ventricular pressure decline with time in control and diabetic rat hearts at 15 cm H2O atrial filling pressure. Negative dP/dt was not
altered by either alloxan or STZ diabetes at 7 days after induction. Hearts isolated from alloxan diabetic rats showed a progressive decline in negative dP/dt with time, starting from 30 days after induction until 240 days which was the longest time point studied. A reduction in negative dP/dt was not observed at 30 days after induction with STZ. STZ-induced diabetes produced a significant reduction in negative dP/dt by 100 days, which persisted until 180 days and then started to rise towards the control value with time. At 360 days after treatment, the negative dP/dt of hearts obtained from STZ diabetic rats was not significantly different from controls. The negative dP/dt in control rat hearts showed a marked decline with increasing age.

RESPONSIVENESS OF CONTROL AND DIABETIC HEARTS TO CARBACHOL:

Carbachol produced a dose-dependent reduction in the basal positive dP/dt of isolated perfused working hearts obtained from control, alloxan and STZ diabetic rats at all the time points studied. At 7 and 30 days after induction of diabetes, carbachol produced similar reductions in the basal positive dP/dt at any dose employed in control as well as diabetic rat hearts (Figures 23 and 24). Data are expressed as percent reduction in basal positive dP/dt produced by various molar concentrations of carbachol. [Basal positive dP/dt values at 7 days were (in mm Hg/sec): control = 2425±97, alloxan diabetic = 2283±132 and STZ diabetic = 2392±122. After the administration of a maximum dose of carbachol these values were: control (10^{-5}M) = 558±48, alloxan diabetic (10^{-5}M) = 566±39 and STZ diabetic (10^{-5}M) = 539±60. Basal positive dP/dt values at 30 days were: control = 2737±59, alloxan diabetic 2285±101 and
STZ diabetic = 2672±57. After the administration of a maximum dose of carbachol these values were: control (10^{-5}M) = 679±62, alloxan diabetic (10^{-5}M) = 619±59 and STZ diabetic 596±52. At 100 days after treatment, carbachol produced significantly smaller reductions in basal +dP/dt development at 10^{-6}, 3 \times 10^{-6} and 10^{-5}M doses in alloxan and STZ diabetic rat hearts as compared to control rat hearts. As a result, the dose response curves to carbachol in the diabetic rat hearts were shifted to the right suggesting that the diabetic rat hearts were subsensitive to carbachol (Figure 25). [Basal +dP/dt values at 100 days were (in mmHg/sec): control = 2584±126, alloxan-diabetic = 2313±160 and STZ diabetic = 2302±138. After the administration of a maximum dose of carbachol these values were: control (10^{-5}M) = 921±106; alloxan-diabetic (3 \times 10^{-5}M) = 927±140 and STZ diabetic (3 \times 10^{-5}M) = 928±133]. STZ diabetic rat hearts at 180 days and alloxan diabetic rat hearts at 240 days exhibited increased sensitivity to carbachol. Reductions in basal +dP/dt development produced by 10^{-6}M and 3 \times 10^{-6}M doses of carbachol were significantly greater in diabetic rat hearts compared to control hearts. This increased sensitivity was reflected by the fact that dose-response curves to carbachol in diabetic rat hearts at these two time points were shifted to the left as compared to control dose-response curve (Figures 26 and 27). [Basal +dP/dt values at 180 days were: control = 2295±73 and STZ-diabetic = 1878±81. After the administration of a maximum dose of carbachol these values were: control (10^{-5}M) = 733±61 and STZ diabetic (3 \times 10^{-6}M) = 709±77. Basal +dP/dt values at 240 days were: control = 2247±130, and alloxan diabetic = 1844±142. After a
maximum dose of carbachol these values were: control (10^{-5}\text{M}) = 531\pm41 and alloxan diabetic = 503\pm77]. At 360 days after treatment, both control and STZ diabetic rat hearts were less responsive to the negative inotropic effect of carbachol (Figure 28). [Basal +dP/dt values at 360 days were: control = 2016\pm105 and STZ-diabetic = 1931\pm54. After a maximum dose of carbachol these values were: control (10^{-5}\text{M}) = 1212\pm282 and STZ-diabetic (10^{-5}\text{M}) = 917\pm124]. The maximal concentration of carbachol (1 \times 10^{-5}\text{M}) employed produced about a 70% reduction in the basal +dP/dt development of control rat hearts at earlier time points, whereas this concentration produced only a 40% reduction in basal +dP/dt development in control hearts at 360 days. Diabetic rat hearts, however, were slightly but not significantly more sensitive and responsive to carbachol at this time point. 

Table IV presents ED_{30} values for carbachol in control and diabetic rat hearts at all the time points studied. At 7 and 30 days of treatment, ED_{30} values for carbachol in diabetic rat hearts were not significantly different from controls. ED_{30} values for carbachol were significantly higher in 100 day diabetic rat hearts when compared to controls. At 180 and 240 days the ED_{30} values for carbachol in diabetic rat hearts were significantly lower than control values. At 360 days the ED_{30} value for carbachol in diabetic rat hearts was slightly but not significantly lower than control. ED_{30} values for carbachol on control rat hearts at various time points were all similar except at 360 days, where the ED_{30} value was slightly but not significantly higher than those at earlier time points.
EFFECT OF ISOPROTERENOL ON POSITIVE DP/DT IN CONTROL AND DIABETIC RAT HEARTS:

Isoproterenol ($1 \times 10^{-10} - 1 \times 10^{-7}$M) produced dose-dependent increases in positive dP/dt of isolated working hearts obtained from control, and alloxan and STZ diabetic rats at all of the time points studied. Maximum changes in positive dP/dt were observed after the administration of $3 \times 10^{-8}$M isoproterenol at all time points. Isoproterenol produced similar increases in positive dP/dt in hearts obtained from 7 and 30 day control and diabetic rats (Figures 29 and 30). At 100 days after the induction of diabetes also, isoproterenol produced similar changes in positive dP/dt in control as well as diabetic rat hearts as depicted in Figure 31. Diabetic rat hearts exhibited slight but not significant depression in basal positive dP/dt. Figures 32 and 33 show the dose response curves to isoproterenol in isolated perfused working hearts from 180 day control and STZ diabetic and 240 day control and alloxan diabetic rats respectively. Basal positive dP/dt was significantly depressed in both 180 day STZ and 240 day alloxan diabetic rat hearts compared to age-matched controls. However the maximum increases in positive dP/dt produced by isoproterenol in 180 and 240 day diabetic rat hearts were of the same magnitude as in age-matched controls. Figure 34 illustrates the dose-response curve to isoproterenol on positive dP/dt in 360 day control and STZ diabetic rat hearts. At this time point also there were no differences in the effect of isoproterenol between control and diabetic rat hearts. The pD2 values for the positive inotropic effect of isoproterenol in diabetic and age-matched control rat hearts are presented in Table V.
The sensitivity of the rat myocardium towards the inotropic effect of isoproterenol was not altered either by diabetes or with age.

**EFFECT OF ISOPROTERENOL ON NEGATIVE DP/DT IN CONTROL AND DIABETIC RAT HEARTS:**

Isoproterenol ($1 \times 10^{-10}$ to $1 \times 10^{-7}$M) produced dose-dependent increases in negative $dP/dt$ of isolated working hearts from alloxan and STZ diabetic and control rats at all of the time points studied. The maximum changes in negative $dP/dt$ were produced after administration of $3 \times 10^{-8}$M isoproterenol in control as well as diabetic rat hearts at all time points. The dose-response curve to isoproterenol on negative $dP/dt$ in 7 day control and alloxan and STZ diabetic rat hearts is presented in Figure 35. Maximum responses in negative $dP/dt$ to isoproterenol were significantly depressed in alloxan diabetic rat hearts. Basal negative $dP/dt$ in both the diabetic groups and maximum responses to isoproterenol in STZ diabetic rat hearts were not altered at this time point. At 30 days after inducing diabetes basal negative $dP/dt$ in control and diabetic rat hearts and maximum responses to isoproterenol in STZ diabetic rat hearts were unaltered (Figure 36). Maximum change produced by $3 \times 10^{-8}$M isoproterenol in negative $dP/dt$ in alloxan-diabetic hearts was significantly depressed compared to that in control. Maximum responses in negative $dP/dt$ to isoproterenol were significantly depressed in both alloxan and STZ diabetic rat hearts at 100 days compared to age-matched controls (Figure 37). Basal negative $dP/dt$ was also significantly depressed in alloxan and STZ diabetic rat hearts at this time point. Figure 38 illustrates the dose-response curves
to isoproterenol in control and STZ diabetic rat hearts at 180
days after induction of diabetes. Both basal negative dP/dt
and maximum responses in negative dP/dt to isoproterenol were
significantly depressed in STZ diabetic rat hearts. Dose-response
curves to isoproterenol on negative dP/dt in 240 day control
and alloxan diabetic rat hearts are presented in Figure 39.
Basal negative dP/dt was significantly depressed in alloxan
diabetic rat hearts. Maximum responses in negative dP/dt to
isoproterenol in alloxan rat hearts were slightly but not
significantly depressed compared to controls. At 360 days after
induction of diabetes both basal negative dP/dt and maximum
changes in negative dP/dt produced by isoproterenol were not
altered in STZ-diabetic rat hearts (Figure 40). Table VI presents
maximum changes in negative dP/dt produced after the administration
of $3 \times 10^{-8}$ M isoproterenol in control as well as diabetic rat
hearts at all of the time points studied. The maximum change
produced by isoproterenol in hearts obtained from 7, 30, and 100
day alloxan and 100 and 180 day STZ diabetic rats was significantly
depressed when compared to that in age-matched control hearts.

TIME COURSE OF THE EFFECT OF ISOPROTERENOL ON CARDIAC CYCLIC AMP
CONTENT, PHOSPHORYLASE ACTIVITY AND INOTROPY OF CONTROL AND DIABETIC
RATS:

Isolated perfused working hearts obtained from 3 day and 100-120
day alloxan and STZ diabetic and control rats were quick frozen before
and at various time points after the administration of isoproterenol and the frozen heart tissue was analyzed for cyclic AMP content and phosphorylase activity.

**EFFECT OF ALLOXAN- AND STZ-DIABETES ON ISOPROTERENOL-INDUCED CHANGES IN RAT MYOCARDIAL cAMP CONTENT AND PHOSPHORYLASE ACTIVITY:**

Figures 41 and 42 present the time course of the effect of an ED<sub>50</sub> concentration of isoproterenol (5 x 10<sup>-9</sup>M) on cardiac cyclic AMP levels in 3 day and 100-120 day control and diabetic rats respectively. Both basal and isoproterenol stimulated levels of cyclic AMP were the same in control and diabetic rat hearts at both time points studied.

Figure 43 presents the time course of the effect of 5 x 10<sup>-9</sup>M isoproterenol on cardiac phosphorylase activity in 3 day control and alloxan- and STZ-diabetic rat hearts. Isoproterenol produced a time dependent activation of phosphorylase enzyme in both control and diabetic rat hearts and maximal activation of phosphorylase was observed at 20 secs after the administration of the drug. Basal phosphorylase activity was slightly but not significantly higher in 3 day alloxan and STZ diabetic rat hearts when compared to controls. Isoproterenol caused significantly greater activation of phosphorylase at 10, 20 and 40 sec time points in 3 day alloxan diabetic rat hearts when compared to controls. In 3 day STZ diabetic rat hearts isoproterenol also produced greater activation of phosphorylase enzyme. However, the changes were significant only at 20 secs after the administration of drug.

The time course of the effect of isoproterenol on cardiac phosphorylase activity in 100-120 day control and diabetic rat
hearts is shown in Figure 44. Basal phosphorylase activity in alloxan and STZ diabetic rat hearts was slightly but not significantly higher than that found in control rat hearts. Isoproterenol produced a time dependent activation of phosphorylase enzyme in control as well as diabetic rat hearts. The activation observed in alloxan and STZ diabetic rat hearts at 20-40 sec after drug administration was significantly greater than in control rat hearts.

Figure 45 presents total phosphorylase activity in control and alloxan and STZ diabetic rat hearts at 3 days and 100-120 days after treatment. Total phosphorylase activity was not significantly different in 3 day diabetic rat hearts from controls. However, 100-120 day diabetic rat hearts exhibited significantly higher phosphorylase activity compared to age-matched control rats.

EFFECT OF ALLOXAN AND STZ DIABETES ON ISOPROTERENOL-INDUCED CHANGES IN RAT HEART INOTROPY:

The time course of the effect of isoproterenol on positive dP/dt in 3 day control and diabetic rat hearts is shown in Figure 46. Changes produced with time in positive dP/dt by isoproterenol (5 x 10^-9 M) in control and alloxan and STZ diabetic rat hearts were similar. Figure 47 shows the time course of the effect of isoproterenol on positive dP/dt in 100-120 control and diabetic rat hearts. Basal positive dP/dt in 100-120 day alloxan and STZ diabetic rat hearts was significantly depressed compared to control rat hearts. The maximal changes produced in positive dP/dt by isoproterenol (at 20 and 40 sec) were not different in 100-120 day diabetic rat hearts as compared to controls. However, the onset of the positive inotropic effect of isoproterenol appears to be
slower in diabetic rat hearts.

The time course of the effect of isoproterenol on negative dP/dt in 3 day control and diabetic rat hearts is shown in Figure 48. Isoproterenol produced similar time dependent changes in negative dP/dt in hearts isolated from 3 day control and alloxan and STZ diabetic rats. Figure 49 illustrates the time course of the effect of isoproterenol on negative dP/dt in 100-120 day control and diabetic rat hearts. Basal negative dP/dt was significantly depressed in 100-120 day alloxan and STZ diabetic rat hearts. However, maximum changes in negative dP/dt caused by isoproterenol in diabetic rat hearts were slightly but not significantly different from those in control rat hearts. The onset of the effect of isoproterenol on negative dP/dt appears to be slower in diabetic rat hearts.

The dose-response curve of isoproterenol on cardiac cyclic AMP content in 100-120 day control and STZ-diabetic rats is shown in Figure 50. Isoproterenol produced similar dose-dependent increases in cardiac cyclic AMP content in control as well as diabetic rats. Figure 51 presents the dose-response curve of isoproterenol on cardiac phosphorylase enzyme in 100-120 day control and STZ-diabetic rats. Basal phosphorylase a activity was slightly but not significantly higher in diabetic rat hearts. Isoproterenol produced significantly higher activation of phosphorylase enzyme in diabetic rat hearts at any dose tested, when compared to that in control rat hearts. The dose-response curve of isoproterenol on positive dP/dt in 100-120 day control and diabetic rat hearts is illustrated in Figure 52. Basal positive dP/dt was significantly depressed in STZ-diabetic rat hearts compared to controls. However, isoproterenol produced similar dose-
dependent changes in positive dP/dt of both control and diabetic rat hearts. The dose-response curve of isoproterenol on negative dP/dt of isolated perfused working hearts obtained from 100-120 day control and STZ-diabetic rats is shown in Figure 53. Isoproterenol produced dose-dependent increases in negative dP/dt of both control and diabetic rat hearts. Basal negative dP/dt was significantly depressed in 100-120 day STZ-diabetic rat hearts compared to controls. Isoproterenol produced similar changes in negative dP/dt in control and diabetic rat hearts.

Effect of PGE$_1$ on cardiac phosphorylase activity in control and diabetic rats:

Effect of $10^{-6}$ M PGE$_1$ on cardiac phosphorylase activity was studied in 3 day and 100-120 day control and STZ-diabetic rat hearts. Isolated perfused working hearts were quick frozen before and 30 sec after the administration of PGE$_1$ and the frozen tissue was assayed for cyclic AMP content and phosphorylase a activity and the results are presented in Table VII. PGE$_1$ significantly increased cyclic AMP content in 3 day and 100-120 day control as well as diabetic rat hearts to a similar extent. However, PGE$_1$ significantly activated phosphorylase a only in diabetic rat hearts.

To test the possibility whether prostaglandin-like substances released by isoproterenol activate phosphorylase in the diabetic heart and thereby potentiate the effect of isoproterenol on phosphorylase, isolated working hearts from 100-120 day control and STZ diabetic rats
were pretreated with $10^{-6}$M indomethacin, a prostaglandin synthesis inhibitor, for 20 minutes before isoproterenol administration. Hearts were frozen 20 secs after the administration of $5 \times 10^{-9}$M isoproterenol and were assayed for phosphorylase activity. Indomethacin pretreatment did not alter the effect of isoproterenol on control cardiac phosphorylase; however, in the diabetic rat hearts indomethacin actually appeared to enhance the isoproterenol effect on phosphorylase. Isoproterenol caused a significantly greater activation of phosphorylase in diabetic rat hearts compared to control rat hearts as shown in Table VIII.

MUSCARINIC RECEPTOR BINDING STUDIES IN CONTROL AND DIABETIC RAT HEARTS:

To determine whether the increased sensitivity to carbachol exhibited by 180 day diabetic rat hearts was associated with changes at the receptor level, muscarinic receptor binding studies were carried out on the ventricular tissue obtained from 180 day control and diabetic rat hearts. The results are summarized in Table IX. There were no significant differences between control and diabetic rat hearts either in the total number of muscarinic receptor sites or in the affinity of these sites for $[^3H]$NMS. Non-radiolabelled NMS competed for the binding sites labelled by $[^3H]$NMS with similar affinities in both the control and diabetic rat hearts. Carbachol exhibited two affinities, one a high affinity and the other a low affinity, for the muscarinic receptor sites. The low affinity sites represented about 60% of the total binding sites. In the presence of GMPPNP, a non-hydrolyzable analog of GTP, the high affinity binding sites were converted to low affinity binding sites. There were no differences between control and diabetic
rat hearts with respect to the affinities of carbachol for the muscarinic receptor sites either in the presence or in the absence of GMPPNP.

LEFT VENTRICULAR NORADRENALINE CONTENT IN 180-DAY CONTROL AND DIABETIC RATS:

Noradrenaline content of the left ventricles obtained from 180-day control and diabetic rats was measured using a HPLC method, in order to determine whether a sympathetic neuropathy has developed in diabetic rat hearts. The results are presented in Table X. There were no significant changes in noradrenaline content in 180-day alloxan and STZ diabetic rat hearts compared to control rat hearts.
Table 1. Certain features of control and diabetic rats.

<table>
<thead>
<tr>
<th>TIME IN DAYS</th>
<th>TREATMENT</th>
<th>SERUM INSULIN µU/mL</th>
<th>SERUM GLUCOSE mg/dL</th>
<th>BODY WEIGHT g</th>
<th>HEART WEIGHT mg/g</th>
<th>HEART WEIGHT/BODY WEIGHT</th>
<th>CORONARY FLOW ml/min/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Control (9)</td>
<td>17.5 ± 2.6</td>
<td>70.8 ± 6.9</td>
<td>210.4</td>
<td>0.94 ± 0.03</td>
<td>4.48 ± 0.11</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Alloxan (9)</td>
<td>7.5 ± 1.7*</td>
<td>200.8 ± 35.5*</td>
<td>201.4</td>
<td>0.87 ± 0.03</td>
<td>4.35 ± 0.16</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>STZ (6)</td>
<td>8.8 ± 1.7*</td>
<td>200.7 ± 34.8*</td>
<td>201.4</td>
<td>0.87 ± 0.02</td>
<td>4.32 ± 0.10</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>30</td>
<td>Control (10)</td>
<td>20.9 ± 2.5</td>
<td>100.3 ± 8.5</td>
<td>236.3</td>
<td>1.03 ± 0.03</td>
<td>4.38 ± 0.16</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Alloxan (6)</td>
<td>13.1 ± 2.2*</td>
<td>383.2 ± 92.9*</td>
<td>214.10*</td>
<td>0.85 ± 0.04*</td>
<td>4.04 ± 0.19</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>STZ (9)</td>
<td>16.7 ± 0.9</td>
<td>252.8 ± 68.4*</td>
<td>222.5</td>
<td>0.88 ± 0.02*</td>
<td>3.96 ± 0.06</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>100</td>
<td>Control (7)</td>
<td>11.2 ± 0.7</td>
<td>88.9 ± 5.0</td>
<td>248.4</td>
<td>1.01 ± 0.03</td>
<td>4.09 ± 0.11</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Alloxan (6)</td>
<td>5.1 ± 1.1*</td>
<td>528.8 ± 108.0*</td>
<td>215.11*</td>
<td>0.80 ± 0.07*</td>
<td>3.72 ± 0.19</td>
<td>4.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>STZ (7)</td>
<td>5.2 ± 1.0*</td>
<td>561.5 ± 26.8*</td>
<td>182.10*</td>
<td>0.79 ± 0.06*</td>
<td>3.32 ± 0.18</td>
<td>5.6 ± 0.8</td>
</tr>
<tr>
<td>180</td>
<td>Control (8)</td>
<td>19.4 ± 1.3</td>
<td>137.0 ± 21.9</td>
<td>312.8</td>
<td>1.41 ± 0.05</td>
<td>4.55 ± 0.10</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>STZ (7)</td>
<td>9.4 ± 1.5*</td>
<td>349.3 ± 32.2*</td>
<td>211.9*</td>
<td>1.10 ± 0.05*</td>
<td>5.23 ± 0.10*</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>240</td>
<td>Control (5)</td>
<td>18.1 ± 2.4</td>
<td>132.6 ± 15.1</td>
<td>324.8</td>
<td>1.41 ± 0.06</td>
<td>4.36 ± 0.16</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Alloxan (8)</td>
<td>8.6 ± 1.9*</td>
<td>391.5 ± 89.7*</td>
<td>239.7*</td>
<td>1.20 ± 0.04*</td>
<td>5.00 ± 0.21*</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>360</td>
<td>Control (7)</td>
<td>18.1 ± 2.0</td>
<td>119.6 ± 8.9</td>
<td>372.13</td>
<td>1.53 ± 0.06</td>
<td>4.16 ± 0.16</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>STZ (6)</td>
<td>10.9 ± 1.6*</td>
<td>345.4 ± 49.7*</td>
<td>249.10*</td>
<td>1.30 ± 0.04*</td>
<td>5.29 ± 0.20*</td>
<td>3.1 ± 0.2</td>
</tr>
</tbody>
</table>

All the values are expressed as mean ± S.E. Numbers in parentheses are the number of rats used at that time point.

Coronary flow was measured during the initial 5 min period of retrograde perfusion at 45 cm H2O aortic perfusion pressure.

* Represents significant difference from control at p < 0.05.
TABLE II. Certain features of control and diabetic rats used in the time course study

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>CONTROL</th>
<th>ALLOXAN</th>
<th>STZ</th>
<th>CONTROL</th>
<th>ALLOXAN</th>
<th>STZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>BODY WEIGHT G</td>
<td>211±3.0</td>
<td>201±3.0*</td>
<td>199±3.0*</td>
<td>268±2.0</td>
<td>217±6.0*</td>
<td>223±5.0*</td>
</tr>
<tr>
<td>Serum Glucose</td>
<td>99±3.3</td>
<td>255±25.0*</td>
<td>188±15.4*</td>
<td>89±3.0</td>
<td>415±30.7*</td>
<td>403±32.7*</td>
</tr>
<tr>
<td>Serum T₄ mg/dL</td>
<td>4.3±1.0</td>
<td>3.1±0.4</td>
<td>5.3±0.6</td>
<td>5.0±0.2</td>
<td>3.9±0.2*</td>
<td>3.9±0.2*</td>
</tr>
<tr>
<td>Serum T₃B %</td>
<td>65.1±1.4</td>
<td>55.7±1.4*</td>
<td>57.3±3.4*</td>
<td>59.7±0.8</td>
<td>47.9±1.1*</td>
<td>49.3±0.9*</td>
</tr>
</tbody>
</table>

Serum glucose, T₄ (thyroxine) and T₃B (triiodothyronine bead uptake) were determined in blood collected at the time of sacrifice from 3 and 100-120 day control and diabetic rats.

All the values are expressed as mean±S.E.M. Numbers in parenthesis denote sample size.

* - denotes significant difference from control at P<0.05.

+ - serum triiodothyronine content was expressed as % bead uptake. The values reported here were similar to those reported by Fein et al. (8), using a similar assay method.
### TABLE III. FEATURES OF CONTROL AND DIABETIC RATS.

<table>
<thead>
<tr>
<th>TITLE OF THE STUDY</th>
<th>DURATION OF DIABETES</th>
<th>BODY WEIGHT (g)</th>
<th>SERUM GLUCOSE mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CONTROL</td>
<td>ALLOXAN</td>
</tr>
<tr>
<td>PGE1</td>
<td>3 days</td>
<td>181±2.0 (6)</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>100-120 days</td>
<td>248±4.0 (6)</td>
<td>--</td>
</tr>
<tr>
<td>INDOMETHACIN</td>
<td>100-120 days</td>
<td>243±6.0 (4)</td>
<td>--</td>
</tr>
<tr>
<td>MUSCARINIC RECEPTOR BINDING</td>
<td>180 days</td>
<td>298±6.0 (6)</td>
<td>--</td>
</tr>
<tr>
<td>NORADRENALINE ESTIMATION</td>
<td>180 days</td>
<td>310±10.0</td>
<td>268±9.0*</td>
</tr>
</tbody>
</table>

All the values are expressed as mean S.E.M. Numbers in parentheses denote sample size.

*Represent significant differences from control (p<0.05).
FIGURE 3:

Left ventricular pressure development at various left atrial filling pressures in isolated perfused working hearts obtained from 7 day alloxan- and STZ diabetic and control rats.

All the values are expressed as the mean±S.E.M. Numbers in parentheses are the number of animals used. LVDP or left ventricular developed pressure is expressed as mm Hg on the y-axis. Left atrial filling pressure is expressed as cm H$_2$O on the x-axis.
7 Days

- ○ ○ CONTROL (8)
- □ □ ALLOXAN (9)
- △ △ STZ (7)

LVDP (mm Hg)

FILLING PRESSURE (cm H₂O)
FIGURE 4:

Rate of left ventricular pressure development at various left atrial filling pressures in isolated perfused working hearts obtained from 7 day alloxan and STZ diabetic and control rats.

All the values are expressed as the mean±S.E.M. Numbers in parentheses are the number of rats used. +dP/dt or rate of left ventricular pressure development is expressed in mm Hg/sec on the y-axis. Left atrial filling pressure is expressed in cm H₂O on the x-axis.
7 Days

- **CONTROL** (9)
- **ALLOXAN** (9)
- **STZ** (7)

**+dP/dt** (mm Hg/sec)

**FILLING PRESSURE** (cm H₂O)
FIGURE 5:

Rate of left ventricular pressure decline at various left atrial filling pressures in isolated perfused working hearts obtained from 7 day alloxan and STZ diabetic and control rats.

All the values are expressed as mean±S.E.M. Numbers in parentheses are the number of rats used. -dP/dt or rate of left ventricular pressure decline is expressed in mm Hg/sec on the y-axis. Left atrial filling pressure is expressed in cm H$_2$O on the x-axis.
7 Days

- $\frac{dP}{dt}$ (mm Hg/sec)

FILLING PRESSURE (cm $H_2O$)
FIGURE 6:

Left ventricular pressure development at various left atrial filling pressures in isolated perfused working hearts from 30 day alloxan and STZ diabetic and control rats.

All the points are expressed as the mean±S.E.M. Numbers in parentheses denote sample size. Asterisks indicate significant differences from control at p<0.05 (Newman-Keuls test). LVDP is expressed in mm Hg on the Y-axis. Atrial filling pressure is expressed in cm H$_2$O on the x-axis.
30 DAYS

- - - CONTROL (9)
- - STZ (10)
- - - ALLOXAN (7)

LVDP mm Hg

FILLING PRESSURE cm H₂O
FIGURE 7:

Rate of rise of left ventricular pressure at various left atrial filling pressures in isolated perfused working hearts from 30 day alloxan and STZ-diabetic and control rats.

All the points are expressed as the mean ± S.E.M. Numbers in parentheses denote sample size. Asterisks indicate significant differences from control at p<0.05 (Newman-Keuls test). Positive dP/dt is expressed in mm Hg/sec on the y-axis. Atrial filling pressure is expressed in cm H₂O on the x-axis.
30 DAYS

○○ CONTROL (9)
△△ STZ (10)
□□ ALLOXAN (7)

+ dP/dt mm Hg.

FILLING PRESSURE cm H₂O
FIGURE 8:

Rate of decline of left ventricular pressure at various left atrial filling pressures in isolated perfused working hearts from 30 day alloxan- and STZ-diabetic and control rats.

All the points are expressed as the mean±S.E.M. Numbers in parentheses denote sample size. Asterisks indicate significant differences from control at p<0.05 (Newman-Keuls test). Negative dP/dt is expressed in mm Hg/sec on the y-axis. Atrial filling pressure is expressed in cm H₂O on the x-axis.
30 DAYS

- $\text{CONTROL (9)}$
- $\text{STZ (10)}$
- $\text{ALLOXAN (7)}$

$-dP/dt$ mm Hg

FILLING PRESSURE cm H$_2$O
FIGURE 9:

Left ventricular pressure development at various atrial filling pressures in isolated perfused working hearts obtained from 100 day alloxan- and STZ-diabetic and control rats.

All the values are expressed as the mean±S.E.M. Numbers in parentheses are the number of hearts used. LVDP is expressed in mm Hg on the y-axis. Filling pressure is expressed in cm H_2O on the x-axis. Asterisks indicate significant differences from control at p<0.05 (Newman-Keuls test).
FIGURE 10:

Rate of left ventricular pressure development at various left atrial filling pressures in isolated perfused working hearts obtained from 100 day alloxan and STZ diabetic and control rats.

All the values are expressed as the mean±S.E.M. Numbers in parentheses denote sample size. Positive dP/dt is expressed as mm Hg/sec on the y-axis. Filling pressure is expressed as cm H₂O on the x-axis. Asterisks indicate significant differences from control at p<0.05 (Newman-Keuls test).
100 DAYS

- **CONTROL** (11)
- **ALLOXAN** (7)
- **STZ** (9)

Graph showing the relationship between filling pressure (cm of H₂O) and dP/dt (mm Hg/sec) with different treatments. The graph includes error bars for each data point.
FIGURE 11:

Rate of left ventricular pressure decline at various left atrial filling pressures in isolated perfused working hearts obtained from 100 day alloxan and STZ diabetic and control rats.

All the values are expressed as the mean±S.E.M. Numbers in parentheses denote sample size. Negative dP/dt is expressed as mm Hg/sec on the y-axis. Filling pressure is expressed in cm H$_2$O on the x-axis. Asterisks denote significant differences from control at p<0.05 (Newman-Keuls test).
100 DAYS

- \( \frac{dP}{dt} \) mm Hg/sec

FILLING PRESSURE cmH\(_2\)O

CONTROL (11)

ALLOXAN (7)

STZ (9)
FIGURE 12:

Left ventricular pressure development at various left atrial filling pressures in isolated perfused working hearts from 180 day STZ-diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote the number of hearts used at this time point. LVDP is expressed in mm Hg on the y-axis and filling pressure in cm H$_2$O on the x-axis. Asterisks denote significant differences from control at $p<0.05$ (one-way ANOVA).
FIGURE 13:

Rate of rise in left ventricular pressure at various left atrial filling pressures in isolated perfused working hearts from 180 day STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote the number of hearts used at this time point. Positive dP/dt is expressed in mm Hg/sec on the y-axis and filling pressure in cm H$_2$O on the x-axis. Asterisks denote significant differences from control at p<0.05 (one-way ANOVA).
180 Days

○ ○ CONTROL (10)
△ △ STZ (11)

+ dP/dt (mm Hg/sec)

FILLING PRESSURE (cm H₂O)
FIGURE 14:

Rate of decline in left ventricular pressure at various left atrial filling pressures in isolated perfused working hearts from 180 day STZ-diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote the number of hearts used at this time point. Negative dP/dt is expressed in mm Hg/sec on the y-axis and filling pressure in cm H_2O on the x-axis. Asterisks denote significant differences from control at p<0.05 (one-way ANOVA).
FIGURE 15:

Left-ventricular pressure development at various left atrial filling pressures in isolated perfused working hearts from 240 day alloxan diabetic and control rats.

All the points represent the mean±S.E.M. Numbers in parentheses denote sample size. LVDP is expressed in mm Hg on the y-axis and filling pressure in cm H$_2$O on the x-axis.
240 Days

- - - Control (7)

○ ○ Alloxan (9)

LVDP mm Hg

Filling pressure cm H₂O
FIGURE 16:

Rate of left ventricular pressure development at various left atrial filling pressures in isolated perfused working hearts from 240 day alloxan diabetic and control rats.

All the points represent the mean±S.E.M. Numbers in parentheses denote sample size. Positive dP/dt is expressed in mm Hg/sec on the y-axis and filling pressure in cm H₂O on the x-axis. Asterisks denote significant differences from control at p<0.05 (one-way ANOVA).
240 Days

- Control (7)
- Alloxan (9)

Filling pressure cm H₂O

+\(dP/dt\) mm Hg/sec.
FIGURE 17:

Rate of left ventricular pressure decline at various left atrial filling pressures in isolated perfused working hearts from 240 day alloxan diabetic and control rats.

All the points represent the mean±S.E.M. Numbers in parentheses denote sample size. Negative dP/dt is expressed in mm Hg/sec on the y-axis and filling pressure in cm H$_2$O on the x-axis. Asterisks denote significant differences from control at $p<0.05$ (one-way ANOVA).
FIGURE 18:

Left ventricular pressure development at various left atrial filling pressures in isolated perfused working hearts obtained from 360 day STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parenthesis denote sample size. LVDP is expressed on the y-axis in mm Hg and filling pressure on the x-axis in cm H$_2$O.
FIGURE 19:

Rate of left ventricular pressure development at various left atrial filling pressures in isolated perfused working hearts obtained from 360 day STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Positive dP/dt is expressed on the y-axis in mm Hg/sec. Filling pressure is expressed on the x-axis in cm H₂O. Asterisks indicate significant differences from control at p<0.05 (one-way ANOVA).
FIGURE 20:

Rate of left ventricular pressure decline at various left atrial filling pressures in isolated perfused working hearts obtained from 360 day STZ-diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Negative dP/dt is expressed in mm Hg/sec on the y-axis and filling pressure in cm H₂O on the x-axis.
360 Days

○ ○ CONTROL (9)
△ △ STZ (9)
FIGURE 21:

Effect of the duration of diabetes on the rate of left ventricular pressure development in isolated perfused working hearts obtained from control and alloxan and STZ diabetic rats, at 15 cm H$_2$O left atrial filling pressure.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Asterisks represent significant differences from control at $p<0.05$ (Newman-Keuls test). Time after treatment with the diabetogenic drug is expressed in days in log scale on the x-axis. Positive dP/dt is expressed in mm Hg/sec on the y-axis.
$+\text{DP/DT AT 15 CM H}_2\text{O FILLING PRESSURE}$

(•) CONTROL (N=9)
(■) ALLOXAN (N=7)
(<•) STZ (N=9)

**Graph:**

- Y-axis: $+\text{DP/DT (MM HG/SEC)}$
- X-axis: TIME AFTER TREATMENT (DAYS)
- Time points: 7, 30, 100, 180, 240, 360
FIGURE 22:

Effect of the duration of diabetes on the rate of left ventricular pressure decline in isolated perfused working hearts obtained from control and alloxan and STZ diabetic rats, at 15 cm H$_2$O left atrial filling pressure.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Asterisks represent significant differences from control at p<0.05 (Newman-Keuls test). Time after treatment with the diabetogenic drug is expressed in days in log scale on the x-axis. Positive dP/dt is expressed in mm Hg/sec on the y-axis.
-DP/DT AT 15 CM H2O FILLING PRESSURE

(*) CONTROL (N=9)
(•) ALLOXAN (N=7)
(*) STZ (N=9)
FIGURE 23:

Dose-response curve of carbachol on basal positive dP/dt in isolated perfused working hearts from 7 day alloxan and STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses indicate sample size. Data are expressed as percent reduction in basal +dP/dt (y-axis) produced by various molar concentrations of carbachol (x-axis).
EFFECT OF CARBACHOL ON ⌧DP/ḊT DEVELOPMENT 7 DAYS

(*) CONTROL (N=9)
(■) ALLOXAN (N=9)
(─) STZ (N=8)
FIGURE 24:

Dose-response curve of carbachol on basal positive dP/dt in isolated perfused working hearts from 30 day alloxan and STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses indicate sample size. Data are expressed as percent reduction in basal +dP/dt (y-axis) produced by various molar concentrations of carbachol (x-axis).
EFFECT OF CARBACHOL ON $+\Delta P/\Delta T$ DEVELOPMENT 30 DAYS

- CONTROL (N=9)
- ALLOXAN (N=7)
- STZ (N=9)

% REDUCTION IN BASELINE

CARBACHOL - (M)
FIGURE 25:

Dose-response curve of carbachol on basal positive dP/dt in isolated perfused working hearts from 100-day alloxan- and STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses indicate sample size. Asterisks denote significant differences from control at p<0.05 (Newman-Keuls test). Data are expressed as percent reduction in basal +dP/dt (y-axis) produced by various molar concentrations of carbachol (x-axis).
EFFECT OF CARBACHOL ON +DP/DT DEVELOPMENT 100 DAYS

(*) CONTROL (N=10)

(*) ALLOXAN (N=8)

(*) STZ (N=10)
FIGURE 26:

Dose-response curve of carbachol on basal positive $dP/dt$ in isolated perfused working hearts obtained from 180 day STZ-diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses indicate sample size. Asterisks denote significant differences from controls at $p<0.05$ (one-way ANOVA). Data are represented as percent reduction in basal $dP/dt$ ($y$-axis) produced by various molar concentrations of carbachol ($x$-axis).
EFFECT OF CARBACHOL ON +dp/dt DEVELOPMENT 180 DAYS

(*) CONTROL (N=10)
(•) STZ (N=7)
FIGURE 27:

Dose-response curve of carbachol on basal positive dP/dt in isolated perfused working hearts obtained from 240 day alloxan diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses indicate sample size. Asterisks denote significant differences from control at p<0.05 (one-way ANOVA). Data are represented as percent reduction in basal +dP/dt (y-axis) produced by various molar concentrations of carbachol (x-axis).
EFFECT OF CARBACHOL ON \( +\text{DP/DT DEVELOPMENT} \) 240 DAYS

(●) CONTROL (N=6)
(■) ALLOXAN (N=9)
FIGURE 28:

Dose-response curve of carbachol on basal positive dP/dt in isolated perfused working hearts obtained from 360 day STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses indicate sample size. Data are presented as percent reduction in basal +dP/dt (y-axis) produced by various molar concentrations of carbachol (x-axis).
EFFECT OF CARBACHOL ON +DP/DT DEVELOPMENT 360 DAYS

(*) CONTROL (N=4)

(*) STZ (N=6)
## TABLE IV. GEOMETRIC MEAN ED<sub>30</sub> VALUES OF CARBACHOL

<table>
<thead>
<tr>
<th>TIME AFTER TREATMENT (DAYS)</th>
<th>CONTROL</th>
<th>ALLOXAN</th>
<th>STZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1.65x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>1.45x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>1.43x10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(1.22-2.23)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(1.16-1.85)</td>
<td>(1.03-1.98)</td>
</tr>
<tr>
<td></td>
<td>(9)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>(9)</td>
<td>(8)</td>
</tr>
<tr>
<td>30</td>
<td>1.67x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>1.82x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>1.57x10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(1.36-2.03)</td>
<td>(0.96-3.45)</td>
<td>(1.11-2.22)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(7)</td>
<td>(9)</td>
</tr>
<tr>
<td>100</td>
<td>1.57x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>5.91x10&lt;sup&gt;-6*&lt;/sup&gt;</td>
<td>6.1x10&lt;sup&gt;-6*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(1.00-2.46)</td>
<td>(2.79-12.5)</td>
<td>(3.08-12.1)</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(8)</td>
<td>(10)</td>
</tr>
<tr>
<td>180</td>
<td>1.85x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>-</td>
<td>7.34x10&lt;sup&gt;-7*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(1.48-2.3)</td>
<td>-</td>
<td>(4.77-11.2)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>-</td>
<td>(7)</td>
</tr>
<tr>
<td>240</td>
<td>1.87x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>8.16x10&lt;sup&gt;-7*&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(1.64-2.13)</td>
<td>(5.97-11.1)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(9)</td>
<td>-</td>
</tr>
<tr>
<td>380</td>
<td>3.02x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>-</td>
<td>1.43x10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(0.65 - 14.1)</td>
<td>-</td>
<td>(0.46-4.4)</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>-</td>
<td>(5)</td>
</tr>
</tbody>
</table>

All the values are expressed as the geometric mean with 95% confidence intervals of the dose of carbachol required to reduce basal dP/dt by 30%. ED<sub>30</sub> values were obtained from individual dose-response curves. Geometric mean and 95% confidence intervals were calculated according to the method of Fleming et al (1972).

<sup>1</sup>95% confidence limits

<sup>2</sup>number of hearts

*significantly different from control at p<0.05
FIGURE 29:

Dose-response curve of isoproterenol on positive dP/dt in isolated perfused working hearts from 7 day alloxan and STZ diabetic and control rats.

All the points are expressed as the mean±S.E.M. Numbers in parentheses denote sample size. Positive dP/dt in mm Hg/sec is plotted against negative log molar concentrations of isoproterenol. C represents basal positive dP/dt prior to isoproterenol administration.
EFFECT OF ISOPROTERENOL ON +DP/DT 7 DAYS

(*) CONTROL (N=9)
(■) ALLOXAN (N=9)
(▲) STZ (N=6)
FIGURE 30:

Dose-response curve of isoproterenol on positive dP/dt in isolated perfused working hearts from 30 day alloxan and STZ diabetic and control rats.

All the points are expressed as the mean±S.E.M. Numbers in parentheses denote sample size. Positive dP/dt in mm Hg/sec is plotted against negative log, molar concentrations of isoproterenol. C represents basal positive dP/dt prior to isoproterenol administration.
EFFECT OF ISOPROTERENOL ON $+\text{DP/DT}$ 30 DAYS

(*) CONTROL (N=10)
(■) ALLOXAN (N=6)
(♦) STZ (N=9)
Figure 31:

Dose-response curve of isoproterenol on positive dP/dt in isolated perfused working hearts from 100 day alloxan- and STZ diabetic and control rats.

All the points are expressed as the mean±S.E.M. Numbers in parentheses denote sample size. Positive dP/dt in mm Hg/sec is plotted against negative log molar concentrations of isoproterenol. C represents basal positive dP/dt prior to isoproterenol administration.
EFFECT OF ISOPROTERENOL ON $\frac{dP}{dt}$ 100 DAYS

- • CONTROL (N=7)
- ■ ALLOXAN (N=6)
- ♦ STZ (N=7)
FIGURE 32:

Dose-response curve of isoproterenol on positive $dP/dt$ in isolated perfused working hearts from 180 day STZ-diabetic and control rats.

All the points are expressed as the mean±S.E.M. Numbers in parentheses denote sample size. Positive $dP/dt$ in mm Hg/sec is plotted against negative log molar concentrations of isoproterenol. C represents basal $dP/dt$ prior to isoproterenol administration. Asterisks indicate significant differences from control ($p<0.05$ one-way ANOVA).
EFFECT OF ISOPROTERENOL ON $+\text{DP/DT}$ 180 DAYS

(●) CONTROL (N=8)
(●) STZ (N=7)
FIGURE 33:

Dose-response curve of isoproterenol on positive $dP/dt$ in isolated perfused working hearts from 240 day alloxan diabetic and control rats.

All the points are expressed as mean±S.E.M. Numbers in parentheses denote sample size. Positive $dP/dt$ in mm Hg/sec is plotted against negative log molar concentrations of isoproterenol. C represents basal positive $dP/dt$ prior to isoproterenol administration. Asterisks indicate significant differences from control ($p<0.05$ one-way ANOVA).
EFFECT OF ISOPROTERENOL ON +DP/DT 240 DAYS

(*) CONTROL (N=5)

(#) ALLOXAN (N=8)
FIGURE 34:

Dose-response curve of isoproterenol on positive dP/dt in isolated perfused working hearts from 360 day STZ diabetic and control rats.

All the points are expressed as mean±S.E.M. Numbers in parentheses denote sample size. Positive dP/dt in mm Hg/sec is plotted against negative log molar concentration of isoproterenol. C represents basal positive dP/dt prior to isoproterenol administration.
EFFECT OF ISOPROTERENOL ON \( +\text{DP}/\text{DT} \) 360 DAYS

(●) CONTROL (N=7)
(●) STZ (N=5)
Table V  Sensitivity of the rat myocardium to the positive inotropic effect of isoproterenol (pD₂ values).†

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time after treatment (days)</th>
<th>7</th>
<th>30</th>
<th>100</th>
<th>180</th>
<th>240</th>
<th>360</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.29±.06</td>
<td>8.32±.12</td>
<td>8.46±.07</td>
<td>8.21±.07</td>
<td>8.27±.10</td>
<td>8.26±.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(10)</td>
<td>(7)</td>
<td>(8)</td>
<td>(5)</td>
<td>(6)</td>
<td></td>
</tr>
<tr>
<td>Alloxan</td>
<td>8.36±.11</td>
<td>8.41±.08</td>
<td>8.43±.10</td>
<td></td>
<td>8.26±.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(6)</td>
<td>(6)</td>
<td></td>
<td>(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STZ</td>
<td>8.34±.07</td>
<td>8.39±.15</td>
<td>8.53±.13</td>
<td>8.15±.04</td>
<td></td>
<td>8.34±.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(9)</td>
<td>(7)</td>
<td>(7)</td>
<td></td>
<td>(6)</td>
<td></td>
</tr>
</tbody>
</table>

All values are mean±S.E.M. Numbers in parenthesis denote sample size.

†pD₂ is the negative log concentration of isoproterenol required to produce 50% of the maximum change observed.
FIGURE 35:

Dose-response curve of isoproterenol on negative dP/dt in isolated perfused working hearts from 7 day alloxan- and STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Asterisks indicate significant differences in the effect of isoproterenol on negative dP/dt from that in control (p<0.05, Newman-Keuls test). Negative dP/dt (mm Hg/sec) is plotted against negative log molar concentration of isoproterenol. C represents basal negative dP/dt prior to isoproterenol administration.
DRC OF ISO ON -DP/DT DEVELOPMENT 7 DAYS

(*) CONTROL (N=9)
(■) ALLOXAN (N=9)
(♦) STZ (N=6)
FIGURE 36:

Dose-response curve of isoproterenol on negative dP/dt in isolated perfused working hearts from 30 day alloxan and STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Asterisks indicate significant differences in the effect of isoproterenol on negative dP/dt from that in control (p<0.05, Newman-Keuls test). Negative dP/dt (mm Hg/sec) is plotted against negative log molar concentration of isoproterenol. C represents basal negative dP/dt prior to isoproterenol administration.
DRC OF ISO ON -DP/DT DEVELOPMENT 30 DAYS

(●) CONTROL (N=10)
(■) ALLOXAN (N=5)
(▲) STZ (N=9)
FIGURE 37:

Dose-response curve of isoproterenol on negative dP/dt in isolated perfused working hearts from 100-day alloxan and STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Asterisks indicate significant differences in the effect of isoproterenol on negative dP/dt from that in control (p<0.05, Newman-Keuls test). Negative dP/dt (mm Hg/sec) is plotted against negative log molar concentration of isoproterenol. C represents basal negative dP/dt prior to isoproterenol administration.
DRC OF ISO ON -DP/DT DEVELOPMENT 100 DAYS

(*) CONTROL (N=7)
(■) ALLOXAN (N=6)
(▲) STZ (N=7)
FIGURE 38:

Dose-response curve of isoproterenol on negative dP/dt in isolated perfused working hearts from 180 day STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Asterisks indicate significant differences in the effect of isoproterenol on negative dP/dt from that in control (p<0.05, one-way ANOVA). Negative dP/dt (mm Hg/sec) is plotted against negative log molar concentrations of isoproterenol. C represents basal negative dP/dt prior to isoproterenol administration.
DRC OF ISO ON -DP/DT DEVELOPMENT 180 DAYS
(●) CONTROL (N=8)
(◆) STZ (N=7)
Dose-response curve of isoproterenol on negative dP/dt in isolated perfused working hearts from 240 day alloxan diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Negative dP/dt (mm Hg/sec) is plotted against negative log molar concentration of isoproterenol. C represents basal negative dP/dt prior to isoproterenol administration.
DRC OF ISO ON -DP/DT DEVELOPMENT 240 DAYS

- CONTROL (N=5)
- ALLOXAN (N=8)

- DP/DT (MM HG/SEC)

ISOPROTERENOL - LOG DOSE
FIGURE 40:

Dose-response curve of isoproterenol on negative dP/dt in isolated perfused working hearts from 360 day STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Negative dP/dt (mm Hg/sec) is plotted against negative log molar concentration of isoproterenol. C represents basal negative dP/dt prior to isoproterenol administration.
DRC OF ISO ON -DP/DT DEVELOPMENT 365 DAYS

(●) CONTROL (N=7)
(○) STZ (N=5)
Table VI: Cardiac relaxant effect of isoproterenol

<table>
<thead>
<tr>
<th>TIME AFTER TREATMENT (IN DAYS)</th>
<th>TREATMENT</th>
<th>MAXIMUM CHANGE IN (-\text{dP/dt})^a (mm Hg/sec)</th>
<th>% OF CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control (9)</td>
<td>2725±300</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>alloxan (9)</td>
<td>1471±317*</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>STZ (7)</td>
<td>2089±274</td>
<td>77</td>
</tr>
<tr>
<td>7</td>
<td>control (9)</td>
<td>2692±330</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>alloxan (6)</td>
<td>1444±275*</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>STZ (9)</td>
<td>1993±256</td>
<td>74</td>
</tr>
<tr>
<td>30</td>
<td>control (7)</td>
<td>1750±185</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>alloxan (6)</td>
<td>825±265*</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>STZ (7)</td>
<td>896±230*</td>
<td>51</td>
</tr>
<tr>
<td>100</td>
<td>control (8)</td>
<td>2207±270</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>STZ (7)</td>
<td>1308±256*</td>
<td>59</td>
</tr>
<tr>
<td>180</td>
<td>control (5)</td>
<td>2293±293</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>STZ (8)</td>
<td>1685±274</td>
<td>74</td>
</tr>
<tr>
<td>240</td>
<td>control (5)</td>
<td>1437±217</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>STZ (5)</td>
<td>1066±232</td>
<td>74</td>
</tr>
</tbody>
</table>

All the values are mean±S.E.M.

Numbers in parenthesis denote sample size

a. maximum changes in \(-\text{dP/dt}\) produced by \(3\times10^{-8}\)M isoproterenol

* Denotes significant difference from control at \(P<0.05\).
FIGURE 41:

Time course of the effect of isoproterenol (5x10^{-9}M) on cyclic AMP content of isolated perfused working hearts from 3 day alloxan- and STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Cyclic AMP content was estimated using a radioimmunoassay method. Cyclic AMP content (femtomoles/mg tissue) is plotted against time (sec).
3 DAYS
(*) CONTROL (N=6)
(*) ALLOXAN (N=6)
(*) STZ (N=6)

$5 \times 10^{-9} \text{M ISO}$

Cyclic AMP Content (Femtomoles/Mg Tissue)

Time (Sec)
FIGURE 42:

Time course of the effect of isoproterenol (5x10^{-9}M) on cyclic AMP content of isolated perfused working hearts from 100-120 day alloxan and STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Cyclic AMP content was estimated using a radioimmunoassay method. Cyclic AMP content (femtomoles/mg tissue) is plotted against time (sec).
100-120 DAYS

(*) CONTROL (N=6)

(*) ALLOXAN (N=6)

(*) STZ (N=6)

$5 \times 10^{-9} \text{M ISO}$
FIGURE 43:

Time course of the effect of isoproterenol (5x10^{-9}M) on phosphorylase a activity of isolated perfused working hearts from 3 day alloxan and STZ-diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Phosphorylase a activity was measured in the direction of glycogen synthesis, and is expressed as a % of total phosphorylase activity on the y-axis. Time is expressed in seconds on the x-axis. Asterisks denote significant differences from control (p<0.05, Newman-Keuls test).
3 DAYS
(•) CONTROL (N=6)
(■) ALLOXAN (N=6)
(♦) STZ (N=6)

5\times 10^{-9} \text{M ISO}

PHOSPHORYLASE ACTIVITY (% OF TOTAL PHOSPHORYLASE)

TIME (SEC)
FIGURE 44:

Time course of the effect of isoproterenol (5x10^{-9}M) on phosphorylase a activity of isolated perfused working hearts from 100-120 day alloxan- and STZ-diabetic and control rats.

Each point represents the mean\pm S.E.M. Numbers in parentheses denote sample size. Phosphorylase a activity was measured in the direction of glycogen synthesis, and is expressed as a % of total phosphorylase activity on the y-axis. Time is expressed in seconds on the x-axis. Asterisks denote significant differences from control (p<0.05, Newman-Keuls test).
100-120 DAYS

(*) CONTROL (N=6)

(*) ALLOXAN (N=6)

(*) STZ (N=6)

$5 \times 10^{-9} \text{M ISO}$
FIGURE 45:

Total phosphorylase activity of isolated perfused working hearts from 3 and 100-120 day alloxan (AXN) and STZ diabetic and control (CONT) rats.

Each bar represents mean±S.E.M. Numbers in parentheses denote sample size. Total phosphorylase activity was measured in the direction of glycogen synthesis in the presence of 5'-AMP and is expressed as μmoles of phosphate liberated per mg tissue per minute (y-axis). Asterisks denote significant differences from control (p<0.05, Newman-Keuls test).
TOTAL PHOSPHORYLASE ACTIVITY
3 DAYS (N=30) 100-120 DAYS (N=30)
FIGURE 46:

Time course of the effect of isoproterenol (5x10^{-9}M) on the rate of left ventricular pressure rise of isolated perfused working hearts from 3 day alloxan- and STZ diabetic and control rats.

Each point represents the mean S.E.M. Number in parentheses denote sample size. Positive dP/dt (mm Hg/sec) is plotted against time (sec). C represents basal positive dP/dt after 15 min equilibration period.
TIME COURSE OF ISO ON RATE OF LV PRESSURE DEVELOPMENT

(*) CONTROL (N=6)
(■) ALLOXAN (N=6)
(※) STZ (N=6)

3 DAYS

$5 \times 10^{-9}$M ISO
Time course of the effect of isoproterenol (5x10^{-9} M) on the rate of left ventricular pressure rise of isolated perfused working hearts from 100-120 day alloxan- and STZ-diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Positive dP/dt (mm Hg/sec) is plotted against time (sec). C represents basal positive dP/dt after 15 min equilibration period. Asterisks denote significant differences from control (p<0.05 Newman-Keuls test).
TIME COURSE OF THE EFFECT OF ISO ON +DP/DT 100-120 DAYS

(•) CONTROL (N=6)
(●) ALLOXAN (N=6)
(♦) STZ (N=6)

5x10^-9 M ISO

+DP/DT (MM Hg/SEC)

TIME (SEC)
FIGURE 48:

Time course of the effect of isoproterenol (5 x 10^{-9} M) on the rate of left ventricular pressure decline of isolated perfused working hearts from 3 day alloxan and STZ-diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Negative dP/dt (mm Hg/sec) is plotted against time (sec). C represents basal negative dP/dt after 15 min equilibration period.
TIME COURSE OF ISO ON RATE OF LV PRESSURE DECLINE

- (•) CONTROL (N=6)
- (■) ALLOXAN (N=6)
- (△) STZ (N=6)

3 DAYS
5 x 10^{-9} M ISO
FIGURE 49:

Time course of the effect of isoproterenol (5x10^{-9} M) on the rate of left ventricular pressure decline of isolated perfused working hearts from 100-120 day alloxan and STZ diabetic and control rats.

Each point represents the mean S.E.M. Numbers in parentheses denote sample size. Negative dP/dt (mm Hg/sec) is plotted against time (sec). C represents basal negative dP/dt after 15 min equilibration period.
TIME COURSE OF THE EFFECT OF ISO ON -DP/DT 100-120 DAYS

(*): CONTROL (N=6)
(■): ALLOXAN (N=6)
(○): STZ (N=6)

5x10^{-9} M ISO
FIGURE 50:

Dose-response curve of isoproterenol on the cyclic AMP content of isolated perfused working hearts obtained from 100-120 day STZ-diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Cyclic AMP content (femtomoles/mg tissue) is plotted against log molar dose of isoproterenol. C represents basal cyclic AMP content.
DRC OF ISO ON CARDIAC CYCLIC AMP CONTENT
(●) CONTROL (N=6)
(△) STZ (N=6)

100 - 120 DAYS

Cyclical AMP Content (Moles/MG Tissue)

2 x 10^-9  5 x 10^-9  10^-8

Isoproterenol (M)
Dose-response curve of isoproterenol on phosphorylase \( a \) activity in isolated perfused working hearts from 100-120 day STZ-diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size.  Phosphorylase \( a \) activity is expressed as a % of total phosphorylase activity and is plotted against log molar dose of isoproterenol. Asterisks denote significant differences from control (p<0.05, one-way ANOVA).  C represents basal phosphorylase \( a \) activity.
DRC OF ISO ON CARDIAC PHOS A ACTIVITY

(●) CONTROL (N=6)
(◆) STZ (N=6)

100 - 120 DAYS

PHOS A % OF TOTAL PHOSPHORYLASE

2 x 10^{-9} M  5 x 10^{-9} M  1 x 10^{-8} M

ISOPROTERENOL (M)
FIGURE 52:

Dose-response curve of isoproterenol on the rate of left ventricular pressure rise in isolated perfused working hearts from 100-120 day STZ diabetic and control rats.

Each point represents the mean±S.E.M. Numbers in parentheses denote sample size. Positive dP/dt (mm Hg/sec) is plotted against log molar concentration of isoproterenol. C represents basal positive dP/dt prior to isoproterenol administration. Asterisk represents significant difference from control (p<0.05 one-way ANOVA).
DRC OF ISO ON RATE OF LEFT VENTRICULAR PRESSURE DEVELOPMENT

- (○) CONTROL (N=6)
- (△) STZ (N=6)

100 - 120 DAYS

ISOPROTERENOL (M)

+dp/dt (mm Hg/sec)
FIGURE 53:

Dose-response curve of isoproterenol on rate of left ventricular pressure decline of isolated perfused working hearts from 100-120 day STZ diabetic and control rats.

Each point represents mean±S.E.M. Numbers in parentheses denote sample size. Negative dP/dt (mm Hg/sec) is plotted against log molar concentration of isoproterenol. C represents basal negative dP/dt prior to drug administration. Asterisks represent significant difference from control (p<0.05 one-way ANOVA).
DRC of ISO on Rate of Left Ventricular Pressure Decline

(●) Control (N=6)
(●) STZ (N=6)

100 - 120 Days

-\( \frac{\text{dP}}{\text{d}t} \) (mm Hg/sec)

\( \text{ISOPROTERENOL (M)} \)

2 x 10^{-9} M 5 x 10^{-9} M 1 x 10^{-8} M
Table VII. Effect of PGE₁ on cardiac cyclic AMP and phosphorylase activity in control and diabetic rats.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>cAMP (femtomoles/mg tissue)</th>
<th>Phosphorylase a (% of total phosphorylase)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO DRUG</td>
<td>PGE₁ 10⁻⁶M</td>
</tr>
<tr>
<td>100-120 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>425±14.0</td>
<td>550±19.0*</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(5)</td>
</tr>
<tr>
<td>STZ DIABETIC</td>
<td>435±24.0</td>
<td>620±27.0*</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>3 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>417±11.0</td>
<td>561±8.0*</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>STZ DIABETIC</td>
<td>414±26.0</td>
<td>571±27.0*</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
</tr>
</tbody>
</table>

Isolated perfused working hearts from 100-120 and 3 day control and STZ diabetic rats were frozen before or 30 secs after the administration of 10⁻⁶ M PGE₁ and the frozen hearts were analyzed for cAMP content and phosphorylase activity.

All values are expressed as mean±S.E.M. Numbers in parenthesis denote sample size.

* denotes significant difference from the basal (no drug) value at p<0.05.
Table VIII. Effect of $10^{-6}$ M indomethacin on isoproterenol-induced cardiac phosphorylase activity in 100-120 day control and diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>PHOSPHORYLASE a ACTIVITY (% of total phosphorylase activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO DRUG</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>8.5±1.8**</td>
</tr>
<tr>
<td>STZ DIABETIC</td>
<td>16.9±3.3</td>
</tr>
</tbody>
</table>

Isolated perfused working hearts from 100-120 day diabetic and control rats were frozen before or 20 sec after the administration of $5\times10^{-9}$ M isoproterenol. Results in the last column were obtained from hearts which were perfused with buffer containing $10^{-6}$ M indomethacin for 20 minutes before administering isoproterenol.

All values are expressed as mean S.E.M. Numbers in parenthesis denote sample size.

* denotes significant differences from basal (no drug) value at $P<0.05$

** denotes significant differences from basal (no drug) value and the corresponding value in control experiments at $P<0.05$. 
Table IX. \([^3H]\) NMS binding in 180 day control and diabetic rat hearts.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>DIRECT BINDING</th>
<th>Cold NMS INHIBITION</th>
<th>CARBACHOL INHIBITION</th>
<th>CARBACHOL INHIBITION IN PRESENCE OF GMPPNP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[R]_t pmol/g</td>
<td>pK</td>
<td>pK_1</td>
<td>pK_2</td>
</tr>
<tr>
<td>CONTROL</td>
<td>57.3±9.8</td>
<td>9.57±1.1</td>
<td>9.3±0.3</td>
<td>6.12±1.1</td>
</tr>
<tr>
<td>DIABETIC</td>
<td>61.8±14.8</td>
<td>9.55±1.1</td>
<td>9.4±0.6</td>
<td>6.23±1.1</td>
</tr>
</tbody>
</table>

[R]_t = Total amount of receptors.
pK = negative log of dissociation constant
pK_1 = high affinity dissociation constant
pK_2 = low affinity dissociation constant
Fr_2 = Fraction of low affinity binding sites
All values are mean±S.E.M. of duplicate determinations
Table X. Left ventricular noradrenaline content in 180 day control and diabetic rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Noradrenaline µg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.48 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
</tr>
<tr>
<td>STZ</td>
<td>0.54 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
</tr>
<tr>
<td>Alloxan</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
</tr>
</tbody>
</table>

All values are mean S.E.M.
Numbers in parentheses denote sample size.
DISCUSSION

Results obtained in the present study demonstrate that injecting rats with either alloxan or STZ results in a diabetic state characterized by hypoinsulinemia, hyperglycemia and weight loss and that these diabetic rats develop cardiac functional and pharmacological abnormalities with time. Cardiac dysfunction observed in diabetic rats included a decreased ability of isolated perfused working hearts to develop peak left ventricular pressure as well as a decrease in maximum rates of left ventricular pressure rise and decline when hearts from diabetic rats were exposed to high work loads. The following pharmacological changes were observed: Diabetic rat hearts exhibited changes in their sensitivity towards the negative inotropic effect of carbachol; a decreased responsiveness towards the cardiac relaxant effect (maximum changes produced in negative dP/dt) of isoproterenol; and an increased sensitivity of the cardiac phosphorylase enzyme to isoproterenol.

THE DIABETIC-RAT MODEL:

Rats treated with either alloxan or STZ developed almost identical diabetic states and exhibited the classical symptoms of IDDM such as hyperglycemia, hypoinsulinemia, glucosuria, polyuria, polydipsia, polyphagia and weight loss. The ability of alloxan and STZ to produce such a diabetic state has previously been reported by Rerup (1970). Insulin levels, measured in the serum of fasted diabetic rats, were reduced to less than approximately 50% of control levels (Table I). The availability of some functional insulin in the diabetic rats eliminated the necessity for insulin therapy in these rats for survival. This untreated diabetic
rat model could possibly represent a poorly controlled IDDM condition. Fasting serum glucose levels were above 200 mg percent and indicated an overt diabetic state with fasting hyperglycemia. In the latter part of this study, insulin levels were not measured and fasting serum glucose levels were used as an indication of the severity of the disease. Throughout this study diabetic rats exhibited lower body and wet heart weights compared to controls. Loss of body weight in the diabetic rats could result from derangements in protein metabolism such as decreased protein synthesis and increased breakdown. All of these features were consistent with those previously reported in a similar diabetic rat model by Penpargkul et al. (1980) and Fein et al. (1980). Diabetic rats exhibited a hypothyroid state as indicated by reduced serum T₃ levels at 3 days and reduced serum T₃ and T₄ levels at 3 and 100 to 120 days after induction of the disease (Table II). It has been previously shown that serum T₃ and T₄ levels are lower in diabetic patients as well as in experimental diabetic rats (Pittman et al. 1979; 1981). A decrease in hepatic T₃ production owing to the inhibition of monodeiodination of serum T₄ in the liver has been demonstrated as early as 3 days after induction of STZ diabetes in the rat (Pittman et al. 1981). Various other studies have also reported depressed thyroid hormone levels in the blood of diabetic rats (Fein et al. 1980; Dillman, 1982).

In the present study 75% of the rats treated with alloxan and 95% of the rats treated with STZ became diabetic. The low incidence of diabetes with alloxan may be due mainly to the ultra short half-life of alloxan in body fluids. Mortality of diabetic rats was seen only
at time points longer than 100 days. It is difficult to attribute the mortality seen in diabetic rats to the severity of the diabetic state since an almost similar incidence of mortality was observed in the control group of rats.

CARDIAC FUNCTION IN DIABETIC AND CONTROL RATS:

Results obtained in the present investigation demonstrate that cardiac functional abnormalities develop in both alloxan and STZ diabetic rats. The prominent abnormalities observed were depressions in peak left ventricular pressure development (100 and 180 days) and decreased rates of left ventricular pressure rise (decreased myocardial contractility, at 30, 100, 180, 240, and 360 days) and left ventricular pressure decline (decreased cardiac relaxation at 30, 100, 180, and 240 days) at high atrial filling pressures (high work loads). Similar cardiac functional alterations have been reported in hearts obtained from 8-week STZ diabetic rats (Penpargkul et al. 1980). In our study alloxan-induced diabetes produced cardiac functional abnormalities as early as 30 days after induction, whereas the alterations produced by STZ diabetes were noticed only 100 days after treatment (Figures 21 and 22). A possible reason why a delayed onset of cardiac functional abnormalities was observed in STZ-induced diabetes may be that, at 30 days in this study, STZ treatment resulted in a relatively mild form of diabetes. STZ treatment at 30 days did not significantly reduce the insulin levels from control levels. However, a significant elevation in the serum glucose levels was observed (Table I). The delayed onset of functional abnormalities in STZ diabetic rats compared with alloxan diabetic rats might not have resulted owing
to the different forms of diabetes induced by these two drugs, but might have resulted owing to a failure of the drug to produce as severe a form of the disease state. Recently Fein et al. (1980) reported that abnormalities in relaxation and velocity of shortening were observed in papillary muscles isolated from 5-week STZ diabetic rats. These authors used rats of the same strain, sex and age and the same dose of STZ as was used in our study. However, a severe form of diabetes was induced by STZ in Fein's study, as evidenced by the high blood glucose values reported by these authors. These results support our assumption that we did not observe functional changes at 30 days in STZ-treated rats owing to the relative mildness of the disease at this time point in this series of animals. Furthermore, Opie et al. (1979a) have recently pointed out that both alloxan and STZ produce almost identical diabetic states in the rat.

Peak left ventricular pressure development was depressed in isolated perfused working hearts obtained from 30- and 100-day alloxan and 100- and 180-day STZ diabetic rats (Figures 6, 9 and 12). Even though hearts obtained from 240- and 360-day diabetic rats developed lower left ventricular pressures as compared to controls, the decreases were not significantly different (Figures 15 and 18). However, the rates at which left ventricular pressure rose in 240- and 360-day diabetic rat hearts and declined in 240-day diabetic rat hearts were significantly slower than in control hearts (Figures 16, 17, 19 and 20). It appears that the functional alterations occurring in the heart in experimental diabetes were more prominent in the rates at which left ventricular pressure developed and declined, than
in the absolute amount of pressure developed.

Depression in the rate of rise of left ventricular pressure by both alloxan and STZ diabetes progressed with time. The decreased ability of the diabetic heart to develop pressure may result from alterations in the contractile proteins and in the sarcoplasmic reticulum (SR) induced by diabetes. Some of these alterations include decreased actomyosin and myosin ATPase and myofibrillar basal and Ca$^{2+}$-stimulated ATPase activities (Dillman 1980; Malhotra et al. 1981; Pierce and Dhalla, 1981), and alterations in ventricular myosin components (Dillman 1980). A diminished Ca$^{2+}$ transport across cardiac SR was also reported in alloxan and STZ diabetic rats (Penpargkul et al. 1981; Lopaschuk et al. 1983). It has been shown that a build-up of intermediates of fatty acid metabolism such as long-chain acylcarnitines occur in the diabetic myocardium (Feuvray et al. 1979). The diminished Ca$^{2+}$ transport and Ca$^{2+}$ ATPase activity of the cardiac SR obtained from diabetic rats has been shown to be mainly due to an increase in the levels of long-chain acylcarnitines associated with the SR membrane (Lopaschuk et al. 1983). We did not observe any alterations in cardiac function at 7 days after induction of diabetes (Figures 3, 4 and 5). Our results do not agree with the results reported by Miller (1979), where alloxan diabetic rat hearts exhibited decreased abilities to develop normal systolic pressure at high atrial filling pressures. In the above study 5 mM glucose was used in the perfusion medium, and when the glucose concentration was raised to 10 mM, diabetic hearts performed similar to controls. Similar effects were observed by Ingebretsen et al. (1980), when they increased the glucose
concentration of the perfusate to 10 mM, in isolated working hearts from 3-week alloxan diabetic rats. In the present study 10 mM glucose was present in the perfusate and might explain why we did not observe changes in the rate of left ventricular pressure development at 7 days.

Depressions in maximum negative dP/dt were observed in alloxan diabetic rats at 30 days and in STZ diabetic rats at 100 days and these alterations progressed with time. Depression in maximum negative dP/dt was most prominent when hearts were perfused at high filling pressures. Regan et al. (1974) reported that in dog, chronic alloxan diabetes resulted in a reduced ventricular compliance and increased ventricular stiffness due to accumulation of glycoprotein in the heart. Stiffness of the ventricle could result in depressed negative dP/dt development. In a parallel study conducted in our laboratory, cardiac SR from 120-day alloxan and STZ diabetic rats exhibited reduced abilities to take up Ca\(^{2+}\), whereas cardiac SR from 7-day alloxan and STZ diabetic rats exhibited normal Ca\(^{2+}\) uptake patterns (Lopaschuk et al. 1983). In the present investigation, no changes in negative dP/dt development were observed in 7-day diabetic rat hearts, and a depressed maximum negative dP/dt development was observed at 15 cm H\(_2\)O and higher filling pressures in hearts isolated from 100-day diabetic rats. The decreased negative dP/dt development could also have resulted from a decreased ability of cardiac SR to accumulate Ca\(^{2+}\).

The rate of decline in ventricular pressure at high filling pressures was depressed in 240- and 360-day control hearts compared with younger control hearts from other time points. Though the maximum negative
dP/dt developments at high atrial filling pressures in 360-day STZ diabetic hearts were lower than the age-matched controls, the changes were not significant. The rates of decline in left ventricular pressure in 360-day STZ diabetic rat hearts were significantly depressed at high filling pressures, when compared with 180-day control rat hearts (heart weight matched controls). A prolongation of cardiac muscle relaxation with age has been reported in the rat (Weisfeldt, 1980). At time points such as 240 and 360 days in our study, depressions in maximum negative dP/dt produced by diabetes were not readily detectable as the cardiac relaxation in controls was also depressed owing to alterations induced by age. Since the changes appearing in control hearts due to age were not totally different from those observed in diabetic hearts at much earlier time points, it is tempting to hypothesize that diabetic rat hearts undergo an early aging process. In fact, premature cellular senescence owing to an underlying cellular abnormality has been proposed to be involved in the pathogenesis of various pathologic manifestations of diabetes (Renold et al., 1978).

Hearts obtained from 360-day STZ diabetic rats appeared to have performed better than those obtained from 180-day diabetic rats, at 15 cm H$_2$O atrial filling pressure (Figures 21 and 22) and at other higher atrial filling pressures. The depression noticed in the cardiac performance in 180-day diabetic rat hearts appeared to have gradually disappeared by 360 days, instead of progressively increasing. About 25% of mortality has occurred both in the diabetic and control groups between 180 and 360 days. One possibility might be that the most severely diabetic animals died before reaching the 360-day mark and
that the remaining survivors, with milder diabetes, exhibited better cardiac performance. However, this possibility appears to be highly unlikely since various features of 360-day diabetic rats were essentially the same as those of the 180-day diabetic rats (Table I).

Hearts obtained from diabetic rats were smaller than control hearts at all time points studied except 7 days after induction (Table I). Alloxan diabetes has been shown to inhibit the rate of protein synthesis in rat hearts (Williams et al. 1980). The depressed cardiac function in the diabetic rats was not due to the smaller size of the heart, as smaller hearts from younger control rats from 7- and 30-day groups performed better than the larger hearts of 240- and 360-day controls. Furthermore, smaller sized hearts were shown to perform better than larger hearts in the isolated perfused working heart system (Neely et al. 1967). It should also be noted that even though hearts obtained from 180-, 240- and 360-day diabetic rats were smaller compared with controls, they were actually larger in size relative to body weight. Coronary flow rates were not altered in the diabetic rat hearts at any time point from control rates (Table I). This observation suggests that the coronary vasculature in the diabetic rat hearts has not been subjected to any major complications. Since the functional alterations observed in the diabetic hearts in this study could have occurred without major vascular complications, these alterations may indicate the development of a diabetic cardiomyopathy. In particular, the findings may indicate the development of the preclinical left ventricular dysfunction phase of the cardiomyopathy. A number of clinical studies have demonstrated preclinical left ventricular
dysfunction in diabetics. Left ventricular dysfunction was observed both in systole and diastole and could represent defects in myocardial contraction as well as relaxation (Shapiro et al. 1981a and b). In the present study, depressions were observed in the rate of left ventricular pressure development and decline of isolated perfused working diabetic rat hearts, and these depressions represent defects in myocardial contraction and relaxation.

Insulin lack and the various metabolic abnormalities associated with insulin lack might primarily be involved in the development of various biochemical and structural alterations that cause myocardial dysfunction in diabetes. Recently, Fein et al. (1981) have demonstrated the ability of 4 weeks of insulin treatment to reverse the functional and biochemical alterations which occur in 6-week STZ diabetic rat hearts. A recent study from our laboratory has shown that insulin treatment could not only reverse the functional alterations in 6-week STZ diabetic rat hearts but could also prevent these alterations from occurring (Tahiliani et al. 1983). In the above two studies, the effect of insulin therapy was studied in diabetic rats at the time of onset of cardiac dysfunction. Studies involving insulin treatment of long-term diabetic animals with completely developed cardiac abnormalities provided conflicting results. While Baandrup et al. (1981) have shown that insulin treatment could prevent the accumulation of connective tissue and multiplication of arterial media cells in hearts obtained from 9-month STZ diabetic rats, Regan et al. (1981) demonstrated the incapability of insulin supplementation to prevent collagen accumulation and cardiac dysfunction from occurring in 1-year alloxan diabetic dogs. Recently, it has been demonstrated in our laboratory that 4-week insulin treatment did not completely reverse the cardiac functional abnormalities
that occur in 5-month STZ diabetic rats (Tahiliani, 1983). The effectiveness of prolonged insulin treatment in reversing the cardiac dysfunction of 5- or 6-month diabetic rats is yet to be studied. From these studies it is apparent that insulin treatment could prolong the onset of cardiac functional abnormalities in diabetic animals and could also reverse these abnormalities back to normal, but only during the early phases of development. However, it should be noted that cardiac complications are observed in treated as well as poorly controlled diabetic patients.

RESPONSIVENESS OF CONTROL AND DIABETIC HEARTS TO CARBACHOL:

Results obtained in the present investigation show that the responses of the diabetic myocardium to carbachol changes with the progression of the disease state. Hearts isolated from short-term alloxan and STZ diabetic rats (7 days and 30 days after induction of the disease, Figures 23 and 24) exhibited a similar responsiveness and sensitivity to the negative inotropic effect of carbachol when compared to control rat hearts. As the disease state progressed, diabetic rat hearts (100 days after induction, Figure 25) became subsensitive to the negative inotropic effect of carbachol. However, this subsensitive phase was transient as hearts obtained from long-term diabetic rats (180-day STZ diabetic and 240-day alloxan diabetic rats, Figures 26 and 27) were supersensitive to the negative inotropic effect of carbachol. Both control and diabetic rat hearts appeared to be less sensitive to carbachol by 360 days (Figure 29). At this time-point diabetic hearts appeared to be slightly more sensitive than control hearts. However, both control and diabetic hearts were slightly less sensitive to carbachol when compared to the respective groups at earlier time-points. Basal positive dP/dt at 15 cm H₂O atrial filling pressure in 100-day diabetic rat hearts in the cardiac function study. Although we did not consistently observe
statistically significant depressions in positive dP/dt and 15 cm H₂O filling pressure in 100-day diabetic rat hearts in these studies, we always observed trends in that direction. Differences in sample size employed in the various experiments probably account for this inconsistency.

To date, there have been few data reported concerning the effects of cholinergic agonists on diabetic myocardium. Foy and Lucas (1976) reported that one-week alloxan- and two-week STZ-induced diabetes produced a reduced sensitivity to the depressor effect of acetylcholine in pithed rats. Very recently, Tomlinson and Yusof (1981) showed that isolated left atria from 7 and 8 month alloxan diabetic rats were supersensitive to the negative inotropic effect of acetylcholine. Results obtained in the present investigation with 7-day and 30-day control and diabetic rat hearts demonstrated no change in the sensitivity of diabetic myocardium towards the negative inotropic effect of carbachol. While our results appear to disagree with those of Foy and Lucas (1976) it should be noted that Foy and Lucas did not directly study the negative inotropic effect of acetylcholine. Results obtained from long-term diabetic rat hearts (180-day STZ and 240-day alloxan diabetic rats) in this study agree with the findings of Tomlinson and Yusof (1981).

The supersensitivity exhibited by the long-term diabetic myocardium might indicate the development of an autonomic neuropathy. Diabetic autonomic neuropathy with early vagal dysfunction has been demonstrated clinically in long-term diabetics (Lloyd-Mostyn and Watkins, 1975; Ewings et al. 1980). Defective parasympathetic innervation of the heart could lead to a lack of the neurotransmitter acetylcholine and
to the development of postjunctional supersensitivity to exogenously administered cholinergic drugs. Partial and occasionally total vagal denervation of the heart has been encountered clinically in chronic diabetic patients (Wheeler and Watkins, 1973; Lloyd-Mostyn and Watkins, 1976). Pathological changes such as severe loss of myelinated axons and excess deposition of collagen have been demonstrated in the vagus nerve during postmortem examination of diabetic patients (Duchen et al., 1980). Similar pathological alterations associated with reduced choline acetyltransferase activity have been reported in cholinergic nerves innervating colons isolated from 5 to 7 month STZ diabetic rats (Schmidt et al., 1981). Tomlinson and Yusof (1981) demonstrated a neuropathy of the cardiac vagus nerve in 7 to 8 month alloxan diabetic rats. These authors showed that stimulation of cholinergic nerves of left atria failed to produce a negative inotropic effect and ultrastructurally demonstrated the absence of cholinergic nerve endings in the right atria.

The decreased sensitivity exhibited by diabetic hearts to carbachol at 100 days after induction of diabetes is difficult to explain. During the initial phase of the disease the diabetic myocardium exhibited no changes in its sensitivity to carbachol suggesting that there were no major alterations in the autonomic regulation of the heart. As the disease progressed, hearts first became transiently subsensitive, before finally becoming supersensitive. The subsensitive phase might represent an earlier stage of a developing autonomic neuropathy. While changes were occurring in the function and structure of autonomic nerves owing to the severe metabolic derangement of
diabetes, physiological compensatory mechanisms might come into play. An excess turnover of the neurotransmitter, acetylcholine, for a short duration resulting in postjunctional receptor desensitization could thus occur. A recently published report by Stuesse et al. (1982) provides some evidence for the above assumption. These authors showed that vagal nerve stimulation resulted in a more pronounced bradycardia in 7 to 9 week alloxan rats when compared to control rats. Activation of a compensatory mechanism in the vagal efferent nerves, resulting in a greater response to nerve stimulation in the diabetic rats, was offered as one of the explanations.

Results obtained in the present investigation demonstrate that the sensitivity of the diabetic myocardium shifts from one extreme to the other as the disease state progressed. Such a phenomenon is not a completely uncommon finding in experimentally-induced diabetic rats. It has been previously shown in STZ diabetic rats that short term diabetes produced hypotension but as the duration of the disease increased rats became hypertensive (Jackson and Carrier, 1981).

Hearts isolated from control and diabetic rats at 360 days were slightly but not significantly less sensitive to carbachol when compared to control and diabetic rat hearts at earlier time points. This apparent decrease in sensitivity exhibited by both control and diabetic rat hearts at this time point might be due to aging. The effect of aging on the responsiveness of the heart to cholinergic agonists has not been well documented although studies on rabbit atria show that the sensitivity to cholinergic agonists does decrease with age (Goldberg and Roberts, 1980).
Differences in mortality did not seem to affect the results. The greatest mortality was seen in the 240-day alloxan treated group (33%) but the same response to carbachol was obtained in the 180-day STZ treated group in which the mortality was zero. At 360 days the mortality in both the STZ and control rats was exactly the same (25%). Therefore the results obtained in the present study are not due to the fact that only the stronger or less diabetic animals survived for longer periods.

In conclusion, results obtained with carbachol in the present study demonstrate changes in the sensitivity of the diabetic myocardium to cholinergic agents which may indicate an autonomic neuropathy of the heart associated with vagal dysfunction.

EFFECT OF ISOPROTERENOL ON CONTROL AND DIABETIC RAT HEARTS:

Results obtained demonstrate that neither the sensitivity nor the responsiveness of the diabetic myocardium to the positive inotropic effect (changes produced in positive dP/dt) of isoproterenol was altered at any stage of diabetes studied (Figures 29 to 34 and Table V). However, the cardiac relaxant effect (changes produced in negative dP/dt) of isoproterenol was altered in diabetic rat hearts. Maximum changes produced in negative dP/dt by isoproterenol were significantly depressed in 7-, 30- and 100-day alloxan and 100- and 180-day STZ-diabetic rat hearts (Figures 35 to 38). Ventricular noradrenaline content did not change in 180-day alloxan- and STZ-diabetic rats when compared to age-matched controls (Table X).

Previous reports concerning the effects of β-adrenergic agonists on the heart in experimentally-induced diabetes are conflicting. Foy
and Lucas (1978) have demonstrated that the inotropic responses to noradrenaline and isoproterenol were reduced while the chronotropic responses to these agonists increased in isolated spontaneously beating atria from rats made diabetic with STZ two weeks prior to use. However, Ingebretsen et al. (1980) reported no change in the inotropic response of isolated perfused working hearts from acute alloxan-diabetic rats to isoproterenol. Furthermore, a very recent report by Tomlinson and Yusof (1981) showed that there were no differences in the response to noradrenaline of isolated atria from 7 to 8 month alloxan-diabetic and control rats. Results obtained in the present investigation showed that the positive inotropic effect of isoproterenol was not altered in isolated working hearts from alloxan- and STZ-diabetic rats at any time point after induction of the disease.

Our results with acute diabetic rat hearts appear to disagree with those of Foy and Lucas (1978). The disagreement could be due to certain differences between these two studies. Foy and Lucas (1978) used isolated spontaneously beating atria from two week diabetic rats, whereas isolated perfused working hearts from 7- and 30-day diabetic rats were used in the present study. Another difference between these two studies is in the glucose concentration of the perfusate employed. Foy and Lucas (1978) used 5.6 mM glucose whereas 10 mM glucose has been used in this study. However, Ingebretsen et al. (1980) have demonstrated that isoproterenol produced normal positive inotropic responses in isolated perfused working diabetic rat hearts perfused with 5.5 mM glucose. Results obtained in this study with 6- and 8-month diabetic rat hearts agree with those reported by Tomlinson and Yusof (1981) using isolated
atria from 7 to 8 month alloxan-diabetic rats. These authors did not find any changes in the responses of diabetic rat atria to noradrenaline. Furthermore, a recent study from our laboratory has demonstrated that left atria obtained from 7-day and papillary muscles from 7- and 70-day diabetic rat hearts exhibited no changes in the maximum inotropic responses to isoproterenol when compared to controls (McCullough and McNeill, 1983).

Apart from producing a positive inotropic effect, catecholamines are known to exert a profound relaxing effect on the heart (Vittone et al., 1981). In the present study, changes produced by isoproterenol in negative dP/dt were measured in control and diabetic rat hearts and were used as an index of the cardiac relaxant effect of isoproterenol. Maximum changes produced in negative dP/dt by isoproterenol were depressed in diabetic rat hearts at various time points after the induction of the disease (Table VI). Recently, Fein et al. (1980) have studied the effect of noradrenaline on time to 1/2 relaxation (time required to reach 50% of total relaxation) of papillary muscles isolated from 10-week diabetic and control rats. These authors reported that the time to 1/2 relaxation in diabetic papillary muscles was longer than controls at all the noradrenaline concentrations tested. However, in that study noradrenaline seems to produce similar changes in time to 1/2 relaxation in control and diabetic papillary muscles as indicated by the parallel upward shift of the noradrenaline dose-response curve in diabetic preparations. In the present study diabetic rat hearts not only had a depressed basal negative dP/dt but also exhibited depressed responses to isoproterenol. Calcium binding to, and calcium uptake by
the sarcoplasmic reticulum have been proposed to be one of the main events that initiate relaxation in the heart (Katz, 1980). Alterations in calcium binding and calcium uptake by the sarcoplasmic reticulum could affect cardiac relaxation. It has been reported previously that the cardiac sarcoplasmic reticulum from chronic diabetic rats exhibited a reduced ability to accumulate and transport calcium (Penpargkul et al. 1981; Lopaschuk et al. 1983). These abnormalities in the sarcoplasmic reticulum might be responsible for the depression observed on the effect of isoproterenol on negative dP/dt. However, decreases in the cardiac relaxant effect of isoproterenol were also observed in 7-day diabetic rat hearts; a time point at which the sarcoplasmic reticular function has been shown to be unaltered (Lopaschuk et al. 1983). Therefore abnormalities in cardiac sarcoplasmic reticular function may not solely be responsible for the observed depressions in the effect of isoproterenol in diabetic rat hearts. Regan et al. (1974) reported that increased ventricular stiffness and decreased ventricular wall compliance to be the major defects in cardiac relaxation in chronic diabetic dogs. Ventricular wall stiffening could also result in the observed depressions in the effect of isoproterenol, but only in chronic diabetic rat hearts. A possibility remains that the cyclic AMP cascade system might be depressed in experimental-diabetes, leading to these alterations. Ingebretsen et al. (1981) have reported a fifty percent reduction in the isoproterenol-induced changes in the cardiac cyclic AMP system in acute diabetic rats. However, these authors also reported that there were no changes in the positive inotropic effect of isoproterenol in diabetic rat hearts. Furthermore, in the present study, isoproterenol
produced similar time-dependent changes in cardiac cyclic AMP levels in acute as well as chronic diabetic rats, when compared to controls (Figures 41 and 42). $\beta$-adrenergic receptor density has been reported to be reduced by 30 percent in diabetic rat hearts (Savarese and Berkowitz, 1979; Ingebretsen et al., 1982). However, a 30 percent reduction in $\beta$-adrenergic receptor density might not result in the observed depression of the effect of isoproterenol because: (1) Reductions in the effect of isoproterenol were only observed in negative $dP/dt$, but not in positive $dP/dt$. If a lower number of receptors were to account for a reduction in the maximum effect to isoproterenol, then these reductions should be seen both in positive and negative $dP/dt$. (2) It has been shown previously that spare $\beta$-adrenergic receptors exist in the heart and that a substantial reduction in the $\beta$-adrenergic receptor density is required in order to cause a shift in the sensitivity to the agonist, without a reduction in the maximum responses (Venter, 1979; Siegl and McNeill, 1982). Therefore, the observed reductions in the maximum negative $dP/dt$ responses to isoproterenol in diabetic rat hearts are probably not due to either a reduction in $\beta$-receptor density or to changes in the cyclic AMP system. The depressed maximum effect of isoproterenol on negative $dP/dt$ in acute diabetic rat hearts might result from the metabolic derangements of acute diabetes leading to altered energy utilization (Feuvray et al., 1979) and decreased ATP production (Allison et al., 1976; Miller, 1979).

Changes produced by isoproterenol in negative $dP/dt$ in 240- and 360-day diabetic rat hearts were slightly, but not significantly, depressed from controls. It should be noted that the maximum changes
produced in negative dP/dt in control hearts at these time points were lower compared to those at earlier time points. An age related decrease in the responsiveness of the heart (Lakatta et al., 1975) and blood vessels (Fleish, 1981) to adrenergic agonists has been previously reported. Since the maximum changes produced by isoproterenol in the negative dP/dt of control rat hearts decreased with age, diabetes-induced reductions in the effect of isoproterenol were not readily apparent at the later time points.

Sympathetic autonomic neuropathy of the heart occurs in long term diabetics, resulting in disturbances of cardiovascular reflexes (Hosking et al., 1978; Clarke et al., 1979). One of the common features of this neuropathy is degeneration of the post-ganglionic nerve terminals leading to depletion of neurotransmitter thus creating a situation similar to denervation (Giachetti, 1981). Noradrenaline content of the heart and arterial system has been reported to be markedly decreased in long-term diabetics (Neubauer and Christensen, 1976). Evidence for degeneration of sympathetic nerves has been presented showing that diabetic patients were supersensitive to exogenous norepinephrine (Moorehouse et al., 1966). In the present study we determined ventricular noradrenaline content in 180-day diabetic and control rats and found that it was unaltered in diabetic rat hearts (Table X). Our results, supported by previously published reports, show no changes in the noradrenaline content of hearts isolated from diabetic rats (Head and Berkowitz, 1979; Kaul and Grewal, 1980). Furthermore, sensitivity of the diabetic myocardium towards isoproterenol was also not altered in the present study. These findings do not support the possibility of the
development of a sympathetic autonomic neuropathy in the heart under the conditions used in the present study.

In conclusion, experimentally-induced diabetes did not alter either the responsiveness or the sensitivity of the myocardium towards the positive inotropic effect of isoproterenol. However, maximum changes produced by isoproterenol in negative dP/dt of isolated working hearts from diabetic rats were significantly depressed at various time points after induction of the disease. Ventricular noradrenaline content in 180-day diabetic rats was unaltered, indicating that there were no pathological changes in sympathetic nerve terminals in the heart. These results indicate the absence of a sympathetic diabetic neuropathy of the heart. Acute metabolic derangements causing changes in energy production and utilization and abnormalities in cardiac sarcoplasmic reticular function may represent the major underlying causes for the depressed cardiac relaxant effect of isoproterenol in diabetic rat hearts.

CARDIAC CYCLIC AMP AND PHOSPHORYLASE SYSTEM IN EXPERIMENTAL DIABETES AND THE EFFECT OF ISOPROTERENOL AND PGE₁:

Results obtained in the present investigation demonstrate that isoproterenol (5x10⁻⁹M) causes a greater activation of the phosphorylase enzyme in isolated perfused working hearts from acute as well as chronic diabetic rats when compared to age-matched controls (Figures 43 and 44). Both acute and chronic diabetic rat hearts exhibited slightly but not significantly higher phosphorylase α activity when compared to control rat hearts, while total phosphorylase activity was significantly higher.
only in chronic diabetic rat hearts (Figure 45). However, maximal changes produced by isoproterenol in cardiac cyclic AMP content, positive and negative dP/dt development in control and diabetic rats were all of similar magnitude (Figures 41, 42, 46, to 49). Further, phosphorylase \(a\) was activated by PGE\(_1\) in both acute and chronic diabetic rat hearts but not in age-matched control rat hearts.

Isoproterenol produced identical dose-dependent changes in cardiac cyclic AMP content and inotropy in 100-120 day diabetic rat hearts compared to controls (Figures 50, 52 and 53). However, isoproterenol produced a significantly higher activation of phosphorylase in 100 to 120-day diabetic rat hearts as compared to control (Figure 51).

Basal cyclic AMP levels were not altered either in 3-day or in 100 to 120-day diabetic rat hearts. This observation does not agree with the findings of Chaudhury and Shipp (1973) that basal cyclic AMP levels were four-fold higher in 48-hour alloxan diabetic rat hearts compared to controls. The discrepancy between these two studies can be to some extent attributed to differences in the methodology employed. Chaudhury and Shipp (1973) studied cyclic AMP content in hearts which were freeze-clamped immediately after sacrifice, whereas in this study cyclic AMP levels were studied in isolated perfused working hearts that were frozen following a 15 minute perfusion. Recently several reports have appeared in the literature showing no changes in the basal cyclic AMP content of isolated perfused hearts (Ingebretsen et al. 1981; Miller et al. 1981), liver (Yamashita et al. 1980) and kidney (Hoskins and Luong, 1981), from acute alloxan and STZ diabetic rats. Furthermore basal adenylate cyclase activity of the hearts from acute alloxan (Menahan et al. 1977)
and STZ diabetic rats (Das, 1973b) has been shown to be unaltered. These reports all support our finding that basal cyclic AMP levels are not altered in acute diabetic rat hearts. The cyclic AMP system in chronic diabetic rat hearts had not been studied previously. We observed that the basal cyclic AMP content was unaltered in isolated perfused hearts from 100 to 120 day alloxan and STZ diabetic rats. Isoproterenol produced similar changes in the cyclic AMP content in isolated perfused hearts from acute as well as chronic diabetic and control rats (Figures 41 and 42). Existing literature on the effect of isoproterenol on cyclic AMP content in acute diabetic hearts is not in agreement. Ingebretsen et al. (1981) have shown that there was a fifty percent reduction in the activation of cyclic AMP and cyclic AMP-dependent protein kinase system by isoproterenol in acute diabetic rat hearts compared to controls; whereas Miller et al. (1981) reported no such reduction in the epinephrine-induced activation of cyclic AMP in acute alloxan diabetic rat hearts. Cardiac β-adrenergic receptor density has been shown to be decreased in diabetic rats (Savarese and Berkowitz, 1977; Ingebretsen et al., 1982). However, Ingebretsen et al. (1981) have reported no changes in maximum positive inotropic effect of isoproterenol in isolated perfused hearts from acute diabetic rats. In the present study we did not observe any significant changes in the isoproterenol-induced maximum increases in positive and negative dP/dt development (Figures 46, 47, 48 and 49) in hearts from acute and chronic diabetic rats and these changes were associated with normal cardiac cyclic AMP responses to isoproterenol. Since the maximum inotropic responses to isoproterenol were not depressed in acute as well as chronic diabetic rat hearts, it is reasonable to assume that various
events occurring between the receptor and the inotropic effect are normal. It seems highly unlikely to have, in diabetic rat hearts, a decrease in the total number of β-adrenergic receptors and a reduction in the β-adrenergic agonist-stimulated activation of the cyclic AMP system and yet have a normal maximum positive inotropic effect. Further supporting our data is the report (Ingebretsen et al., 1982) showing that the basal myocardial adenylate cyclase activity and its responses to isoproterenol were unaltered in acute alloxan diabetic rats.

Very few studies have been published in which the glycogen phosphorylase enzyme in hearts isolated from diabetic rats has been studied. Das (1973a) reported that there were no changes in the cardiac basal phosphorylase a and total phosphorylase activities in STZ diabetic rats. Miller et al. (1981) showed that acute alloxan diabetes did not alter basal cardiac phosphorylase activity but resulted in a significantly greater activation of phosphorylase by epinephrine. In the present study we observed that isoproterenol caused a 2 to 3-fold higher activation of phosphorylase a enzyme in acute and chronic diabetic rat hearts, as compared to age-matched control rat hearts (Figures 43 and 44). Our observations on cardiac phosphorylase enzyme levels from acute diabetic rats thus agree with those reported by Miller et al. (1981) and we have now extended these observations to chronic diabetic rat hearts as well. Total phosphorylase activity was significantly greater in 100 to 120 day diabetic rat hearts as compared to age-matched control hearts (Figure 45), whereas basal phosphorylase a activity was slightly but not significantly higher in diabetic rat hearts. The greater activation of phosphorylase a enzyme by isoproterenol
could partly result from the increase in total phosphorylase activity. However, this is not the case with acute diabetic hearts as total phosphorylase activity in these hearts was not significantly different from control. Phosphorylase can be activated by calcium (Friesen et al., 1969) as well as by cyclic AMP. Ca\textsuperscript{2+} uptake by sarcoplasmic reticulum has been shown to be depressed in chronic diabetic rat hearts (Penpargkul et al., 1981; Lopaschuk et al., 1983). This may lead to a situation where a build up of cytoplasmic free Ca\textsuperscript{2+} concentration over normal concentrations could occur in the myocardium, particularly when the heart was stimulated by a β-adrenergic agonist. The increase in cytoplasmic free Ca\textsuperscript{2+} could then cause an activation of phosphorylase which would be most apparent following isoproterenol induced increases in intracellular calcium. Alterations in cyclic AMP-mediated calcium flux in the diabetic myocardium as suggested by Miller et al. (1981) are yet another possibility contributing to the phosphorylase response in chronic diabetic rat hearts.

The nature of the increased sensitivity of phosphorylase to isoproterenol in acute diabetic rat hearts is more difficult to explain. Ca\textsuperscript{2+} transport across the sarcoplasmic reticulum has been shown to be unaltered in acute diabetic rat hearts (Lopaschuk et al., 1983). Hence the contributory effect of an elevated cytoplasmic Ca\textsuperscript{2+} concentration may not occur. Miller et al. (1981) suggested that a diabetes-related α-effect, which was not present in the normal heart, may play a contributory effect but since isoproterenol is essentially a pure β-agonist this possibility is also unlikely. Another possibility could be that changes occur in the phosphorylase enzyme caused by acute metabolic derangement produced by the induction of a diabetic
state. The increased sensitivity of phosphorylase enzyme to isoproterenol may thus be related to the severity of the acute diabetic phase. Figure 43 shows that isoproterenol-activated phosphorylase was significantly greater in hearts obtained from acute alloxan diabetic rats at 10, 20 and 40 seconds after administration as compared to control rat hearts. In hearts obtained from acute STZ diabetic rats significantly greater activation was seen only at 20 seconds. This difference between alloxan and STZ treated rats may be due to the severity of the induced diabetic state. In this study alloxan resulted in a more severe acute diabetic state as indicated by the fasting serum glucose levels (Table II). Since similar increases in cyclic AMP, in acute as well as chronic diabetic rat hearts, resulted in a significantly greater activation of phosphorylase compared to that in control rat hearts; the possibility remains that the sensitivity of the phosphorylase enzyme to cyclic AMP-mediated activation could have been increased due to diabetes, as has been suggested previously (Ingebretsen et al., 1981). Furthermore, the sensitivity of phosphorylase kinase enzyme from acute diabetic rat hearts to epinephrine-induced activation has been shown to be increased when compared to that in controls (Miller et al., 1981).

PGE₁ elevated cyclic AMP content in control and diabetic rat hearts to a similar extent, but activated phosphorylase a in a diabetic rat hearts only (Table VII). It has been shown previously that PGE₁ can elevate cyclic AMP content without altering phosphorylase a activity in perfused rat hearts (Keely, 1977; Vadlamudi and McNeill, 1981). Hypothetical compartmentalization of the cardiac cell with respect to cyclic nucleotide action has been proposed to be responsible for such a specific effect of PGE₁ (Hayes and Brunton, 1982). The experimentally-induced diabetic state seems to have disrupted these hypothetical compartments in the myocardium resulting in the activation of phosphorylase
by PGE₁. Such an effect might also be responsible for the enhanced activation of phosphorylase by isoproterenol in the diabetic myocardium. The observation that indomethacin did not block the enhanced activation of phosphorylase by isoproterenol in diabetic rat hearts rules out the possible involvement of prostaglandin-like substances, released by β-adrenergic receptor stimulation, in activating phosphorylase. A very recent report by Shaffer and Malik (1982) has shown that β₁-adrenergic receptor stimulation indeed caused a release of prostaglandins in isolated perfused rabbit hearts and also that these prostaglandins did not influence the mechanical effects produced by the adrenergic stimuli. Indomethacin, in fact, appeared to enhance the effect of isoproterenol activating phosphorylase in diabetic but not in control hearts. There is no known interaction between the two drugs and the enhancement is thus difficult to explain.

Thyroid function has been shown to be depressed in experimentally-induced diabetes (Pittman et al. 1981). As shown in Table 2, T₃ levels were depressed in acute as well as chronic diabetic rats but T₄ levels were depressed only in chronic diabetic rats. These results agree with previously published results (Fein et al. 1981; Dillman 1982). Altered thyroid function has been shown to cause various changes in the responsiveness of the myocardium towards the effects of catecholamines (Kunos, 1981). Myocardial adenylate cyclase activity, and its responsiveness to agonists, was shown to be depressed in hypothyroid animals (Levey et al. 1969; Brodde et al. 1979). Metabolic effects of catecholamines have been reported to be depressed in hypothyroid rat hearts (Fain, 1981). The changes due to hypothyroidism seem to be in the
opposite direction to those observed in this study. Hence, the changes observed in the cardiac glycogen phosphorylase system in experimentally-induced diabetes are almost certainly not the result of the hypothyroid state produced by diabetes.

In conclusion, experimentally-induced diabetes did not alter either the basal or the isoproterenol-stimulated cyclic AMP levels in the heart. However, the responsiveness of the cardiac phosphorylase enzyme towards isoproterenol was increased in diabetes. This effect was observed as early as 3 days after induction of diabetes and persisted in 100-to-120-day diabetic rats. Furthermore, the cardiac phosphorylase enzyme in diabetic rats was activated by PGE₁, a compound which does not activate phosphorylase in normal rat hearts. The nature and physiological significance of this increased responsiveness of phosphorylase enzyme in diabetic rat hearts is yet to be determined. Severe metabolic derangements and alterations in Ca²⁺ homeostasis produced by diabetes may be the underlying causes for this effect.

MUSCARINIC RECEPTORS IN DIABETIC RAT HEARTS:

Results from muscarinic receptor binding studies demonstrated no changes either in the number or the affinity of muscarinic receptors in the hearts obtained from 180-day STZ diabetic rats compared to controls. These results suggest that the supersensitivity exhibited by the 180-day diabetic myocardium towards the negative inotropic effect of carbachol is not a result of the increased number of receptors. The supersensitivity of the diabetic myocardium could result from changes in post-receptor events. Unfortunately, the consequences of
muscarinic receptor stimulation in the heart are not well understood. Therefore it is very difficult to make any assumptions on the nature of the changes occurring in post-muscarinic receptor events.

Recently, Latifpour et al. (1983) have demonstrated a 30% decrease in the number of muscarinic receptors in ventricles obtained from 180-day diabetic rats. These results disagree with the findings of this study and also make it difficult to explain the supersensitivity exhibited by diabetic rat hearts towards carbachol. However, a number of methodological differences exist between the two binding studies. We used pooled ventricular preparations obtained from diabetic and control hearts that were perfused on working heart apparatus, frozen and stored at -70°C for over 8 months, for the binding studies and Latifpour et al. (1983) used fresh, non-perfused, individual ventricles in their binding studies. The radioligands, incubation conditions, and methods used to separate bound radioactivity from free were all different in these two studies. These methodological differences could partly explain the differences in results between these two studies. In any case, both studies agree that muscarinic receptor number and/or affinity are not increased in ventricles from 6-month diabetic rats.

CONCLUSIONS:

Experimentally-induced diabetes in the rat produced various time dependent changes in cardiac function, pharmacological responses and biochemical events.

Cardiac function was not altered in 7-day alloxan and STZ diabetic
rats compared to control rats. Cardiac function was depressed in 30-, 100- and 240-day alloxan diabetic and 100-, 180- and 360-day STZ diabetic rats when compared to age-matched controls. Cardiac functional abnormalities observed in diabetic rats included a decreased ability to develop peak left ventricular pressure and a decrease in maximum rates of left ventricular pressure rise and decline, when isolated perfused working hearts from diabetic rats were exposed to high atrial filling pressures. These functional abnormalities may reflect defects in myocardial contractility and relaxation induced by the diabetic state. These findings may also represent the development of the preclinical left ventricular dysfunction phase of diabetic cardiomyopathy, which is often observed in chronic diabetic patients.

Responsiveness of the diabetic myocardium to the negative inotropic effect of carbachol was not altered at 7 and 30 days after induction of diabetes. A subsensitivity to carbachol was exhibited by diabetic rat hearts at 100 days, which was ultimately converted to a supersensitivity by 180 days and this supersensitivity persisted in the diabetic myocardium thereafter. The changes in the sensitivity of diabetic rat hearts to the negative inotropic effect of carbachol may represent various stages in the development of a parasympathetic diabetic neuropathy.

Diabetic rat hearts did not exhibit any changes in either the sensitivity or in the responsiveness to the positive inotropic effect of isoproterenol. However, maximum changes produced by isoproterenol in negative dP/dt (cardiac relaxant effect) were depressed in diabetic rat hearts at various time points after the induction of diabetes. The change in the responsiveness of the diabetic myocardium to the cardiac
relaxant effect of isoproterenol might result from metabolic derangements in the heart causing changes in energy production and utilization and/or abnormalities in cardiac sarcoplasmic reticular function.

Left ventricular noradrenaline content was unchanged in chronic diabetic rat hearts indicating the absence of any sympathetic nerve damage owing to the development of a sympathetic neuropathy.

Basal cyclic AMP content and phosphorylase α activity were not altered in acute as well as chronic diabetic rat hearts. Isoproterenol (5 x 10^{-9}M) produced similar time- and dose-dependent changes in cyclic AMP content and positive and negative dP/dt in isolated perfused working hearts obtained from 3 and 100 to 120 day alloxan and STZ diabetic rats as compared to controls. However, isoproterenol caused a significantly greater activation of phosphorylase in hearts obtained from 3 and 100 to 120 day diabetic rats, when compared to age-matched controls. Diabetic rat hearts from 100 to 120-day animals had significantly higher total phosphorylase activity as compared to age-matched controls. PGE_{1}, a drug which increases cyclic AMP content without altering phosphorylase α activity in perfused rat hearts, increased phosphorylase α activity in acute as well as chronic diabetic rat hearts but not in control rat hearts. The nature and physiological significance of the enhanced responsiveness of the phosphorylase enzyme in diabetic rat hearts is unclear. Acute metabolic derangements and alterations in Ca^{2+} homeostasis caused by diabetes could possibly result in the enhanced responsiveness of phosphorylase to agonists.

Muscarinic receptor binding studies performed on ventricles isolated from 180-day control and diabetic rats revealed no changes
either in the total density or in the affinity of muscarinic receptors in 180-day diabetic rat hearts. These results suggest that the supersensitivity exhibited by the 180-day diabetic myocardium to carbachol is not a result of increased density of muscarinic receptors in the heart, but could result from alterations in events beyond the receptor level.
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ABSTRACTS:


