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The Ohio State University Ph.D. 1984

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THE EFFECTS OF MATERNAL DIABETES MELLITUS ON CARDIAC DEVELOPMENT IN THE CD-1 MOUSE FETUS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Graduate School of The Ohio State University

BY:

PAUL BRUCE BARLETT, B.Sc., M.Sc.

The Ohio State University
1984

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INTRODUCTION

Birth defects in the human have been attributed to several different agents in our environment. These agents include a variety of physical, biological and chemical factors. One of these biological factors is the disease diabetes mellitus. Soler (1976) stated that a malformation rate of 8.1% exists in infants of diabetic mothers and that this rate was three to four times higher than the rate in the general population. The most frequently observed malformations are of the cardiovascular system, in particular the heart. Jervell (1980) found that the cardiovascular malformation rate was five times higher in infants of diabetic mothers when compared to the general population. The relationship between maternal diabetes and fetal malformations goes back to the discovery of insulin in 1922.

Banting and Best, in their 1922 article, gave a brief summary of the research on the pancreas that lead them to postulate the existence of the hormone insulin and its control over glucose levels in the blood. In 1889, Mering and Minkowski totally removed the pancreas from dogs and found that this resulted in severe and fatal diabetes. Following this, many different investigators experimented with animals of various species and found in all types examined a glycouria and fatal cachexia after this operation. Opie and Scobolew, in 1901, were first to
postulate that the islets of Langerhans were involved in pancreatic diabetes. This postulate lead Minkowski to utilize the pancreas in studying defects of carbohydrate metabolism. He attempted to feed the pancreas to subjects with diabetes, but had no success in the treatment of the disease. Murlin and Kleiner, 1913 and 1914 respectively, prepared extracts from pancreatic tissue and after injection of this solution, secured a reduction in sugar excreted in diabetic animals. This lead Banting and Best (1922), studying dogs, to postulate the existence of the hormone insulin, its production in the islets of Langerhans, and its importance in the regulation of blood sugar levels.

With the discovery of insulin by Banting and Best, insulin therapy became an important weapon in the fight against diabetes. Sisson (1940) points out that up to this time, diabetic women either did not become pregnant or they failed to give birth to live children. He also states that since the insulin era, the fecundity of diabetic women has increased and the juvenile diabetic patient can now reach child bearing age, which lead to an increase in the number of infants of diabetic mothers.

The fact that cardiac defects in neonates are associated with diabetes mellitus is not a new discovery. Miller (1943) observed, by roentgen examination, an increase in the size of the hearts of the infants born to diabetic mothers. The diagnosis of an enlarged heart was based on the comparison of the greatest transverse diameter of the heart to that of the thorax. A cardiothoracic ratio of fifty-five or greater was arbitrarily accepted as evidence of cardiac enlargement. It was also noted that after the first ten days of life the heart
decreased in size and returned to a normal size. He also observed an increase in the amount of glycogen upon histological examination of the heart tissue.

Miller (1945) published observations relating to the postmortum examination of infants who died due to complications associated with the influence of maternal diabetes. In all cases, he reported an increase in heart size and weight by both direct observation and roentgen examination. He also noted that during histological observation of the myocardial tissue of these infants, there were hypertrophied cell of the muscle fibers.

It was not until 1954 that Pedersen theorized a cause for these defects. Studying twenty-seven mothers with diabetes mellitus and their infants, Pedersen (1954) suggested that there is a relationship between maternal glucose levels and fetal development. He found that maternal hyperglycemia passed through the placenta and was added to the fetal circulation. This, in turn, would cause hyperactivity of the fetal islet cells, thus increasing the fetal production of insulin. It appeared to Pedersen that the insulin was the important factor and not necessarily the glucose. He found that after birth the condition changed. The blood sugar levels of the infant were now determined by the fetal liver threshold which was very low. This agreed with his report that in the first twenty-four hours of life, the infant's blood sugar levels is inversely correlated with the maternal pregnancy level. He noted that the effect only lasted for a few days. Therefore, to Pedersen, the primary teratological agent is the insulin which is produced by a secondary agent, the glucose.
Kucera (1971) examined malformation types in fetuses of diabetic mothers by geographic area. His data indicated that the most common types of defects are those involving the heart. He also showed that the most common malformations in the United States are those involving the cardiovascular system.

Rowland (1973) reviewed 470 cases of infants born to diabetic mothers and classified these cases into groups according to the forms of congenital heart disease seen. He reported cases of transposition of the great arteries, ventricular septal defects and coarctation of the aorta as the most frequently observed malformations. Upon autopsy of these infants, Rowland found that the heart weights may exceed two standard deviations above the normal weights. He did not find an increase in glycogen deposits, but did discover an increase in the amount of both myocardial nuclei and sarcoplasm. Thus suggesting an anabolic effect of hyperinsulinemia.

As improvements were made in diagnostic techniques, more and more cardiac malformations were recognized. Gutgesell (1976), using hemodynamic, angiographic and echocardiographic methods, reported important findings on three infants that had congestive heart failure. He associated the heart failure with a condition called hypertrophic subaortic stenosis. This condition is characterized by asymmetric hypertrophy of the ventricular septum. All these infants were born to mothers with diabetes mellitus and the condition appeared to be resolved during the first six months of life. Wolfe and Way (1977), observing twenty-eight infants of diabetic mothers, noted that fifty percent or
more of these infants had either radiographic cardiomegaly or clinical features suggesting congestive heart failure.

In 1978, controversy arose with respect to the association of heart defects and maternal diabetes. Maron (1978) reported a disproportionate ventricular septal thickening in developing normal human heart. Maron observed normal human heart specimens from five weeks of gestation to after birth both grossly and histologically. He saw a thickening of the ventricular septum with respect to the left ventricular freewall. Histologically, the vast majority of the cardiac muscle cells in the ventricular septum were normally arranged. He did observe small foci in which adjacent cardiac muscle cells were arranged obliquely and perpendicularly to each other. So the question arose: Were the defects seen by earlier investigators really associated with maternal diabetes or were they just looking at normal development in a slightly immature heart?

In order to respond to the question raised by Maron, a new resurgance in research began. Gutgesell (1978) reported that in twenty-seven infants of diabetic mothers, twenty-five had cardiomegaly or electrocardiographic abnormalities. Histologically, hypertrophic fibers, some in a whorling pattern, lie adjacent to necrotic foci in the septum. He concluded that despite clinical and histological similarities, the hypertrophic cardiomegaly of infants of diabetic mothers differs from other forms of obstructive cardiomegaly in that it is transient and non-familial. Way (1979) reported on his study of eleven infants of diabetic mother. He used a variety of testing procedures which included echocardiography, pressure studies and
electrocardiography. He observed a disproportionate septal hypertrophy with subaortic obstruction. He concluded that this condition was benign with a resolution of symptoms in two to four weeks and a resolution of the septal hypertrophy within two to twelve months postnatally.

Using echocardiographic techniques, Mace (1979) compared septal thickness with body weight. Using three groups: 1) those infants in which congestive heart failure predominates, 2) those infants in which respiratory distress predominates and 3) those infants that were asymptomatic. It was shown that infants of diabetic mothers generally have thickened septa in excess of the expected value on the basis of body weight and those infants with congestive heart failure have the thickest septa.

Inspecting hearts of infants of diabetic mothers histologically, Halliday (1981) reported that there were some vacuolar and hydrospic changes but these were nonspecific and did not amount to any clear cardiomegaly. There was no disorganization of myocardial fibers nor inflammatory cell infiltration and even special staining failed to show excessive deposition of glycogen within the cellular cytoplasm. In a case study, Leslie (1982) reported on a female fetus born to a diabetic mother. She found septal hypertrophy defined as septal freewall ratio greater than 1.3 when compared to the left ventricle. The heart was enlarged with a deviation of the interventricular septum. The septal wall had a shelf-like protrusion in the subaortic area. Microscopically, the heart showed increased nuclear density and myocardial hyperplasia, but the fibers seemed to be well organized.
In order to correlate the effects of maternal diabetes to other diseases, Breitsweser (1980) studied infants of diabetic mothers and an infant with nesidioblastosis. Nesidioblastosis is a condition of ductoinsular cell proliferation with hyperplasia of the islets of Langerhans. He reported that there were identical cardiac defects in both groups and therefore deduced that insulin, and not glucose, was the most important teratological agent in infants of diabetic mothers.

Animal studies by Solomon (1959) using a high dosage of Alloxan on pregnant rats, reported an increased birth weight and an increased number of stillborns in the fetuses. Deuchar (1977) employing 60 mg/kg of Alloxan in one rat study and 40 mg/kg of streptozotocin in another rat study, found an increased incidence in both groups' fetuses of: nonclosure of the neural tube, deformities of the heart chambers and a variety of skeletal defects. Monkey studies by Susa (1979) using implanted insulin pumps in the fetuses found an increased fetal body weight and enlarged livers, placentas, hearts and spleens. Animal studies to date have not fully explored the developmental aspects of cardiac defects caused by diabetes in the pregnant dam.

Previous investigations have dealt primarily with retrospective studies on human newborn infants of diabetic mothers. There have been limited studies in both human and animal models of the cardiac defects caused by diabetes. Since diabetes is widely present in the general population of women of child-bearing age, continued investigation of the mechanisms of teratogenicity of diabetes and attempts to lessen such teratogenicity is warranted.
This proposed study will administer a measured dose of streptozotocin to pregnant CD-1 mice in order to establish an animal model in which to study the effects of diabetes on the development of the interventricular septum. (The choice of drug-induced diabetes over genetically diabetic animals was made because the proposed study will mimic the gestational diabetic condition and not the overt, long-term diabetic.)

Although animal models do not reflect perfectly the human condition, the data collected from this experiment may help predict the effect of maternal diabetes on the developing fetal heart as well as establish an animal model for the study of mechanisms of formation of developmental defects in this select population. It is hoped that physicians can use these results to provide diabetic women with reliable information concerning the effects of diabetes on the developing heart.
MATERIALS AND METHODS

Adult CD-1 mice obtained from Charles River Animal Laboratories for use in this experiment were housed in polypropylene cages in a temperature, humidity and light-controlled (12 hour light-dark) environment. Water and Purina Lab Chow were given ad libitum.

Mice, weighing approximately 30 grams, were mated overnight (6:00 p.m. - 6:00 a.m.) in a ratio of three females to one male and the presence of a vaginal plug was considered day 0 of gestation. All pregnant animals were weighed daily and the amount of the drug given adjusted to the changing weights.

Design of Experiment (Appendix 1)

I. Drugs

On day nine of gestation, two groups of ten pregnant females each were injected intraperitoneally with 30 mg/kg and 40 mg/kg respectively, of streptozotocin (Upjohn Co.) dissolved in distilled water. A third group of ten female animals served as controls and were injected with distilled water.

II. Maternal Pancreas - Verification of the Diabetic State

On day nineteen of gestation, each pregnant mouse was weighed and sacrificed by cervical dislocation. At this time, the maternal pancreas was removed from each animal by a transverse incision below the
diaphragm. The pancreas was fixed in 10% neutral buffered formalin. It was imbedded in paraffin and sectioned on an American Optic 820 microtome at 7 μm. Sections were placed on glass slides and stained for beta-cell content of the islets of Langerhans using the Rhodocyan Technique of Glenner and Lillie (1957). The sections were observed with an American Optic microscope and photographs taken using Kodacolor VR ASA 100 film. The data obtained was compared with the maternal pancreas from the control group which was processed in the same manner.

III. Examination of the Fetuses

After removal of the maternal pancreas the uteri were exposed. Implantation sites were counted, and recorded as live, dead and resorbed fetuses. Each fetus was removed, weighed and examined for external gross malformations.

One-half of the live fetuses were fixed in Bouin's fixative and examined using Wilson's technique (1965). The other one-half of the live fetuses were fixed in 95% ethyl alcohol and stained for cartilage and bone using the Inouye technique (1976).

IV. Examination of the Hearts

Hearts were removed from the fetuses as part of the evisceration in preparation for the Inouye technique for cartilage and bone staining. These hearts were weighed and a cut was made along the midtransverse plane of the ventricles. These hearts plus the Wilson thorax sections through the ventricular septum were examined under a Wild Photomakroskop dissecting microscope and a photograph was taken using Kodacolor VR ASA 100 film with a final magnification of thirty-two times. From these photographs, measurements were taken of the free right ventricular wall,
the free left ventricular wall, the interventricular septum and overall ventricular diameter. These values were compared with the values obtained from control animals.

V. Statistical Analysis

An Analysis of Variance was carried out on fetal weights, heart weights, heart ventricular diameter and interventricular septal diameter to measure the effects of drug dosage levels. Within each dosage group, the mean, standard deviation and normal biological distribution curve of the above parameters were used to study the extremes found in each group.

The use of linear regression was used to study the relationship between the interventricular septal diameter and the left ventricular free wall diameter. And lastly, the Fischer exact probability test was used to investigate the number of fetal deaths with respect to dosage level.
RESULTS

Pancreatic Analysis

The maternal Islets of Langerhans from the control group showed cells with well defined borders. These cells were uniform in size, dark staining nuclei and visible cytoplasm. Islets from the 30 mg/kg and 40 mg/kg group exhibited cells with well-defined borders, dark-staining nuclei and vacuolated cytoplasm. The number of vacuolated cells appears to increase as the dosage increases with the greatest number of vacuolated cells in the 40 mg/kg group. In the three groups examined, the number of islet groups are not changed (Figure 2).

Fetal Deaths

The effect of the dosage levels of streptozotocin on fetal deaths is summarized in Appendix 1. In the control group, there were no fetal deaths. One fetal death was recorded in the 30 mg/kg group and four dead fetuses were found in the 40 mg/kg group.

Although there appears to be a dose-response relationship in fetal deaths, the results were found not to be statistically significant by the Fischer's exact probability test.

Fetal Weights

The effect of the drug dosage levels on mean fetal weights is shown in Table 1 and Figure 3. The mean fetal weights and standard deviations for each group are as follows: Control mean = 1.70 with a standard
deviation of 0.11. 30 mg/kg mean = 1.66 with a standard deviation of 0.06. 40 mg/kg mean = 1.95 with a standard deviation of 0.10. If one compares the individual weights within each group by use of the mean, standard deviation and the normal biological curve, it shows that there is no significant difference in the control and 40 mg/kg groups within two standard deviations. Within the 30 mg/kg group, there are three points that are over the normal expectation of values within two standard deviations. If one compares the three groups using one way analysis of variance, there is a statistical significant difference between the 40 mg/kg group and the other two groups (p ≤ .05).

**Skeletal Analysis**

Examination of the fetal thoracic skeleton revealed no gross abnormalities in any of the dosage groups.

**Heart Weights**

The effects of dosage levels of the drug on the mean heart weights is summarized in Table 2 and Figure 4. The control mean was 19.77 with a standard deviation of 1.16, the 30 mg/kg mean was 19.91 with a standard deviation of 1.16 and the 40 mg/kg mean was 19.92 with a standard deviation of 0.86.

Using the mean, standard deviation and normal biological curve, the control and 30 mg/kg groups showed no significant difference when looking at individual weights within the two groups. The 40 mg/kg group had four weights in excess of what would be predicted using the same method. With a one way analysis of variance among the three treatment groups with the extreme points removed, no statistically significant difference was found.
Heart Diameter

The effects of the drug dosage levels and mean heart diameter is summarized in Table 3 and Figure 5. The control mean was 49.88 with a standard deviation of 0.99. The 30 mg/kg mean was 49.78 with a standard deviation of 1.13. The 40 mg/kg mean was 49.82 with a standard deviation of 1.13. When one compares the individual diameters within a group using the mean, standard deviation and normal biological curve, there is no statistically significant difference in the control and 30 mg/kg groups but there are three points in excess of predicted values in the 40 mg/kg group. One way analysis of variance on the three groups with the extreme points removed from the 40 mg/kg group showed no statistical significance.

Interventricular Septum

The effects of the dosage levels of streptozotocin on the mean interventricular septal dimension is summarized in Table 3 and Figures 6, 7, 8, and 9. Since the two methods of fixation gave significantly different results, the two methods were treated independently. The control group of Wilson technique hearts had a mean of 9.78 with a standard deviation of 0.87. The 30 mg/kg Wilson's group had a mean of 9.91 with a standard deviation of 0.71. The 40 mg/kg Wilson's group had a mean of 9.47 with a standard deviation of 0.79. Using the mean, standard deviation and normal biological curve, the individual measurements in all three groups showed no significant difference. There was a statistically significant difference (p < .05) between the three groups using one way analysis of variance. In order to
isolate the specific difference between the three groups, Scheffe procedure was performed and the statistically significant difference ($p \leq .05$) was found to be between the 30 mg/kg and 40 mg/kg groups. No statistically significant difference was found at the .01 level.

The control mean for the hearts fixed in 95% alcohol was 16.15 with a standard deviation of 0.45, the 30 mg/kg mean was 16.28 with a standard deviation of 0.42 and the 40 mg/kg group mean was 16.27 with a standard deviation of 0.37. No statistically significant difference was found in the individual dimensions in the control and 30 mg/kg groups using the mean, standard deviation and normal biological curve. The 40 mg/kg group had two measurements in excess of predicted values using the mean, standard deviation and normal biological curve. One way analysis of variance in both fixation groups showed no statistical significant differences when the extreme points were removed.

**Interventricular Septum vs. Left Ventricular Wall**

The relationship between the interventricular septum and left ventricular wall are summarized in Table 4 and Figure 10. The data from all the groups shows a high linear correlation ($r = .75$ to $.98$). Using the alpha and beta for the Wilson Technique hearts, one obtains a prediction line of $y = .61x + .85$, where $y$ = the mean of the left ventricular wall dimension and $x$ = the mean of the interventricular septal dimension. The 95% alcohol fixation group had a prediction line of $y = 1.93x - .68$, where $y$ = the mean of the left ventricular wall dimension and $x$ = the mean of the interventricular septal dimension. With these two prediction lines, there are three points that fall outside the lines and these are considered to be abnormal hearts.
DISCUSSION

This study demonstrated that the CD-1 mouse fetus is sensitive to chemically induced maternal diabetes mellitus. Teratogenic effects were noted with respect to fetal death, fetal weight and heart development. These same three areas of developmental abnormalities have been described in human infants of diabetic mothers.

Streptozotocin was chosen for this study as the diabetic inducer substance. Streptozotocin has been shown to be beta-cell toxic but animals that receive injections of the drug remain alive until term or until sacrificed without requiring insulin therapy although they were overtly diabetic (Sybulski et al., 1971). It was shown by Karunanayake et al. (1976) that streptozotocin is cleared from the rat system in approximately six hours. But it was shown by Deuchar (1978) not to affect the heart development (Deuchar, 1978). The beta cells from the control group of maternal animals in this study showed homogenous cytoplasm. Vacuolated cytoplasm of the beta cells in the 30 and 40 mg/kg groups was observed. There appears to be a dose-related increase in vacuolated cells in the 40 mg/kg when compared to the 30 mg/kg group. Lazarus (1962) showed the same type of cells in human patients with diabetes mellitus caused by a variety of reasons. He concluded that these vacuoles were caused by the degranulation of the beta cells in the islets of Langerhans.
Although there was an increase in the fetal death rate with respect to the streptozotocin dosage levels, this was found to be not statistically significant. Adashi et al. (1979) reported that in a series of 133 human infants of diabetic mothers, there was no fetal deaths. In a study in which insulin pumps were implanted in the fetuses of rhesus monkeys thus mimicking the hyperinsulinemic state manifested in infants of diabetic mothers, Susa et al. (1979) concluded that the physiologic stress of hyperinsulinemia per se does not further jeopardize the fetus to early fetal loss. However, this finding was in contrast to studies done by Eriksson (1982) on mildly diabetic rats in which no live fetuses were found. In the human population, Pedersen et al. (1964) reported an increase in fetal deaths of seven times when compared to infants of nondiabetic mothers. Rubin and Murphy (1958) found in their human study that the mortality rate of infants of diabetic women is three to five times higher than the rate in infants of nondiabetic women. It appears that the approximate rate of deaths in the human population among infants of diabetic mothers is 3.8% (Soler, 1976). In this study in the 40 mg/kg dosage group the death rate was similar to the human population.

In the present study, there was a statistically significant difference between the fetal weights of the control and 30 mg/kg group when compared with the 40 mg/kg group by use of the one way analysis of variance. Also, within the 30 mg/kg group, there were three points in excess of the predicted value using the mean, standard deviation and normal biological curve. These results correspond well to those found in the human population. Macrosomia has long been associated with
infants of diabetic mothers. Miller and Wilson (1943) found infants of diabetic mothers had birth weights of over 3900 grams. Birth weights of these infants usually exceed average values by one standard deviation and can go as high as exceeding the average by two standard deviations (Bjorntorp et al., 1974). It was shown by O'Sullivan et al. (1978) that women who have large babies or those giving histories of a previous large baby births will include persons with transient gestational hyperglycemia and other risk factors that will be related to an increase frequency of later diabetes. It appears that the hyperinsulinemia within the fetus results in an increased fetal body fat content which accounts for the cherubic appearance of these infants at birth and possibly complicates vaginal delivery due to dystocia (Sklyer et al., 1980; Oppermann et al., 1980; and Stevenson et al., 1982). Because of this increase in weight and size of these infants, Haukkamaa et al. (1980) found that the Caesarian section rate is from 55.3% to 66.6% for these infants.

Other primates show the same characteristic increase in body weight as humans. Chez (1980) found that the body weight at birth in a control group of monkeys was from 330 grams to 375 grams, while the streptozotocin induced diabetic monkeys was found to be from 380 grams to 655 grams. Schwartz et al. (1980) noted the same effect on rhesus monkey fetuses when they were injected directly with insulin and concluded that the macrosomia observed was similar to that found in infants of diabetic mothers. This led him to postulate that insulin is probably the main fetal growth promoting hormone. In a study on rhesus monkeys, Mintz et al. (1972) found that all the fetal birth weights were
above two standard deviations when compared to the average birth weights of the control animals. Solomon (1959) also observed an increased fetal birth weight in diabetic rats when compared to the control group.

In contrast to these studies, Eriksson et al. (1982) found that the average fetal weights decreased in severely diabetic rats. These animals showed retarded growth in many of their body systems, particularly the skeletal system.

Several different parameters were used in order to establish that the infants from this study had cardiac abnormalities. The first parameter was heart weight. From the current study, it was found that the 40 mg/kg dosage group contained four weights that were in excess of predicted values by use of the mean, standard deviation and normal biological curve. Using the one way analysis of variance statistical test with the extreme points removed, there was no significant difference in the results of each dose group. Human data on heart weights was from autopsy material. Miller et al. (1943) found eight out of nine hearts weighed over 29 grams. Scammon (Wilson, 1943) found that the absolute values for cardiac weights in the newborn infant should be between 20 and 25 grams. Miller stated that he found two out of eight hearts were two times heavier than normal and two out of eight hearts were three times heavier than normal. In a follow-up study (Miller, 1945), he reported four hearts at autopsy that were heavier than normal. Rowland (1973) found that the heart weights at postmortem examination may exceed two standard deviations above the normal in as many as one half of these infants of diabetic mothers without congenital heart disease. Animal studies provide more conclusive data. Susa et al.
(1979) found that there was a significant difference between treated and untreated groups of rhesus monkeys. He reported that the control heart weight mean was 1.94 grams while the treated heart weight mean was 3.94 grams. Also studying monkeys, Schwartz et al. (1980) found that there was a significant difference in heart weights between the control animals and fetuses that were under the influence of high insulin concentrations.

The second parameter observed was heart diameter. This method was first used clinically in order to investigate the cardiomegaly associated with infants of diabetic mothers. In the present study, there was no significant difference in the heart diameters in the control and 30 mg/kg dosage groups using the mean, standard deviation and normal biological curve. There were three diameters that were in excess of predicted values in the 40 mg/kg dose group using the same method. With one way analysis of variance, there was no significant difference between the three groups with the extreme weights removed. Miller et al. (1943) found that a roentgenographic examination of infants of diabetic mothers showed characteristic large hearts. He based a diagnosis of an enlarged heart on a comparison of the greatest transverse diameter of the heart compared to that of the thorax. A cardiothoracic ratio of 55 or greater was arbitrarily accepted as evidence of cardiac enlargement. He found twenty out of twenty-one infants of diabetic mothers had these signs of cardiac enlargement. He reported much the same type of results in his study on prediabetic mothers (Miller, 1945). He found that two out of seven infants had the
same signs of cardiac hypertrophy as infants of diabetic mothers (five out of seven did not live).

The third parameter, and the most studied parameter, is the dimension of the interventricular septum. This study showed that there was no significant difference in the measurements obtained on Wilson's technique hearts using the mean, standard deviation and normal biological curve. There was, however, a significant difference between the 30 mg/kg and 40 mg/kg group when the one-way analysis of variance test was used. Using the mean, standard deviation and normal biological curve on individual measurements in the 95% alcohol fixed group, only the 40 mg/kg dose showed a statistically significant difference with two measurements in excess of predicted values. One way analysis of variance showed no significant difference between the three groups when the extreme points were removed. Many investigators have shown that hypertrophy of the ventricular septum is associated with infants of diabetic mothers (Soler et al., 1976; Mills et al., 1979; and Chung et al., 1975). In a study conducted on 237 Pima Indian women, there was a high incidence of cardiovascular defects found in the infants, among these were ventricular septal defects. The authors concluded that this was a result of the high percentage of diabetes in the Pima Indian population (Comess et al., 1969). With the use of echocardiography, Gutgesell (1976) showed that this septal defect was a hypertrophy of the ventricular septum with subsequent subaortic stenosis. It was also shown that this condition was transient and disappeared in one year or less (Wolfe et al., 1977). Gutgesell et al. (1978) examined twenty-seven infants of diabetic mothers and found seven with systolic
ejection murmurs, thirteen with radiographic evidence of cardiomegaly and twelve with electrocardiographic abnormalities. Echocardiography has become the tool of choice in order to investigate septal defects. Mace et al. (1979) found that hypertrophy of the septum could be asymptomatic, or associated with respiratory distress or with congestive heart failure. Breitweser (1980) in a study to determine the cause of the septal swelling found strong evidence to suggest that it is associated with the hyperinsulinemic state of the infant due to the maternal diabetic state.

Animal studies on interventricular septal hypertrophy are few. Deuchar (1977) reported that, in rats, the heart malformations found were distortions or reductions in size of one or more chambers. This suggests that possibly the septum was growing larger and narrowing the chambers. It appears that infants of diabetic mothers can have an interventricular septal hypertrophy with subaortic stenosis. This occurs in a very small number of individuals.

In contrast, Maron et al. (1978) questioned the existence of hypertrophic cardiomegaly in infants of diabetic mothers. He suggests that there is a thickening of the ventricular septum during normal development after observing 151 normal human hearts at various stages of development. He postulates that what investigators are seeing in infants of diabetic mothers is an immature state of the heart due to a problem in obtaining the correct gestational age of these infants. Halliday (1981) in a study of twelve infants of diabetic mothers could find no subaortic stenosis by use of echocardiography.
The final parameter looked at in this study was a comparison of the interventricular septal dimension to the left ventricular wall dimension. In all groups looked at there was a high linear correlation. When linear regression was conducted, two prediction lines were obtained. From these lines, there were three hearts that did not fall on the line and these are considered abnormal hearts. Numerically, these same three hearts had an interventricular septal:left ventricular wall ratio of greater than 1.3. In the human, using echocardiography, Way et al. (1979) defined disporportionate septal hypertrophy as that ratio of interventricular septum to the posterior left ventricular wall that exceeds 1.3. In a case study done by Leslie et al. (1982), an infant was born to a diabetic mother and exhibited signs of septal hypertrophy with subaortic stenosis. Upon echocardiographic examination, it was found that the septum:left ventricular wall ratio exceeded 1.3. This was considered to be an abnormal heart with an enlarged septal dimension.

This study suggests that the CD-1 mouse mimics the human diabetic condition and is therefore an ideal animal model in which to study the effects of maternal diabetes mellitus on cardiac development.
CONCLUSION

1. Streptozotocin was shown to be a beta cell toxin.
2. Examination of 200 CD-1 mouse fetuses revealed that maternal diabetes mellitus is teratogenic and embryotoxic in varying degrees of expression.
3. Analysis of Variance showed a significant increase in mean fetal weights in the 40 mg/kg group compared to the control and 30 mg/kg groups.
4. The number of dead fetuses within the three dosage groups proved to be statistically nonsignificant.
5. There was a statistically significant increase in heart weights in four hearts (about 10%) in the 40 mg/kg group using the mean, standard deviation and normal biological curve. One way analysis of variance with extreme points removed showed no statistically significant difference between the three groups.
6. There was a statistically significant increase in heart diameter in three hearts (about 6%) in the 40 mg/kg group using the mean, standard deviation and normal biological curve. One way analysis of variance with extreme points removed showed no statistically significant difference between the three groups.
7. There was a statistically significant increase in the interventricular septal dimension in two hearts (about 4%) in the 40
mg/kg dose group in the hearts fixed in 95% alcohol. One way analysis of variance in the 95% alcohol group showed no significant difference in the three dose groups when the extreme points were removed. In the Wilson's technique group, there was a statistically significant difference between the 30 and 40 mg/kg dosage groups using the one way analysis of variance. There were no extreme measurements in this group.

8. Comparison between the interventricular septal and left ventricular wall showed a strong linear correlation. There were three hearts (about 3%) that did not fall on the prediction lines and these hearts had a septum:left ventricular wall ratio greater than 1.3 and were considered abnormal.

9. There was a dose-related increase in cardiac defects.
TABLE 1

SUMMARY OF MEAN FETAL WEIGHTS*

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Fetal Weight</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>1.70 (n° = 26)</td>
<td>0.11</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>1.64 (n = 73)</td>
<td>0.07</td>
</tr>
<tr>
<td>40 mg/kg</td>
<td>1.87 (n = 101)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

* Fetal Weights in Gram

** \( \bar{x} \) = Mean Fetal Weight

° n = Number of Fetuses Examined

§ SD = Standard Deviation

c = Control
### TABLE 2
SUMMARY OF MEAN HEART WEIGHTS*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Weight</th>
<th>Sample Size (n)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x}_{uc}$</td>
<td>19.77</td>
<td>13</td>
<td>SD° = 1.16</td>
</tr>
<tr>
<td>$\bar{x}_{30 \text{ mg/kg}}$</td>
<td>19.36</td>
<td>38</td>
<td>SD = 1.16</td>
</tr>
<tr>
<td>$\bar{x}_{40 \text{ mg/kg}}$</td>
<td>20.30</td>
<td>49</td>
<td>SD = 0.86</td>
</tr>
</tbody>
</table>

*Heart Weights in Milligrams

uc = Untreated Controls (Distilled Water)

° SD = Standard Deviation
<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>30 mg/kg</th>
<th>40 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>$\bar{x}_w=9.78$ n=13 SD=.87</td>
<td>$\bar{x}_w=9.91$ n=35 SD=.71</td>
<td>$\bar{x}_w=9.48$ n=48 SD=.79</td>
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<tr>
<td></td>
<td>$\bar{x}_h=16.15$ n=13 SD=.45</td>
<td>$\bar{x}_h=16.28$ n=37 SD=.42</td>
<td>$\bar{x}_h=16.38$ n=49 SD=.37</td>
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<tr>
<td></td>
<td>$\bar{x}_t=12.96$ n=26 NC</td>
<td>$\bar{x}_t=13.05$ n=72 NC</td>
<td>$\bar{x}_t=12.96$ n=97 NC</td>
</tr>
<tr>
<td>LVW</td>
<td>$\bar{x}_w=8.82$ n=13 SD=.53</td>
<td>$\bar{x}_w=9.01$ n=35 SD=.64</td>
<td>$\bar{x}_w=8.79$ n=48 SD=.80</td>
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<tr>
<td></td>
<td>$\bar{x}_h=12.81$ n=13 SD=.39</td>
<td>$\bar{x}_h=13.08$ n=37 SD=.36</td>
<td>$\bar{x}_h=13.13$ n=49 SD=.27</td>
</tr>
<tr>
<td></td>
<td>$\bar{x}_t=10.81$ n=26 NC</td>
<td>$\bar{x}_t=11.10$ n=72 NC</td>
<td>$\bar{x}_t=10.98$ n=97 NC</td>
</tr>
<tr>
<td>RVW</td>
<td>$\bar{x}_w=7.63$ n=13</td>
<td>$\bar{x}_w=8.29$ n=35</td>
<td>$\bar{x}_w=8.21$ n=48</td>
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<td>$\bar{x}_h=10.51$ n=13</td>
<td>$\bar{x}_h=10.44$ n=37</td>
<td>$\bar{x}_h=10.41$ n=49</td>
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<tr>
<td></td>
<td>$\bar{x}_t=9.07$ n=26</td>
<td>$\bar{x}_t=9.39$ n=72</td>
<td>$\bar{x}_t=9.32$ n=97</td>
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<tr>
<td>HEART DIAMETER</td>
<td>$\bar{x}_w=49.69$ n=13 NC</td>
<td>$\bar{x}_w=49.85$ n=35 NC</td>
<td>$\bar{x}_w=49.86$ n=48 NC</td>
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<td>$\bar{x}_h=50.06$ n=13 NC</td>
<td>$\bar{x}_h=49.71$ n=37 NC</td>
<td>$\bar{x}_h=50.01$ n=49 NC</td>
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<tr>
<td></td>
<td>$\bar{x}_t=49.88$ n=26 SD=.99</td>
<td>$\bar{x}_t=49.78$ n=72 SD=1.13</td>
<td>$\bar{x}_t=49.94$ n=97 SD=1.13</td>
</tr>
</tbody>
</table>

*Dimensions in Millimeters, IS=Interventricular Septal Diameter, LVW=Left Ventricular Wall Diameter, RVW=Right Wall Diameter, $\bar{x}_w$=Mean of Hearts from Wilson's Technique, $\bar{x}_h$=Mean of Hearts from 95% Alcohol Group, $\bar{x}_t$=Mean of All the Hearts, NC=Not Calculated.
## TABLE 4

SUMMARY OF THE COMPARISON OF INTERVENTRICULAR SEPTUM DIAMETER AND LEFT VENTRICULAR WALL DIAMETER

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>SD&lt;sub&gt;x&lt;/sub&gt;</th>
<th>SD&lt;sub&gt;y&lt;/sub&gt;</th>
<th>SD&lt;sub&gt;xy&lt;/sub&gt;</th>
<th>x̄&lt;sub&gt;x&lt;/sub&gt;</th>
<th>x̄&lt;sub&gt;y&lt;/sub&gt;</th>
<th>r</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Wilson)</td>
<td>13</td>
<td>.87</td>
<td>.53</td>
<td>.64</td>
<td>9.78</td>
<td>8.82</td>
<td>.89</td>
<td>3.54</td>
<td>.54</td>
</tr>
<tr>
<td>Control (Heart)</td>
<td>13</td>
<td>.42</td>
<td>.39</td>
<td>.35</td>
<td>16.11</td>
<td>12.81</td>
<td>.75</td>
<td>1.62</td>
<td>.69</td>
</tr>
<tr>
<td>30 mg/kg (Wilson)</td>
<td>35</td>
<td>.50</td>
<td>.40</td>
<td>.40</td>
<td>9.91</td>
<td>8.99</td>
<td>.91</td>
<td>.94</td>
<td>.81</td>
</tr>
<tr>
<td>30 mg/kg (Heart)</td>
<td>37</td>
<td>.42</td>
<td>.36</td>
<td>.35</td>
<td>16.28</td>
<td>13.08</td>
<td>.82</td>
<td>1.78</td>
<td>.69</td>
</tr>
<tr>
<td>40 mg/kg (Wilson)</td>
<td>48</td>
<td>.79</td>
<td>.80</td>
<td>.79</td>
<td>9.47</td>
<td>8.78</td>
<td>.98</td>
<td>.68</td>
<td>1.00</td>
</tr>
<tr>
<td>40 mg/kg (Heart)</td>
<td>46</td>
<td>.37</td>
<td>.28</td>
<td>.29</td>
<td>16.27</td>
<td>13.13</td>
<td>.82</td>
<td>3.01</td>
<td>.62</td>
</tr>
<tr>
<td>Hearts</td>
<td>96</td>
<td>.40</td>
<td>.34</td>
<td>.33</td>
<td>16.25</td>
<td>13.07</td>
<td>.80</td>
<td>1.93</td>
<td>-.68</td>
</tr>
<tr>
<td>Wilson</td>
<td>96</td>
<td>.79</td>
<td>.72</td>
<td>.73</td>
<td>9.68</td>
<td>8.87</td>
<td>.93</td>
<td>.61</td>
<td>.85</td>
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</tbody>
</table>

n = Number of Fetuses, SD<sub>x</sub> = Standard Deviation of x, SD<sub>y</sub> = Standard Deviation of y, SD<sub>xy</sub> = Standard Deviation of xy, x̄<sub>x</sub> = Mean of x, x̄<sub>y</sub> = mean of y, r = Correlation coefficient, a = Linear regression Alpha-Intercept, b = Linear Regression Beta-Slope, Wilson = Measurements Obtained in the Wilson Technique Hearts, Heart = Measurements Obtained in the 95% Alcohol Hearts.
Figure 1. Chemical structure of the drug: streptozotocin

2-deoxy-2-[(methylnitrosoamino)carbonyl]amino]-alpha (and beta)-D-glucopyranose
(MW = 265.2)
Figure 2A. Islet of Langerhans from an adult CD-1 mouse from the control group. Beta cells (arrows). Glenn and Lillie Rhodocyan 125 X.

Figure 2B. Islet of Langerhans from an adult CD-1 mouse treated with 30 mg/kg streptozotocin. Vacoulated beta cell (dark arrow). Glenn and Lillie Rhodocyan 250 X.

Figure 2C. Islet of Langerhans from an adult CD-1 mouse treated with 40 mg/kg streptozotocin. Vacoulated beta cell (dark arrow), cellular distruction (open arrow). Glenn and Lillie Rhodocyan 250 X.
Figure 3. The effect of the dosage of streptozotocin on fetal weight in the 19 day old CD-1 mouse fetus.
Figure 4. The effect of the dosage of streptozotocin on the cardiac weight in the 19 day old CD-1 mouse fetus.
Figure 5A. The effect of the dosage of streptozotocin on cardiac diameter in the 19 day old CD-1 mouse fetus.
Figure 5B. The effect of the dosage of streptozotocin on cardiac diameter in the 19 day old CD-1 mouse fetus.
Figure 5C. The effect of the dosage of streptozotocin on cardiac diameter in the 19 day old CD-1 mouse fetus.
Figure 6. The effect of the dosage of streptozotocin on the interventricular septal dimension in the 19 day old CD-1 mouse fetus prepared for Wilson's technique.
Figure 7. The effect of the dosage of streptozotocin on the interventricular septal dimension in the 19 day old CD-1 mouse fetus prepared for the whole heart examination.
Figure 8A. A transverse section through the ventricular area of the heart prepared for Wilson's technique of a 19 day old CD-1 mouse fetus. Interventricular septum untreated (I), Left ventricular wall (arrow). 32 X.

Figure 8B. A transverse section through the ventricular area of the heart prepared for Wilson's technique of a 19 day old CD-1 mouse fetus treated with 30 mg/kg of streptozotocin. Interventricular septum (I), Left-ventricular wall (arrow). 32 X.

Figure 8C. A transverse section through the ventricular area of the heart prepared for Wilson's technique of a 19 day old CD-1 mouse fetus treated with 40 mg/kg of streptozotocin. Interventricular septum (I), Left ventricular wall (arrow). 32 X.
Figure 9A. A transverse section through the ventricles of the whole heart of an untreated 19 day old CD-1 mouse fetus. Interventricular septum (I), Left ventricular wall (arrow). 32 X.

Figure 9B. A transverse section through the ventricles of the whole heart of a 19 day old CD-1 mouse fetus treated with 30 mg/kg streptozotocin. Interventricular septum (I), left ventricular wall (arrow). 32X.

Figure 9C. A transverse section through the ventricles of the whole heart of a 19 day old CD-1 mouse fetus treated with 40 mg/kg streptozotocin. Interventricular septum (I), left ventricular wall (arrow). 32 X.
Figure 10. The relationship between the interventricular septal and the left ventricular wall dimensions in the 19 day old CD-1 mouse fetus.
# APPENDIX 1
## THE DESIGN OF THE EXPERIMENT

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>30 mg/kg</th>
<th>40 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Live Fetuses</td>
<td>26</td>
<td>73</td>
<td>101</td>
</tr>
<tr>
<td>Heart Weights and Inouye Method</td>
<td>13</td>
<td>37</td>
<td>49</td>
</tr>
<tr>
<td>Wilson's Method</td>
<td>13</td>
<td>35</td>
<td>48</td>
</tr>
<tr>
<td>Dead</td>
<td>0</td>
<td>1</td>
<td>4</td>
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