SEXUAL BEHAVIOR IN MALE OFFSPRING OF FEMALE DIABETIC RATS

Thesis for the Degree of M. A.
MICHIGAN STATE UNIVERSITY
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1976

ABSTRACT

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By

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Diabetes mellitus currently afflicts an estimated 5 million Americans. Despite treatment with insulin and oral antidiabetic agents, many of the concomitants of diabetes remain problematic. Clinical studies have shown that 50 percent of diabetic males are impotent, although the etiology of this disturbance remains undetermined. Also, despite better prenatal and postnatal care, diabetic pregnancy continues to pose a greater risk for mother and offspring due to the metabolic imbalances of gestation. The present study was an attempt, using the rat as a model, to determine whether male offspring subjected to the metabolic alterations of a diabetic pregnancy would show altered sexual behavior in adulthood.

Long Evans females (60-70 days) were adapted to the lab and then given a single intravenous injection of either a diabetogenic agent, streptozotocin, or a control vehicle, buffered citrate. Females were then mated and weight and urine sugar were measured on days 1, 7, 14, and 20 of

pregnancy. Pups were weighed on the day of birth and litters were culled to 8 pups; some litters were cross fostered and others were reared by their natural mothers. Weight and urine sugar were measured in the male offspring at 50 and 90 days.

Beginning at 60 days, each male was given three weekly pretests for sexual behavior with a receptive female; this established a baseline of sexual activity. Then 3 weekly sexual behavior tests, also 30 minutes each, were given and frequencies and latencies for mounts, intromissions and ejaculations were recorded. Ten days to 3 weeks after the last test, each male received a satiation test, with a criterion of 30 minutes without an intromission for termination of the test.

Male offspring of diabetic mothers, either reared by their own mothers or cross fostered to control mothers, showed a shortened latency to the initiation of sexual behavior. A change in temporal pacing resulted in significantly shorter latencies to intromission and ejaculation. The decreased intromission latency shown by these males when compared to controls indicates a lowering of the copulatory threshold for the initiation of sexual behavior. The decrease in ejaculation latency and decrease in intromission frequency in offspring of diabetic mothers indicates a concomitant lowering of the ejaculatory threshold.

The pattern of sexual behavior shown by male offspring of diabetic mothers is also seen in adult male rats subjected to various manipulations, all of which increase arousal level: castration, adrenalectomy, audiogenic stimulation, handling of rats during copulation, peripheral electrical shock and rewarding or aversive brain stimulation. Changes in patterning in sexual behavior are maladaptive in decreasing reproductive efficiency. A species specific pattern of number and spacing of intromissions is needed to initiate neuroendocrine reflexes for the initiation of pregnancy. It is possible that behavior shown by offspring of diabetic mothers would be detrimental in this regard.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF ARTS

Department of Psychology

1976

ACKNOWLEDGMENTS

I would like to express appreciation to my committee members Dr. Lynwood G. Clemens, Dr. Lawrence I. O'Kelly, and Dr. Glenn I. Hatton for their individual and thoughtful contributions to the shaping of this thesis.

The author was supported by a NIMH Traineeship, 5 T01 MH10611, and the research was supported by NIH, HD 06760-09 to Dr. Lynwood G. Clemens.

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INTRODUCTION

Complications of diabetes mellitus encompass a wide spectrum of pathology, ranging from the obvious disruption of carbohydrate metabolism to retinopathy and neurological lesions. One concomitant of diabetes mellitus frequently seen in the clinic is impotence in adult males. While the average incidence of this disorder is 50 percent in diabetic males, its etiology remains undetermined. In male rats made diabetic in the laboratory there are disturbances in testicular functioning similar to those seen in the human, and fertility is reduced. However, there have been no published studies on the patterning of sexual behavior in these males. In a preliminary investigation in Dr. Clemens' laboratory, Mary Hoover (unpublished) studied the sexual behavior of male rats made diabetic with streptozotocin (STZ) injections. She found an increased number of mounts (poorly oriented), and a decreased frequency of intromissions and ejaculations. Latency to erection was also increased.

Offspring of a diabetic mother might also be expected to have impaired sexual functioning in adulthood.

Although the offspring themselves are not overtly diabetic, the metabolic stress of hyperglycemia and hyperinsulinemia in utero might impair the development of normal male sexual potential. In this thesis, I aim to study this problem by making female rats diabetic with STZ injections, impregnating them and testing the sexual behavior of their male offspring in adulthood.

In the remainder of this section, I shall elaborate on the rationale for this experiment. The sections are as follows: first a brief description of naturally occurring diabetes mellitus, and the disturbances of sexual functioning in the human male. Following is a summary of the incidence of experimental diabetes in male and female rats, and the effect on male reproductive capacity. Then I will describe the diabetogenic action of streptozotocin, the dynamics of diabetic pregnancy in the rat, and the development and expression of the normal patterning of sexual behavior in the male rat.

Diabetes Mellitus

Diabetes is a metabolic disorder currently affecting five million Americans, and is the fifth leading cause of death by disease in the United States today. The highest incidence rate is for the obese or those with a family history of diabetes. The disease can be roughly divided into two divisions: growth onset, occurring before puberty, and maturity onset. The growth onset type is usually more

severe; insulin must be used for maintainence rather than the oral hypoglycemic agents and these patients are more prone to ketosis and other complications of diabetes.

The course of the disease in an individual usually progresses through four stages: prediabetes, latent chemical diabetes, chemical diabetes, and overt diabetes. Diabetes is based on abnormal blood glucose concentrations either in the fasting state or after a glucose stimulation test. Onset in younger patients is usually rapid and is accompanied by polyuria, thirst, weight loss, increased appetite and visual disturbances. If these early symptoms are not recognized and treated, acidosis and coma may result. In gradual onset diabetes, the symptoms are fewer and complaints of nocturia, muscle cramps or impotence may lead to diagnosis of diabetes (Gorman, 1973).

The mechanisms responsible for diabetes mellitus are still not fully understood; models of insulin action in the normal individual and the hormonal interrelationships contributing to hyperglycemia are currently being revised and reformulated. Insulin seems to achieve its anabolic actions by binding to the cell membrane and changing its structural conformation, resulting in release of calcium ions from high affinity binding sites. The increased concentration of intracellular calcium then functions as a second messenger to alter the sensitivity of protein kinase to cAMP, eventually resulting in activation of glycogen synthetase for conversion of glucose to glycogen. The same

mechanism would result in activation of pyruvate dehydrogenase to stimulate lipogenesis (Kisselbah, Tulloch, Hope-Gill, Clarke, Vydelingum & Fraser, 1975; Larner, 1975; Lazarus & Davis, 1975). In diabetes mellitus, an insufficiency of insulin results in high levels of glucose in the blood and urine, breakdown of protein with urinary excretion of nitrogen, and breakdown of lipids and fatty acids resulting in accumulation of ketone bodies.

Complications of diabetes mellitus can result from vascular or metabolic abnormalities. Acute metabolic complications are reversible by a readjustment of the insulin supply to the body. Currently two approaches are being investigated to improve long term control of blood sugar. The first involves transplantation of donor or artificial pancreases, or transplantation of islets of Langerhans. These attempts have not been successful to date. The second approach aims at modulating the hormonal imbalances in the system.

Insulin deficiency alone is not responsible for the hyperglycemia of diabetes. The current hypothesis is that the metabolic abnormalities result from an absolute or relative hypoinsulinemia and an absolute or relative hyperglucagonemia. A deficiency of insulin is responsible for underutilization of available glucose and the excess production of free fatty acids. The increased glucagon secretion is responsible for hepatic overproduction of glucose (Unger & Orci, 1975). This was discovered through

the use of somatostatin, a polypeptide isolated from the hypothalamus which inhibits the release of growth hormone from the pituitary, and also lowers plasma insulin and glucagon levels when infused. During the period of infusion, plasma glucose is also lowered. Somatostatin in itself has no effect on muscle glucose uptake or hepatic glucose production; reduction in plasma glucose was attributed then to the inhibition of glucagon stimulation of hepatic glucose production (Alberti, Christensen, Christensen, Hansen, Iversen, Lundbaek, Seyer-Hansen, & Orskov, 1973; Koerker, Ruch, Chideckel, Palmer, Goodner, Ensencke, & Gale, 1974; Gerich, Lorenzi, Hane, Gustafson, Guillemin & Forsham, 1975; Sakurai, Dobbs & Unger, 1975). Analogues of somatostatin are being synthesized for clinical use in the hope that a better regulation of blood glucose can minimize the complications of diabetes.

Diabetes still presents special problems for the pregnant woman. Although fertility is normal in diabetic females, fetal mortality is high. Complications are usually related to the severity of the diabetes, and the probability of fetal viability is classified as follows:

Class A: Mild chemical diabetes (fetal survival approaching normal)

Class B: Overt diabetes of less than 10 years which developed in adult life--no complications (67% fetal survival)

Class C: Diabetes beginning between 10 and 19 years or diabetes of 10 to 19 years duration--no vascular disease (48% fetal survival)

Class D: Diabetes of more than 20 years with a high probability of vascular disease; diabetes beginning before 10 years or diabetes associated with mild retinopathy or leg-vessel calcification (32% fetal survival)

Class E: Pelvic vessel calcification on X-ray (13% fetal survival)

Class F: Diabetic nephropathy (3% fetal survival)

Class R: Severe diabetic retinopathy (high fetal loss)

Effects on the fetus include a slightly increased risk of malformations, hypertrophy of the islets of Langerhans under the stimulation of maternal hyperglycemia, and a large body size resulting from excessive accumulation of fat and fluid, but also larger organ size (Gorman, 1973).

Impotence in Males

The average incidence of this disorder is estimated to be 50% of diabetic males (Ellenberg, 1971; Faerman, Vilar, Rivarola, Rosner, Jadzinsky, Fox, Perez-Lloret, Bernstein-Hahn & Saraceni, 1972; Kolodny, Kahn, Goldstein & Barnett, 1974). When this disorder does develop, the symptomology follows a characteristic pattern. Impotence usually occurs within five years of the occurrence of diabetes (Rubin & Babbott, 1958; Schoffling, Federlin, Ditschuneit & Pfeiffer, 1963; Kolodny et al., 1974); in some cases the impotence may precede the diagnosis of diabetes. The onset of impotence is usually gradual with decreased firmness of erection within six months to one year; 53% of the impotent males in one study had no morning erections and were unable to masturbate although libido

persisted, indicating that the impotence is not of psychogenic origin (Rubin et al., 1958). Nocturnal emissions are usually absent also. No correlations have been found with the age at onset, nor in some studies with the complications of the disease, either vascular or neurological.

The etiology of impotence remains unknown. Schoffling et al. (1963) has been a main influence attributing impotence and sterility to secondary hypogonadotropic hypogonadism. They found reduction in testicular size and consistency, decreased prostate size and volume of seminal fluid, abnormal sperm counts, decreased seminal fluid fructose, decreased gonadotropins and atrophy of the seminiferous tubules with basement membrane thickening. With combination therapy with chorionic gonadotropin and testosterone, the impotence disappeared and semen analysis, sperm counts and prostate size improved. This would indicate that the impotence was a result of hormonal deficiency (Schoffling, 1965). However, later studies have found no difference in plasma testosterone concentrations between potent and impotent diabetics and normals (Ellenberg, 1971; Faerman, et al., 1972; Kolodny, et al., 1974) although slight to marked hypospermatogenesis was observed. Testosterone therapy alone or in combination with chorionic gonadotropin in a range of doses and treatment regimes failed to ameliorate the condition. A possible explanation of the discrepancy is that Schoffling used a very narrow range of

normal values as indices of function but did not test for plasma testosterone.

A link has been made, however, between impotence and neuropathy in diabetes (Ellenberg, 1971; Faerman, et al., 1972; Kolodny, et al., 1974). In a survey of diabetic males, Ellenberg found that 59% were impotent and of these, 88% had some accompanying neuropathy. Of the 41% diabetics with normal potency, only 12% had some neuropathological disorder. This pathology consists of diminished nerve conduction velocity, abnormal deep tendon reflexes and especially bladder dysfunction. Since autonomic pathways for micturition and erection are identical, involving the second, third, and fourth components of the spinal cord, a neurological lesion might be responsible for impairment of both functions. This has not yet been demonstrated conclusively. Recently, Faerman, Glocer, Fox, Jadzinsky and Rapaport (1974) studied the nerve fibers of the corpora cavernosa in five impotent and five non-impotent diabetics. Four of the five impotent and none of the control diabetics showed irregularities consisting of diffuse thickenings of the nerve fiber. This would indicate that the reflex arc of erection was damaged at a peripheral level.

Sex Differences in the Diabetic Rat

Diabetes has been studied in the rat as a phenomenon induced by sub-total (95%) pancreatectomy, alloxan, STZ and

other preparations. Disturbed sexual functioning, i.e., reduced fertility has been noted in noted in male rats following these treatments. Foglia, Schuster and Rodriguez (1947) first described a sex difference in the occurrence of diabetes after sub-total pancreatectomy in the rat. Following a seven hour fast, 92% of the males and only 28% of the females had blood sugar levels over 150 mg%. Females after surgery showed a slow linear increase in the number of diabetics up to six months, while the males showed an abrupt increase between one and two months, continuing at a high number to six months. Paired feeding of males and females showed that the higher incidence of diabetes in males was not due to a higher food intake which might aggravate the condition.

Castration of males simultaneously with pancreatectomy caused a slight reduction in the incidence of diabetes, however, castration after the onset of diabetes was ineffective. Testosterone in doses of 1-5 mg/day for 10 days had no effect in either the prediabetic or diabetic phase (Foglia, et al., 1947). Angerwell, Hesselsjö, Nilsson and Tisell (1967) treated alloxan diabetic castrates and non-diabetic castrates with testosterone propionate (TP), .5 mg/day for two weeks. Animals were castrated at a weight of 62 grams and alloxanized 30 days later. Prior to TP treatment, both groups of castrates showed inhibition of the ventral prostate, dorsolateral prostate, coagulating glands and seminal vesicles; there was no difference in

this reduction between alloxan diabetic and non-diabetic castrates, indicating that insulin deficiency was not a factor. While TP treatment markedly stimulated the growth of these organs in both groups, the increase was of lower magnitude in the diabetics, indicating slightly less secretory capacity. This could be a direct result of insulin deficiency or a reduced output of other synergistic hormones, such as growth hormone or prolactin.

Female rats who were pancreatectomized and ovariectomized eight to 10 days later showed a higher incidence of diabetes than after pancreatectomy alone (Foglia, et al., 1947). Androgenized females who were given TP, 1 mg/rat on day 3, and then pancreatectomized on day 60 had a higher fasting blood sugar level at 30 and 60 days post-pancreatectomy than controls (Foglia, Basabe, & Chieri, 1969). Neonatal castration of males and females decreased the incidence in males and increased the incidence in females of overt diabetes; this effect was more pronounced than in adult castrations, with the incidence intermediate between that of normal males and females (Foglia, Borghelli, Chieri, Fernandez-Collazo, Spindler, & Wesely, 1963). Estradiol benzoate (EB) given in doses of 2, 4, or 15 ug/day for six months exerted a protective effect, i.e., normal beta cell granulation, on both castrated and intact pancreatectomized males and females (Foglia, et al., 1947). Using a criterion of three months normal blood sugar without the use of insulin after the cessation of EB treatment,

Rodriguez (1954) found that with prior treatment with EB alone, 47% were normalized and in conjunction with insulin 69% were normalized. This effect was achieved in females whose blood sugar was less than 250 mg%.

The above results seem to indicate that the ovary rather than the testis is instrumental in the normalization of diabetes; removal of the testes in the adult male only slightly decreases the incidence of diabetes while removal of the ovary in the adult female greatly increases the incidence. EB exerts a protective influence not only on the female but also on the male. Whether this estrogenic influence represents an effect on pituitary secretions, the adrenal cortex, metabolic action on the liver or a direct effect on the islet cells themselves is not known. Electron micrographs have established that besides mature secretory granules in the pancreas (260 nm) which are affected by STZ, there remain some immature granules which persist (65 nm) (Arison, Ciaccio, Glitzer, Cassaro, & Pruss, 1967). These could represent sites of estrogen facilitation of insulin release. Goodman and Hazelwood (1974) injected alloxan diabetic rats for 10 days with 10 ug EB per day to assess its effect on diabetes. In the minimally diabetic, urinary glucose excretion was decreased although plasma glucose was not changed. They also found increased plasma growth hormone and corticosteroid levels with slightly enlarged pancreatic islets. It seems that given a sufficient number of beta cells, estrogen can stimulate these cells

directly, although a modification of hypophyseal release of growth hormone and adrenocorticotropin could also be involved.

What are the implications of these findings for the sexual behavior of the diabetic rat? Foglia et al. (1963) found that in the period of manifest diabetes, the ability of males to impregnate females drastically decreased depending on the severity and duration of the diabetes. Males with blood sugar levels of 150 to 200 mg/ml impregnated 45% of the females they were mated with; this compares with 50% for the laparotomized controls. Rats with higher fasting blood glucose levels only impregnated 12% of the females. Similarly, with a zero to two week duration of diabetes, 49% of the females were impregnated by diabetic males but by 41 to 44 weeks of diabetes none of the males were able to impregnate females. In conjunction with this sterility, diabetics with blood glucose levels over 300 mg/ml showed reduced body weight, testis, prostate, seminal vesicles and coagulating gland weight. This was accompanied by tubular alterations, with a delay in spermatogenesis. Fecundity was reduced to a greater extent if these lesions were present.

In immature rats (40-44) days, alloxan diabetes reduced testis diameter and tubule diameter, prostate and seminal vesicles secretion and delayed descent of the testes depending on the severity of the diabetic state. Permanent diabetes resulted in sterility but treatment with insulin,

TP or chorionic gonadotropin, alone or in combination were able to normalize prostate and seminal vesicle secretions, and to a lesser extent, the testicular and tubule diameters (Hunt & Bailey, 1961). Schoffling, Federlin, Schmitt and Pfeiffer (1967) found no change in spermatogenesis with mild alloxan diabetes with blood sugar from 151 to 250 mg/ml. Moderate diabetes interfered with spermatogenesis and severe (351-450 mg/ml) caused absence of Leydig cells, decrease in the volume of germinal epithelium and absence of spermatazoa and spermatids. There is no evidence to date on the pattern of sexual behavior in these rats which would be correlated with disturbed endocrine functioning and sterility.

Streptozotocin

Streptozotocin, an antibiotic isolated from Streptomyces achomogenes, was reported by Rakieten, Rakieten and Nadkarni (1963) to have diabetogenic properties along with its anti-tumor activity. Since then a number of studies have shown the uniformity and specificity of STZ action. It is superior to alloxan in inducing experimental diabetes in having greater selective toxicity for beta cells, a larger dose range and an insensitivity to nutritional state in its action (Junod, Lambert, Stauffacher & Renold, 1969).

The major action of STZ, as studied by light and electron microscopy, is a rapid and irreversible degranulation of the beta cells of the islets of Langerhans (Rakieten

et al., 1963; Arison et al., 1967; Junod, Lambert, Orci, Pictet, Gonet & Renold, 1967). Rakieten and Junod attributed this to necrosis of the beta cell seen within seven hours whereas Arison found degranulation only indicating that insulin secretion or production was inhibited. Whether actual necrosis accompanies degranulation is still an open question.

The effect of STZ is achieved within 48 hours of intravenous administration. The minimum effect dose is 35 mg/kg and LD_{50} is 137.7 mg/kg, with increased glycosuria and ketonuria with increased dosage (Rakieten et al., 1963; Junod et al., 1969). The initial effect of moderate doses of STZ, 50 to 65 mg/kg, is a marked increase in blood glucose two hours post-injection along with a rise in free fatty acids and blood ketone bodies. This is followed by a marked hypoglycemia at seven to 10 hours, with a decrease in blood ketone bodies and free fatty acids. This hypoglycemic response is directly related to an increase of insulin released from the pancreas as a result of beta cell degranulation. Between 24 and 48 hours, a permanent hyperglycemia is established with a return to normal fasting blood ketone levels; STZ does not produce ketonuria at low doses. Pancreatic insulin levels are decreased to 5% of normal after 24 hours and after one month remain at less than 2% of normal. Glycosuria begins on the first day and increases throughout the first week. This is accompanied by polydipsia and polyuria, with urine volume

increasing from control levels of 1-5 ml/24 hours to 100-150 ml/24 hours (Junod et al., 1967; Schein, Alberti & Williamson, 1971).

Diabetic Pregnancy

Pregnancy involves a state of metabolic stress for the normal female, and this condition is exacerbated in the diabetic female. Normal pregnancy in the rat is characterized by a fall in blood sugar starting between days 10 and 15 of pregnancy, with an increase in the insulin content of the pancreas and a marked increment in the response of beta cells to induced glucose stimulation (Malaisse, Malaisse-Lagae, Picard & Flament-Durand, 1969; Rishi, Golob, Becker & Shah, 1969). The total pancreatic insulin content in late pregnancy is 53% greater than that of non-pregnant controls. Malaisse et al. found a smaller increase of 15%. These results indicate increased secretory capacity during late pregnancy.

Although insulin does not cross the placental barrier, during this time the fetal pancreas is developing its own secretory capacity. On the twelfth day of gestation the islet tissue of the fetal pancreas is undifferentiated but by day 21 maximum growth and differentiation has been achieved. By day 18, the duct cells represent 40% of the pancreatic volume and less than 1% is islet tissue. On day 22, the proportions of 2% islet tissue and 1% duct tissue resemble those of the adult (Rishi, et al., 1969; Hegre, McEvoy, Bachelder & Lazarow, 1973).

This secretory pattern is drastically altered both in the mother and in the fetus by the administration of diabetogenic agents. Common results of such treatments are sterility, abortion, reduced litter size, fetal abnormalities and either an increase or decrease in fetal weight. Most studies report higher fetal mortality (Kim, Runge, Wells & Lazarow, 1960; Golob, Rishi, Becker & Moore, 1970; Lazarow & Heggestad, 1970; Pitkin, Plank & Filer, 1971; Sybulski & Maugham, 1971). This is not due solely to hyperglycemia since it was also found in studies of sub-diabetic rats (Lazarow, Kim & Wells, 1960). At least one study has shown a predominance of female offspring with 46% in normal litters and 64% in litters of pancreatectomized mothers (Foglia, 1970). This effect is also seen in sub-diabetic female rats but is not observed in offspring of diabetic fathers, indicating that it is environmentally induced.

Some studies report fetuses with decreased birth weight (Golob, et al., 1970; Sybulski, et al., 1971) while others report cases of fetal giantism (Lazarow, et al., 1960; Foglia, 1970; Pitkin, et al., 1971). Lazarow and Heggestad (1970) suggest this discrepancy is due to differences in gestational age; when litters are delivered at 519 hours (the earliest estimated time for spontaneous delivery) they tend to be lighter, but when spontaneous delivery occurs, usually after a prolonged gestational period, the fetuses are heavier. Pitkin and VanOrden (1974) attribute the differences in weight, still a

controversy, to the severity of the diabetes, with higher glycosuria associated with smaller fetuses.

What effect does maternal hyperglycemia have on the fetal pancreas? This effect follows a pattern of maternal hyperglycemia and fetal insulinemia as described by Pedersen (1967). The fetal beta cells are subjected to high blood sugar levels in utero, resulting in degranulation of the beta cells and insulin depletion with glycogen infiltration. When pancreatic segments from normal rat embryos are grown in vitro, the number of beta granules is inversely proportional to the level of glucose in the media. When fetal pancreases from diabetic mothers are explanted at day 19.5 or 21.5, they contain only 5% granulated beta cells versus 75% for controls. On a low glucose media, however, rapid granulation can be observed in two days (Wells, Schweisthal, Marx, McKay, Saccoman & Lazarow, 1967; Wells & Lazarow, 1967). In vivo, blood sugar returns to normal levels in the neonate within 24 hours postpartum, although the appearance of the beta cells is not completely normalized for three to 10 days. Although none of the offspring studied developed overt signs of diabetes, they exhibited abnormal glucose tolerance curves for the two years they were studied (Lazarow, et al., 1970).

Masculine Sexual Differentiation and Behavior in the Rat

The development of normal sexual capacity in the male rat is contingent upon the presence of androgen during the critical perinatal period of sexual differentiation. Fetuses are bisexual at 17 days of gestation; the anlagen of both male and female reproductive structures are present. Under the influence of the sex chromosomes in the male, through an as yet unexplained inductive substance, cortexin, the cortex of the undifferentiated gonad develops into a testis and the medulla regresses. In the presence of the fetal testes, the Wolffian ducts develop the male accessory sex organs, and the Muellerian ducts are inhibited. In the final step of morphological development, the genital tubercle develops as the penis (Jost, 1972). During this period, the potential for developing masculine behavior patterns is established.

The plasticity of the behavioral system allows for a period of up to 10 days postnatally for behavioral differentiation to occur. In the presence of androgen during this period, both genetic males and females will show a high probability of exhibiting a male pattern of sexual behavior. Conversely, in the absence of androgens or other gonadal steroids, both genetic males and females will show a high probability of showing lordosis, the female pattern of receptivity to the male. Beyond this period of maximal sensitivity to the action of steroids,

removal of endogenous androgen does not inhibit masculine behavior potential, given a sufficient supply of androgen in adulthood. However, androgen deprivation perinatally results in a deficiency of male sexual functioning in adulthood regardless of the level of circulating androgen in the adult system.

According to this formulation, then, two periods of androgen action are critical: perinatally to organize behavioral patterns and in adulthood to exhibit the behavior (Young, Goy & Phoenix, 1964). In this theory of androgenization of behavior, the hormones are presumed to act in an organizational way on undifferentiated neural tissues as they do on the genital tract. This would result in the development of an acyclic pattern of gonadotropin release characteristic of the male. In the adult, gonadal hormones would act via excitation or inhibition to regulate the gonadotropin secretion and thus, the hormone dependent patterns of sexual behavior (Harris, 1964).

Experimental evidence supports the necessity of early androgen. When male rats are castrated on day 1, they are incapable of showing the ejaculatory response in adulthood. When castration occurs on day 5, from 8.3% to 25% of the males show an ejaculatory response while 100% of intact males ejaculate (Grady, Phoenix & Young, 1965; Gerall, Henricks, Johnson & Bounds, 1967). Nadler (1969) has also administered an anti-androgen, cyproterone acetate, to pregnant female rats and then castrated their offspring on

day 1 to eliminate both prenatal and postnatal effects of androgen. When tested with testosterone propionate in adulthood, none of these males ejaculated. In both types of studies, males exhibited high levels of lordosis behavior when treated with appropriate hormones; this provides corroboration that lack of androgen exposure during development leads to a high probability of female behavior patterns. However, it is significant to note that males deprived of early androgen also have reduced penis length which could also alter the sensory feedback derived from copulatory behavior. Beach, Noble and Orndoff (1969) have shown that unless androgen is present before day 10, the penis will not grow in response to later androgen treatment. Therefore, they offer an alternative hypothesis to the organizational action of androgen action on neural structures. Mechanisms for masculine behavior patterns may be determined by prenatal androgen but in the absence of normal penile development this behavior cannot be normally expressed. The crucial experiments have not been done to determine peripheral and central factors.

Expression of sexual behavior in adulthood follows a stereotypic temporal patterning. Initial encounters between a male and female usually involve exploratory anogenital sniffing as a component of precopulatory behavior. This is followed by a mount, i.e., the male clasps and palpates the sides of the female with his fore-limbs while thrusting the pelvic region. These movements

can be aborted by unreceptive female, and in any event, the male slides weakly off her back. With intromission, palpation and thrusting culminate in a deep thrust penetrating the vagina which terminates in the male lunging backwards and ending contact with the female. An ejaculation can be differentiated from an intromission in that the final lunge is not executed; the male remains clasped to the female and then slowly raises his forelegs and drops off. Preceding each segment of activity, the male chases the female who is actively hopping and darting as a means of solicitation, and she then remains immobile for the duration of the mount, intromission or ejaculation (Young, 1961).

Each intromission lasts approximately .5 seconds with an interval of 30 to 60 seconds between intromissions. The first ejaculation occurs after approximately eight to 15 intromissions while four to seven intromissions are required for subsequent ejaculations in a series. The post-ejaculatory interval, the period following an ejaculation during which the male is unresponsive to further sexual stimulation, lasts approximately five minutes after the first ejaculation. Studies of sexual exhaustion or satiation have shown that the post-ejaculatory interval increases with subsequent ejaculatory series. The ejaculation latency, intromission frequency and inter-intromission interval decline after the first ejaculation and then rise as satiation is approached. A recovery

period of about two weeks is required for the male to return to the initial temporal patterning of the first ejaculation (Beach & Jordan, 1956; Larsson, 1956; Bermant, 1967; Dewsbury, 1968).

Statement of the Problem

In this introduction, I have elaborated on the factors which might influence the sexual behavior of male rats born to diabetic mothers. Evidence was presented indicating a correlation between the occurrence of diabetes and sterility in the adult male. This could be the result of a hormonal deficiency in either of two ways. A direct effect could be a decrease in the levels of circulating testosterone or an insensitivity of the target tissues to the androgen. Secondarily, androgen sensitive reflex systems might be compromised due to vascular and neurological complications of the diabetic state.

The perinatal period has also been demonstrated to be sensitive to androgen deficiency. Males exposed to the gestational period of a diabetic pregnancy are vulnerable in two ways. First, there might be a direct decrease in the testosterone production of the fetal testis as a result of exposure to the diabetogenic condition of the mother. This would parallel the exposure of the adult male in which the expression rather than organization of sexual behavior is inhibited. Also, differentiation of sexual behavior might be impaired by the diffuse effects of

continued metabolic stress of hyperglycemia and hyperinsulinemia experienced throughout the pregnancy by both the mother and her pups.

The problem I wish to consider in this study is whether the process of sexual differentiation in male rats will be disrupted by the hyperglycemia and hyperinsulinemia experienced by the offspring of a diabetic mother. It has been shown that organization of male sexual behavior is impaired by imbalances of gonadal steroids, and also that expression of sexual behavior is disrupted by diabetes. This study will determine if the same processes which can affect sexual behavior in adulthood can also affect the organization of sexual behavior neonatally.

The following hypotheses are offered concerning the sexual behavior of the male offspring:

1. Adult behavior may be disrupted in offspring of diabetic mothers either directly through an insufficient supply of androgen during the period of sexual differentiation or indirectly through the deleterious effects of prenatal metabolic stress. Disruption of sexual behavior would be characterized by a high number of mounts (reflecting an inability to achieve vaginal penetration), a decreased inter-intromission interval (reflecting the decreased excitatory value of sexual stimulation) and a decreased number of ejaculations to satiation (reflecting decreased sexual motivation).

2. An alternative hypothesis is that male offspring of diabetic mothers will exhibit normal sexual functioning in adulthood despite the aberrant intrauterine environment. Previous studies on the effects of stress during pregnancy have been confined to the study of emotional responses with the exception of one study on the effect of exogenous stress on sexual behavior. Since there are no systematic data available on the effects of stress on sexual differentiation it is probable that fetuses are able to compensate in varying degrees to different categories and intensities of trauma. Prolonged hyperglycemia may create an altered homeostatic condition for the fetus. Pups might also differentiate normally given decreased testosterone levels if the amount produced is still in excess of the sensitivity of the target tissues.

METHOD

Animals

Long Evans (Charles River) virgin female rats, aged 70 to 90 days, were used as mothers in this experiment after an adaptation period to the laboratory of approximately one week. They were housed individually in cages measuring 36x30.5x17 cm. Purina Lab Chow and water were available ad lib, and a constant temperature was maintained. All animals were under a 14-10 hour reversed light-dark cycle, with lights off at 1100 hours. Pups were weaned at 21 days, and the males were housed by litter in cages measuring 15x35.5x22.5 cm. During urine collection, animals were housed in individual metabolic cages under the same conditions.

Treatment

All females were placed in metabolic cages for a 24 hour urine pretest. Urine samples were tested for glucose using two indices. Clinitest (Ames Company) which indicates the presence of reducing substances, resonds to minimum levels of 150 mg/ml. Testape (Eli Lilly Company), a specific test for glucose, can discriminate between 0, 100, 250, 500, and 2000 mg/ml. Acetest (Ames Company)

was used to test for ketonuria, an index of the severity of diabetes. A minimum positive reaction indicates the presence of at least 10-20 mg/ml acetoacetic acid. A moderate reaction indicates twice that concentration, and a strong reaction indicates over 50 mg/ml acetoacetic acid (Pyke, 1968).

Following this procedure, females were anesthetized, ear tagged, weighed, and given an intravenous injection of 40 mg/kg STZ. The STZ (National Cancer Institute) was administered in a 4% solution in 0.4M citric acid with the pH adjusted to 4.0 with sodium hydroxide. Controls received an equivalent volume of buffered citrate solution. All solutions were prepared at the time of injection. After a two day recovery period, females were placed daily with a male until mating took place. This was considered day 1 of pregnancy. Only females who were severely diabetic as judged by maximum readings throughout pregnancy on the two indices of urine sugar were included in the study.

On days 1, 7, 14, and 20 of pregnancy, glycosuria and acetonuria were measured from urine samples. Weights also were recorded. Starting on day 21, all cages were checked daily for the presence of litters. The date of birth, and the number, sex and weight of the offspring were recorded. All litters were culled to eight pups, a mixture of both males and females, between the first and third day. Some litters were cross fostered to assess possible effects of postnatal maternal influences.

Testing

Males were weighed and tested for glycosuria and acetonuria at 50 and 90 days, and following the final behavioral test. Litters of experimental and control animals were numbered randomly so the behavioral tests were conducted without experimenter knowledge of the treatment group of the animal. Testing for male sexual behavior began at 90 days of age. Testing was conducted in clear plexiglass arenas (59x46.5x51.5 cm) in a dimly lighted room between 1300 and 1700 hours. Males were placed in the arena for a five minute adaptation period before testing. At the start of the test period a receptive female (screened with a normal male) was placed in the test arena. Receptivity was induced in the females with 12 ug estradiol benzoate for three days followed by .5 mg progesterone on the fourth day, four hours prior to testing. Mounts (with pelvic thrusting), intromissions (with vaginal penetration) and ejaculations were recorded on an Esterline Angus Event Recorder.

Each weekly test was conducted for 30 minutes. Three weekly pre-tests were followed by three tests in which the following behaviors were scored: mount latency, intromission latency, ejaculation latency, mount frequency, intromission frequency, inter-intromission interval, and post-ejaculatory interval for the first series. Total number of ejaculations per test also was recorded. Ten days after the last weekly test, each animal was tested for

satiation. This was defined as one half hour after the test had begun without an intromission. The total number of ejaculations preceding satiation was a measure of the upper limit of the animal's performance.

All data were analyzed non-parametrically by either a Kruskal-Wallace Test or a Mann-Whitney U Test.

RESULTS

Pregnancy

Present findings confirm those reported in the literature in that diabetic females had a slightly prolonged gestational period. Also, while all of the control mothers reared their litters to weaning, pups from four diabetic mothers had to be sacrificed prior to weaning because of their emaciated condition. These diabetic mothers showed maternal behavior by aggregating the young in a nest and crouching over them, but it is likely that the diabetic condition interfered with milk letdown.

Appendix A contains the weights, urine sugar and ketosis measurements for both severely diabetic and control mothers on days 1, 7, 14, and 20 of pregnancy. Severely diabetic mothers showed urine sugar measurements of 1 to 2% throughout pregnancy. This was the maximum reading on both of the semi-quantitative indices of urine sugar, Clinitest and Testape. Control mothers showed minimal levels on these indices; this could be attributed to normal fluctuation or to some unavoidable contamination of the urine with the food pellets. No animals showed a positive reaction with Acetest at any time. The mean weight gain during pregnancy

was 159.7 gms for the diabetic mothers and 143.4 gms for the control mothers; this difference in weight gain was not significant. Table 1 indicates the mean number of pups per litter, mean weight of the litters, and the percentage of males in each litter at birth for both diabetic and control mothers. Only mothers whose offspring were included in the study are represented.

Table 1.--Mean Litter Size, Mean Weight Per Litter, and Mean Percent Males Per Litter From Diabetic and Control Mothers.

Group	N*	Litter Size	Litter Wt. (gms.)	% Males
Diabetic Mothers	3	13.0	6.1	51.2
Control Mothers	4	11.0	5.9	49.0

*N = number of mothers in each group.

Offspring

The mean weight just before testing at 90 days is presented in Table 2 for male offspring in the four groups; differences between groups were not significant. Urine sugar, ketosis and weights were also recorded at 50 and 90 days. These data are presented in Appendix B. Offspring of both control and diabetic mothers showed minimal levels of sugar in the urine; positive results were not found with Acetest on any test.

Table 2.--Mean Weight of Male Offspring at 90 Days.

Group	Weight (gms.)
OSD*	384.2
OC*	390.0
OSDXC*	384.4
OCXSD*	387.7

*OSD = offspring of severely diabetic mothers; OC = offspring of control mothers; OSDXC = offspring of severely diabetic mothers cross fostered to control mothers; OCXSD = offspring of control mothers cross fostered to severely diabetic mothers.

Male Behavior Tests

Copulators during the weekly mating tests and on the satiation test were determined by two criteria. Scores from males that ejaculated on two of the three weekly mating tests were included in the data analysis. Scores from animals that ejaculated on the satiation test, independent of the other tests, were included in the satiation data. These criteria were adopted because inclusion of data from inconsistent and non responders skewed the scores of the group as a whole: as a result, the data did not reflect the performance of the consistent copulators. Table 3 presents the proportion of males in each group who met the criterion in the weekly tests and on the satiation test.

Means and standard errors for the behavioral measures recorded in the three weekly tests are presented

Table 3.--Proportion of Copulators During Weekly Mating Tests and Satiation Test.

Group	Weekly Tests**	Satiation***
OSD*	4/5	3/5
OC*	8/8	7/8
OSDXC*	8/9	8/9
OCXSD*	7/11	7/11

*OSD = offspring of severely diabetic mothers; OC = offspring of control mothers; OSDXC = offspring of severely diabetic mothers cross fostered to control mothers; OCXSD = offspring of control mothers cross fostered to severely diabetic mothers

**Criterion for copulators = ejaculation on 2 of 3 weekly tests.

***Criterion for copulators = ejaculation on satiation test only.

in Table 4, scores for the satiation test are presented in Table 5. These are measures for the first series on the tests indicated. In addition, the mean number of ejaculations on the satiation test is given for each group.

A Kruskal-Wallace test was used to determine if there were any overall differences between groups. On Test 1 there was an overall difference in ejaculation latency ($p<.05$) and intromission frequency ($p<.05$). On Test 2, the difference in ejaculation latency was significant ($p<.05$); on Test 3 the difference in intromission frequency was significant ($p<.05$). On the satiation test, the difference in intromission latency ($p<.05$) and inter-intromission interval ($p<.05$) was significant.

Table 4.--Mean Behavioral Scores on Weekly Mating Tests.

	OSD*			OC*		
	1	2	3	1	2	3
ML° (sec)	24.3±15.9[+]	24.0±18.3	85.3±77.9	73.5±29.5	50.4±38.1	197.6±127.0
IL° (sec)	35.0±22.4	79.3±73.3	87.8±77.1	291.5±98.2	138.3±71.8	289.5±137.0
EL° (sec)	251.8±47.2	263.3±22.9	297.0±32.2	504.8±142.2	632.3±63.9	486.4±122.1
MF°	3.8±1.5	3.8±1.8	3.8±1.3	12.5±3.4	11.3±4.0	11.1±2.1
IF°	8.5±1.0	7.5±1.6	6.5±0.6	10.8±1.3	10.8±0.8	8.9±1.5
III° (sec)	28.9±1.0	49.6±13.2	54.6±3.9	57.3±20.8	69.4±10.8	59.8±14.2
PEI° (sec)	343.3±29.0	361.0±54.1	363.0±21.6	164.6±64.2	401.9±42.3	322.6±86.2

Table 4.--Continued.

	OSDXC*			OCXSD*		
	1	2	3	1	2	3
ML° (sec)	20.3±14.6+	6.1±1.0	4.6±0.9	93.0±82.0	154.4±130.5	32.9±22.4
IL° (sec)	98.9±60.2	52.3±37.9	36.6±18.8	176.0±86.5	189.1±128.0	73.3±38.4
EL° (sec)	418.6±60.0	412.4±57.5	359.9±73.1	488.0±47.2	567.0±90.0	439.7±137.5
MF°	6.6±1.2	6.8±0.8	5.0±1.1	9.3±3.2	4.7±1.8	3.7±1.4
IF°	12.6±1.5	11.8±1.0	10.5±1.7	14.9±1.0	10.6±0.6	9.3±1.9
III° (sec)	39.4±6.8	40.8±6.7	32.6±6.1	36.2±4.0	60.5±10.9	43.3±11.1
PEI° (sec)	349.5±18.7	367.8±22.8	322.9±54.6	361.1±18.3	309.9±53.9	297.9±57.3

*ODS = offspring of severely diabetic mothers; OC = offspring of control mothers; OSDXC = offspring of severely diabetic mothers cross fostered to control mothers; OCXSD = offspring of control mothers cross fostered to severely diabetic mothers.

°ML = mount latency; IL = intromission latency; EL = ejaculation latency; MF = mount frequency; IF = intromission frequency; III = inter-intromission interval; PEI = post ejaculatory interval.

+Mean ± SE.

Table 5.--Mean Behavioral Scores on Satiation Test.

	OSD*	OC*	OSDXC*	OCXSD*
ML° (sec)	60.0±54.5+	266.7±123.0	52.6±26.5	267.4±128.3
IL° (sec)	109.7±37.3	499.3±175.9	104.0±30.8	486.3±143.5
EL° (sec)	390.7±72.7	1759.1±818.4	424.0±45.4	1088.7±430.0
MF°	9.7±4.3	13.3±4.4	4.5±1.0	8.0±2.9
IF°	7.7±0.9	9.9±1.0	11.8±1.1	10.3±1.3
III° (sec)	64.1±21.6	200.2±76.3	42.3±6.5	120.4±40.4
PEI° (sec)	437.3±38.0	662.3±120.7	359.8±24.9	422.0±32.5
Total E	5.3±0.3	5.4±0.4	6.6±0.5	6.1±0.8

*OSD = offspring of diabetic mothers; OC = offspring of control mothers; OSDXC = offspring of severely diabetic mothers cross fostered to control mothers; OCXSD = offspring of control mothers cross fostered to diabetic mothers.

°ML = mount latency; IL = intromission latency; EL = ejaculation latency; MF = mount frequency; IF = intromission frequency; III = inter-intromission interval; PEI = post ejaculatory interval.

+Mean ± SE.

Due to the small number of animals in some groups, some of the measures approached but did not reach significance. A second analysis was done with a Mann-Whitney U Test. First, tests were made to see if there was any difference between offspring of severely diabetic mothers and those cross fostered with controls; a similar analysis was made for offspring of control mothers and those cross fostered with severely diabetic mothers. Only one measure turned out to be significant from all behavior tests. On Test 2 and 3 and on the satiation test, offspring of severely diabetic mothers reared by their own mothers showed fewer intromissions preceding ejaculation ($p<.05$) than offspring of severely diabetic mothers who were cross fostered. Therefore, on all measures except intromission frequency, data from the offspring of both diabetic groups were pooled as one group. Similarly, scores from the two control groups were also pooled.

Table 6 shows the significance levels on the Mann-Whitney U Test for the pooled groups. As a general trend, latencies to the initiation of sexual behavior were shortened in males who had diabetic mothers.

Table 6.--Levels of Significance on Mann-Whitney U Test for Behavioral Measures on Weekly Mating Tests and Satiation Test.

	OSD* + OSDXC*		vs.	OC* + OCXSD*	
	Test 1	Test 2		Test 3	Satiation
ML° (sec)		p<.05			p<.05
IL° (sec)	p<.05	p<.05			p<.05
EL° (sec)	p<.05	p<.05			p<.05
MF°					
III° (sec)					p .05
PEI° (sec)					

*OSD = offspring of severely diabetic mothers; OSDXC = offspring of severely diabetic mothers cross fostered to control mothers; OC = offspring of control mothers; OCXSD = offspring of control mothers cross fostered to severely diabetic mothers.

°ML = mount latency; IL = intromission latency; EL = ejaculation latency; MF = mount frequency; III = inter-intromission interval; PEI = post ejaculatory interval.

DISCUSSION

The sexual behavior of male offspring from diabetic mothers was characterized by a shortened latency to the initiation of sexual behavior. A change in temporal pacing resulted in significantly shorter latencies to intromission and ejaculation. Mount latency was reduced on one of the weekly tests, and also on the satiation test. The reduction in latencies was not attenuated by repeated testing.

The reduction in latencies appears to result from the prenatal diabetic environment since no difference was found in latencies to sexual behavior between offspring of diabetic mothers reared by their own mothers and those cross fostered to control mothers. The only difference attributed to cross fostering was an increase in intromission frequency in cross fostered offspring of diabetic mothers when compared to offspring of diabetics reared by their own mothers. On all other measures, there was no difference between pups that were reared by their own mothers and those which were cross fostered. Although differences in maternal behavior may have been present,

differential caregiving in general did not influence sexual behavior.

Sexual Arousal

Measures of copulatory behavior can be divided into two categories: latency to copulate and rate of copulation. Beach (1956) has proposed that the male's sexual excitement in the presence of a female increases to a point at which he initiates copulation, i.e., a copulatory threshold has been reached. A decrement of this threshold is facilitated by visual and olfactory cues from the female as well as her own solicitation behavior. The summation of this stimulation will trigger a mount or an intromission by the male; in the case of non copulators, this threshold is not reached and the male habituates to the presence of the female.

Once the copulatory threshold has been reached, a second hypothetical mechanism, the intromission and ejaculatory mechanism, is initiated. Stimulation provided by copulation modifies the internal state of the animal to bring the male to the ejaculatory threshold. The trigger for the ejaculatory mechanism is controversial. One possibility is that each successive intromission builds up a level of excitement until the threshold for ejaculation is reached. Another possibility is that the initial intromission triggers a timing mechanism and successive

intromissions maintain a constant level of input to the timer until the culmination of the interval in ejaculation.

Male offspring of diabetic mothers in the present study showed changes in both aspects of this dual system of sexual motivation. The decreased intromission latency shown by these males when compared to controls indicates a lowering of the copulatory threshold for the initiation of sexual behavior. The decrease in ejaculation latency and decrease in intromission frequency in offspring of diabetic mothers indicates a concomitant lowering of the ejaculatory threshold. The latency differences suggest a difference in arousal level between males born to diabetic mothers and males born to control mothers.

One other measure of temporal pacing, the post ejaculatory interval, did not differ between groups. Also, there was no difference in the number of ejaculations to satiation between any of the groups; this would indicate that sexual exhaustion, or lowering of sexual arousal to the point that no intromissions occurred in a half hour interval, followed the same total number of ejaculations in all groups.

The performance of normal males on a sexual exhaustion test is characterized by a U shape pattern of response (Karen and Barfield, 1975). Facilitation of sexual behavior, i.e., shortened ejaculation latency, shortened inter copulatory intervals, longer durations per intromission, and fewer intromissions to achieve ejaculation

characterize the intermediate stages in exhaustion tests. Longer inter copulatory intervals and a greater number of intromissions preceding ejaculation are seen in the first and last series of a satiation test. In this context, male offspring of diabetic mothers showed an initial facilitation compared to the behavior of control males. Additional tests would indicate whether a U shape curve in satiation would also be obtained for offspring of diabetic mothers, i.e., whether the decreased latencies relative to controls would be maintained in successive series. Although satiation data were obtained in the present study, refusals on the part of some females in the later series distorted latency measures. In some instances females showed lordosis only after persistent attempts to mount by the male. These refusals may have been preferentially directed towards experimental males; however, since female behavior was not monitored throughout the tests, no conclusions can be drawn.

Reduction in ejaculation latency, and in intromission frequency, has been found in contexts other than satiation tests. This result is found following castration (Davidson, 1966), adrenalectomy (Bloch and Davidson, 1968), handling of rats during copulation (Larsson, 1963), peripheral electric shock (Barfield and Sachs, 1968), and rewarding or aversive brain stimulation (Caggiula, 1970; Eibergen and Caggiula, 1975). These diverse treatments have in common their ability to increase levels of

behavioral arousal whether through the diffuse effects of surgical trauma or localized stimulation in the brain. Since peripheral stimulation is not always specific to copulatory behavior, it probably reflects diffuse activation of arousal mechanisms in the brain; in this study, reactiveness to other types of stimulation was not studied to determine if the pattern of behavior in male offspring of diabetic mothers was specific to sexual stimulation or whether it represented a generalized increase in responsiveness to external stimulation.

Adaptive Significance

At first glance, decrease in intromission frequency and ejaculation latency seem to suggest a greater efficiency in sexual behavior; the ejaculatory response can be achieved in less time with less work. However, this atypical patterning of sexual behavior may decrease the reproductive efficiency of these animals. Diamond (1970) discussed a species specific vaginal code where number and spacing of intromissions were critical factors in initiating neuroendocrine reflexes in the female necessary for pregnancy to occur. Multiple intromissions result in facilitation of sperm transport and luteal activation; three intromissions are sufficient for sperm transport whereas more than five are necessary for luteal activation. This corresponds to behavioral studies where only 20% of female rats receiving three or less intromissions became

pregnant but 90% of those receiving approximately nine intromissions became pregnant (Wilson, Adler and Le Boeuf, 1965; Adler, 1969; Chester and Zucker, 1970). Thus, a male rat not conforming to the normal pattern of sexual behavior in his species reduces his chances of reproductive success. It is very possible that the behavior shown by offspring of diabetic mothers could lead to this result.

Etiology

It is difficult to assign a mechanism to the altered pattern of sexual response seen in male offspring of diabetic mothers, although the results do suggest an analogy with the effects of certain stressful conditions on temporal patterning in adult sexual behavior. Only one study has been reported on the effects of prenatal stress (constraint under glaring light) on sexual behavior (Ward, 1972); results indicated a decrease in ejaculations in intact males and an increase in feminine responses when given appropriate hormones. This was interpreted as resulting from increased secretion of a weak androgen, androstenedione, from the adrenal cortex as a concomitant of stress. Consequently, the organization of sexual behavior would be impaired paralleling the situation where males are deprived of testosterone during development. The effects of long term stress in adult males also results in decreased plasma testosterone (Milkovic and Milkovic, 1966); studies have not been done on sexual

behavior in chronically stressed adult males but they would be expected to show decreased ejaculatory capacity.

One possibility is that maternal diabetes in this study functioned to produce a steady but low level of stress in which corticosteroids were activated without also producing large amounts of adrenal androgens. Levine and Mullins (1966) proposed a model whereby early stress (handling of rats in infancy) could permanently alter the set point for adrenocortical response, resulting in a more graded response to stressful stimuli in adulthood rather than an all or none activation. Corticosteroids would have to be measured in both the mother and the offspring at birth and maturity to determine if there was an altered adrenal output either prenatally to alter the hypothalamo-pituitary-adrenal system, or in adulthood to alter the arousal value of sexual stimuli.

Conclusion

The effects of maternal diabetes on sexual behavior of male offspring reflect patterns seen in conditions of increased arousal or stress, i.e., decreased latencies to ejaculation and decreased intromission frequency. Other behavioral tests would be needed to determine whether this is specific to sexual behavior or whether it represents a phenomenon of general excitability. Studies are being conducted presently to determine the ability of these males to impregnate females; it is predicted that ejaculation in

these males will not lead to fertilization because the intromission frequency and pacing will not function appropriately to initiate reproductive processes in the female. Finally, the important question of mechanisms can only be answered by assays of hormonal changes in the diabetic mother and her fetuses, and also by a wider range of studies on the effects of prenatal stress on behavior, so that meaningful comparisons can be made.

APPENDICES

APPENDIX A

Table 7.--Urine Sugar and Body Weight for Mothers.

	Day 1				Day 7				Day 14				Day 20			
	Wt. (gms.)	CT* (%)	TT* (%)	AT* (%)	Wt.	CT	TT	AT	Wt.	CT	TT	AT	Wt.	CT	TT	AT
Control																
13	230.3	0	0	0	236.8	1	0	0	293.3	1/4	0	0	362.2	1/4	0	0
15	199.0	1/4	0	0	220.8	0	1/10	0	290.6	0	0	0	362.2	1/4	0	0
19	178.1	0	0	0	264.1	0	0	0	264.7	1/4	0	0	343.5	1/4	0	0
21	218.6	0	0	0	242.9	0	0	0	255.1	0	0	0	331.5	1/2	0	0
Severe Diabetes																
11	227.4	1	2	0	240.9	2	2	0	274.9	1	2	0	373.1	2	2	0
18	199.0	2	2	0	212.1	2	2	0	265.3	2	2	0	300.2	3/4	2	0
23	175.2	2	2	0	185.5	2	2	0	190.1	2	2	0	307.4	2	2	0

*CT = Clini-Test, TT = Tes-Tape, AT = Acetest.

APPENDIX B

Table 8.--Urine Sugar and Body Weight for Male Offspring.

Rat	Day 50				Day 90			
	Wt. (gms)	CT* (%)	TT* (%)	AT* (%)	Wt.	CT	TT	AT
OSD+								
7	231.0	1/4	0	0	390.0	0	0	0
8	212.1	1/4	0	0	380.0	1/4	0	0
9	206.5	1/4	0	0	355.0	0	0	0
10	230.4	1/2	0	0	405.0	1/4	1/10	0
11	186.7	3/4	0	0	395.0	0	0	0
12	216.5	1/4	1/4	0	400.0	0	0	0
OSDXC+								
39	247.0	1/4	1/10	0	400.0	1/2	1/4	0
40	214.7	1/2	1/10	0	370.0	1/4	1/10	0
41	246.5	1/4	1/10	0	350.0	1/4	0	0
42	253.3	0	1/10	0	395.0	1/4	1/10	0
43	229.5	0	1/10	0	330.0	0	1/10	0
44	264.5	0	1/10	0	430.0	1/4	0	0
46	265.0	1/4	1/10	0	410.0	0	1/10	0
47	211.2	0	1/10	0	360.0	0	1/10	0
48	238.4	1/4	1/10	0	415.0	1/4	1/10	0
OC+								
19	293.5	1/4	0	0	460.0	0	0	0
20	279.8	1/4	0	0	410.0	0	1/10	0
21	229.5	1/2	1/10	0	370.0	1/4	0	0
22	254.4	0	0	0	400.0	1/4	1/10	0
24	266.3	1/4	0	0	370.0	1/4	1/10	0
56	262.0	1/4	1/10	0	360.0	0	0	0
57	255.6	1/4	1/10	0	370.0	0	0	0
58	252.4	3/4	1/10	0	405.0	1/4	1/10	0
59	222.3	3/4	1/10	0	365.0	0	0	0
OCXSD+								
34	242.8	1/2	1/10	0	320.0	1/2	1/10	0
35	241.2	1/4	1/10	0	310.0	1/2	1/10	0
36	227.9	1/2	0	0	410.0	1/4	0	0
37	214.9	1/2	1/10	0	350.0	0	0	0
38	223.4	0	1/10	0	350.0	1/2	1/4	0

Table 8.--Continued.

Rat	Day 50				Day 90			
	Wt. (gms)	CT* (%)	TT* (%)	AT* (%)	Wt.	CT	TT	AT
50	233.2	1/2	1/10	0	410.0	1/4	1/10	0
51	263.3	0	0	0	400.0	1/4	1/10	0
52	328.1	1/4	1/10	0	450.0	1/2	1/4	0
53	276.1	1/4	1/10	0	430.0	1/2	0	0
54	307.0	1/4	0	0	415.0	1/4	0	0
55	274.1	1/4	1/10	0	420.0	0	0	0

*CT = Clini-Test; TT = Tes-Tape; AT = Acetest

+ODS = offspring of severely diabetic mothers; OSDXC = offspring of severely diabetic mothers cross fostered to control mothers; OC = offspring of control mothers; OCXSD = offspring of control mothers cross fostered to severely diabetic mothers.

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