THE EFFECT OF 5-THIO-D-GLUCOSE ON REPRODUCTION IN FEMALE MICE

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree of

Master of Science in Biology

by

Roslyn S. Gray

July, 1982

ACKNOWLEDGMENT

I would like to thank Dr. Brinn, Dr. Daugherty, and Dr. Ito for serving on my committee and giving of their time and knowledge. I am very grateful to Dr. Simpson for his enthusiasm, interest, time, and assistance on the research project and writing of the thesis.

Also, I wish to thank Bob for his support.

ABSTRACT

Roslyn S. Gray. THE EFFECT OF 5-THIO-D-GLUCOSE ON REPRODUCTION IN FEMALE MICE. (Under the direction of Dr. Everett C. Simpson). Department of Biology, July, 1982.

DUB:ICR mice were divided into three treatment categories and administered 5-thio-D-glucose in the treated groups and D-glucose in the control groups. Treatment I had a fourteen day pre-treatment period prior to breeding, continuing with a breeding period and ending with a fourteen or eighteen day length of gestation. Its daily dosage was 33 mg/kg. Treatment II had no pre-treatment period and a dosage of 33 mg/kg. It had treated and control groups gestating either fourteen or eighteen days. Treatment III had no pre-treatment period, a daily dosage of 45 mg/kg and a gestation length of fourteen days.

The experimental parameters in this investigation were maternal weight gain, ovarian weights, number of fetuses and resorptions, fetal crown-rump lengths and fetal weights. Pre-treatment with 5-thio-D-glucose caused changes in the majority of the experimental parameters. Treatment I was significantly different at the 5% level for maternal weight gain, ovarian weight, the number of fetuses and the number of resoprtions. It was not significant for fetal crown-rump length and fetal weight. Treatment II was not significantly different at any level for any of the experimental parameters. Treatment III had a significant decrease in the number of control fetuses.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

5-Thio-D-glucopyranose (hereafter referred to as 5-TDG) is an analogue of D-glucose, competitively inhibits D-glucose transport (Hoffman and Whistler, 1968) and has antispermatogenic properties (Zysk et al., 1975; Homm et al., 1977; Fick, 1979). 5-TDG has produced complete reversible sterility (Zysk et al., 1975) and shown promise as a nonhormonal male contraceptive (Maugh, 1974). Additional studies using rats and mice produced permanent sterility (Homm et al., 1977) or partial sterility among the experimental animals (Lobl and Porteus, 1978).

Unlike the male, no research has been done with 5-TDG on female fertility and 5-TDG's effect on the female reproductive organs. 5-TDG's effect on female reproductivity was, therefore, investigated because of the possible production of temporary sterility without the use of hormones or toxic chemicals. During this investigation, reproductive rates were monitored, as well as, body and ovarian weights and fetal development as measured by fetal weights and crown-rump lengths.

REVIEW OF LITERATURE

5-TDG was first prepared by Feather and Whistler (1962). It differs from D-glucopyranose by the substitution of a sulfur atom in place of the oxygen in the pyranose ring. This single change, and the physical and chemical properties of the resulting sugar, make 5-TDG the nearest analogue of D-glucose available to date (Feather and Whistler, 1962). Studies have shown that 5-TDG has a LD_{50} of 14 g/kg; thus, it is classified as non-toxic (Hoffman and Whistler, 1968). It tastes sweet and has a sweetness value approximately that of D-glucose. In molarity ratios of 5-TDG, a ratio as low as 0.03 effectively interfered with the utilization of D-glucose for the development of Drosophila melanogaster. Molarity ratios of 0.31 above prevented development from the larval to the pupal stage. This inhibition was attributed to interference of enzyme(s) and/or transport of D-glucose across cell membranes due to the structural similarity of the two sugars (Shankland et al., 1968).

Rats, intraperitoneally administered with 5-TDG in isotonic saline, rapidly develop glucosuria and hyperglycemia. With a dose of 50 mg/kg, the blood D-glucose rises to approximately 300 mg/100 ml within 2.5 hours, then drops to 160 mg/100 ml within 6 hours. Analysis of the urine showed that 97.3 ± 5.7% of the administered 5-TDG was recovered unaltered in the urine 24 hours after injection; therefore, little metabolism of 5-TDG occurred. Rats given booster doses of 5-TDG every hour maintain blood D-glucose levels of 300 mg/100 ml. The same effect is seen in rats with ligated kidneys given a single 50 mg/kg dose. Administration of insulin completely nullifies the diabetogenic effect (Hoffman and Whistler, 1968).

Livers from rats fasted for 20 hours had 50% less glycogen 2.5 hours after injection of 50 mg/kg of 5-TDG than saline injected controls. 5-TDG inhibited D-glucose uptake in liver, kidney and diaphragm tissue in vitro when molarity ratios, of 5-TDG to D-glucose, from 0.5 to 1.0 are present in initial incubation media at 37°C. 5-TDG did not inhibit the metabolism of D-glucose in kidney homogenates in vitro but did slightly inhibit glycolysis. After a series of three injections of 5-TDG in vivo at 2 hour intervals, there is a 43% increase in the total catecholamine content of the urine which correlates with the increased glycogen breakdown. A 73% increase in blood non-esterified fatty acids of rats fasted for 16 hours was observed 0.5 hour after a single 150 mg/kg dose of 5-TDG. The non-esterified fatty acid level returns to a normal fasting level within 2 hours. 5-TDG is neither a substrate nor an inhibitor of D-glucose oxidase which is used to analyze for D-glucose. 5-TDG does not inhibit yeast hexokinase but acts as a poor substrate (Hoffman and Whistler, 1968).

It is apparent that 5-TDG has a physiological effect when administered orally or intraperitoneally to rats. The immediate effect is the rise in blood D-glucose with simultaneous complementary glucosuria developing. From the results of experiments with kidney and diaphragm tissue sections and kidney homogenates, the conclusion could be drawn that 5-TDG inhibits membrane transport of glucose. Evidence indicates that 5-TDG is bound to a D-glucose transport carrier in these tissues. In liver sections, the effect of 5-TDG seems to be increased D-glucose production. Here, 5-TDG may inhibit glycogen synthesis or stimulate glycogen breakdown (Hoffman and Whistler, 1968).

5-TDG affects active and facilitated diffusion transport processes. It

is readily transported in rabbit kidney-cortex slices and shows typical saturation kinetics with a Km value of 2.4 mM and Vmax value of 70 umol/h per gram of cell water (Whistler and Lake, 1973). Values for Km Vmax and Ki for 5-TDG are the same order as those found for D-glucose and other sugars (Kleinzeller et al., 1967a; Kleinzeller, 1970). Uptake of 5-TDG is phlorrhizin-sensitive, Na^+ dependent and energy-dependent. D-galactose and methyl-d-D-glucopyranoside transport is competitively inhibited by 5-TDG with Ki values of 4.8 and 9.4 mM respectively. Since 5-TDG is rapidly accumulated against the concentration gradient, the sugar is acting as a true substrate for the transport carrier. Thus, in kidney cortex, 5-TDG shows all the active transport characteristics. In rat kidney cortex, transport of neutral amino acids is inhibited by the sulfur analog of D-glucose (Whistler and Lake, 1972). Mutual inhibition of transport exists between amino acids and hexoses that use the D-glucose-D-galactose transport systems in kidney cortex (Thier, et al., 1964; Segal et al., 1962). The inhibition between amino acids and hexoses is non-competitive (Genel et al., 1971).

5-TDG is freely taken up by the facilitated diffusion transport system in rat diaphragm muscle. The analog(ue) inhibits the transport of D-xylose in this tissue but has no effect on D-arabinose uptake probably because D-arabinose enters the intracellular space by diffusion rather than facilitated transport. It was concluded that the ring heteroatom is not an effector of binding in the transport processes examined and causes no important alteration in the sugar's conformation, even though the ring oxygen is replaced by a larger and less negative sulfur atom. Both sugars (D-glucose and 5-TDG) exist in the same shape with the same conformation

where all ring hydrogens can be equatorially oriented (Suzuki and Whistler, 1972). The diabetogenic action produced by 5-TDG is due, in part, to the ability of the analog(ue) to interfere with cellular transport processes that use D-glucose. The (pseudo) diabetogenic effect occurs with large does of 5-TDG but is short-lived since about 97% of the analog-(ue) is excreted within 6 hours. At lower doses, normal blood sugar levels are maintained (Whistler and Lake, 1972).

Critchley et al., (1970) and Barnett et al., (1970) used 5-TDG to examine the transport of the sulfur sugar in hamster small intestine. It exhibited all of the criteria to be accepted as a substrate of the D-glucose active-transport system. Critchley (1970) also found the specific binding of D[^{3}H] glucose to the intestinal brush border was inhibited by 5-TDG.

Paranjpe and Jagannathan (1971) reported that 5-TDG inhibits ox heart hexokinase but does not inhibit the metabolism of D-glucose in kidney homogenates (Whistler and Hoffman, 1968).

5-TDG and its l-phosphate interfere with D-glucose-6-P formation. 5-TDG serves as a substrate for yeast hexokinase and competitively inhib-its D-glucose phosphorylation. 5-Thio-D-glucose-1-P is a competitive inhibitor of D-glucose-1-P conversion to the 6-P, thus, affecting glucose metabolism (Chen and Whistler, 1975).

5-TDG is little metabolized. On intravenous administration of ^{14}C labeled sugar, 1% is oxidized to $^{14}CO_2$, 93% excreted in the urine, and 1.6% retained in the carcass. Orally, 1% goes to CO_2, 90% excreted in feces and urine, and 4% in the carcass after 72 hours (Chen and Whistler, 1975).

In the pancreatic islets, 5-TDG inhibits the uptake of D-glucose by

30% and is an inhibitor of D-glucose stimulated insulin release. It was concluded that the sulfur atom substitution for oxygen in the sugar analog(ue) caused the apparent loss of insulin releasing action. Also, 5-TDG interferes with the metabolism of D-glucose. The sulfur analog(ue)'s diabetogenic actions is probably a combination of its effect on membrane transport, insulin release and D-glucose metabolism (Hellman et al., 1972). 5-TDG inhibits cancer cell growth. A drug concentration of 2.6×10^{-3} to 6.3×10^{-4}M produced 60 to 70% reduction in growth while higher concentrations prevented growth (Bushway et al., 1977).

When mature mice were fed 5-TDG, their sperm development was inhibited within three weeks at daily dose levels greater than 30 mg/kg. There was no impaired libido. Removal of the analog(ue) resulted in resumed sperm development and fertility (Zysk et al., 1975). This study was done due to known effects that D-glucose has on sperm development (Davis, 1969). Plus, D-glucose is essential for normal sperm development as demonstrated by the decreased fertility of male diabetic rats (Folgia, 1963). Reproductive function in the human male is markedly affected by the diabetic state; for instance, there is increased incidence of impotence (Schoffling et al., 1963) decreased sperm count (Babbott et al., 1958) poor sperm motility (Klebanow and MacCleod, 1960) and atrophic changes in the germinal epithelium of the testis (Babbott et al., 1958).

Just as the diabetic state affects (human) male reproductive function, the (human) female reproductive function is also affected. According to Eastman (1946) uncontrolled diabetes mellitus and pregnancy are basically incompatible. Prior to the advent of insulin in 1921, the majority of diabetic women were sterile. Those few who did become pregnant showed an

increased tendency to abort. The underlying cause of infertility during the pre-insulin era was probably due to a number of factors most of which are poorly understood.

5-TDG's effect on female reproductivity is being investigated because of the possible production of temporary sterility without the use of hormones or toxic chemicals. Unlike the male, no research has been done with 5-TDG on female fertility and its effect on the female reproductive organs. Since 5-TDG acts as a competitive inhibitor on D-glucose transport carrier systems, produces a diabetogenic effect and inhibits tumor cell growth, it may interfere with normal oogenesis, fertilization and/or implantation.

Since the early stages of mammalian development are not supported by D-glucose, the use of 5-TDG would appear to have little effect on fertilization and the early blastocyst stages. Culture experiments with chemically defined media have shown that the human and mouse oocyte (Banks et al., 1976), one-cell fertilized egg (Biggers et al., 1967) and two-cell stage (Whitten, 1957) cannot develop in the presence of D-glucose alone; in contrast, the eight cell stage will develop in the presence of D-glucose alone (Whitten, 1956); Brinster and Thomson, 1966). Human and mouse oocyte maturation is supported by a pyruvate (or oxaloacetate) medium (Banks et al., 1976). Rabbit embryos will not develop in the presence of glucose alone until the blastocyst stage (Daniel, 1967). A possible explanation for these results is that glucose is not taken up by the mammalian embryo prior to the eight cell stage; however, in the mouse, the permeability of the two cell and eight cell stages to glucose are almost the same, and the unfertilized egg is impermeable to glucose. After fertilization, more of the sugar can enter per unit of time (Wales and Brinster, 1968). In

addition, in all pre-implantation stages of the mouse, it has been shown that U^{14}C-glucose is metablized to lactate (Wales, 1969) and CO_2 in embryos at all stages rather than being held in the plasma membrane. The evidence suggests that glucose is unable to provide sufficient energy for early mammalian embryos and at some point the necessary metabolic pathways are activated. Additional evidence is provided by lactate production which is low until the eight cell stage but then rapidly increases (Wales, 1969). The change in the metabolic capabilities of the rat embryo, in its ability to use glucose as an energy source, is seen in the enhanced oxygen consumption produced by glucose at the blastocyst stage (Sugawara and Umezu, 1961; Boell and Nicholas, 1948). Thus, the necessary substrate(s) to support the early blastocyst would be available even though glucose utilization is impaired, but the later stages of the blastocyst which use glucose could be affected.

5-TDG's inhibition of cancerous tumor growth (Bushway and Whistler, 1975) and inhibition of Drosophila melanogaster's development (Shankland, 1968) is indicative of interference with cellular D-glucose usage during high rates of glycolysis. It is known that the rate of aerobic glycolysis is high in cells and organs of the developing mammalian embryo (Needham, 1931). For implantation to occur, both the blastocyst and uterus must undergo proliferation which requires elevated glucose usage due to high rates of cell division. As a consequence, implantation and subsequent development of the embryo may be (adversely) affected by 5-TDG resulting in lower reproductive rates.

MATERIALS AND METHODS

Animals

Albino nulliparous female mice of the DUB: (ICR) strain were used in this investigation. At the start of the investigation, all the female mice were approximately 60 days of age. This strain is known to have a normal 21 day gestation period.

Sexually mature mice of the same strain were used for breeding purposes. The animals were housed in plastic containers with wire tops. Two sizes of containers were used. One was 20 x 31 x 13 cm which housed six or fewer mice. The other container was 24 x 43 x 15 cm which housed between seven to fifteen mice. All animals were kept in a room with approximately 10 hours of light and 14 hours of darkness and in a near constant temperature of 22°C. All animals had free access to water and received a mixture of Wayne Lab Blox and Wayne Mouse Breeder Blox ad libitum.

Vaginal lavages for all females in the investigation were obtained daily between 1200 hours and 1600 hours. During this same time period, the appropriate drug was given by intubation.

Prior to being assigned to their respective treatments, the sexually mature females of this study had to demonstrate three or more consecutive estrous cycles. Once treatment was initiated, the females continued to be checked daily through day ten of gestation to insure that the mice were pregnant.

Experimental Design

This investigation involved three experimental categories referred to as Treatment I, Treatment II, and Treatment III (Table 1). Each category

Table 1. Schematic of the three treatments which were administered either 5-thio-D-glucose or D-glucose.

Treatment	Group	Designation	Days of Gestation	Sugar	Dosage
I (Pre-treated 14 days)	A	Treated	14	5-TDG	33 mg/kg
	B	Control	14	D-Glucose	
	C	Treated	18	5-TDG	
	D	Control	18	D-Glucose	
II (No Pre-treatment)	E	Treated	14	5-TDG	33 mg/kg
	F	Control	14	D-Glucose	
	G	Treated	18	5-TDG	
	H	Control	18	D-Glucose	
III (No Pre-treatment)	I	Treated	14	5-TDG	45 mg/kg
	J	Control	14	D-Glucose	

contained at least one group of treated mice which was administered 5-TDG and at least one group of control mice which was administered D-glucose. 5-TDG and D-glucose was given by stomach intubation. Within the treated and control groups of each experimental category, there were subdivisions based on the gestation length. The gestation lengths studied were either fourteen or eighteen days of a normal twenty-one day gestation length. Day one of gestation was designated by the observation of a vaginal copulatory plug.

Treatment I consisted of the daily administration of D-glucose and 5-TDG during a fourteen day pretreatment period prior to breeding, continuing with a breeding period and ending with fourteen or eighteen days of gestation. Of the four groups in Treatment I, the two treated groups,

hereafter called Group A and C, were intubated between the hours of 1200 to 1600 hours with 5-TDG dissolved in water at a dosage of 33 mg/kg/day. The two control groups, hereafter called Group B and D, were duplicates of Groups A and C except D-glucose was administered during intubation at a dosage of 33 mg/kg/day. Group A (treated) and Group B (control) were sacrificed on day fourteen of gestation, and Group C (treated) and Group D (control) were sacrificed on day fourteen of gestation, and Group C (treated) and Group D (control) were sacrificed on day eighteen of gestation.

During Treatment II, drug administration was initiated after breeding had occurred. As with Treatment I, day one of gestation was established by the presence of a vaginal copulatory plug. In Treatment II, the drug treatments were confined to either fourteen or eighteen days of gestation. Treatment II had four groups of animals. The two treated groups, hereafter called Groups E and G, were intubated between the hours of 1200 to 1600 hours with 5-TDG dissolved in water at dosage of 33 mg/kg/day. The control groups, hereafter called Groups F and H, were treated exactly the same except D-glucose was given instead of 5-TDG at the same concentration and during the same time period. Groups E (treated) and F (control) were sacrificed on day fourteen of gestation. Groups G (treated) and H (control) were sacrificed on day eighteen of gestation.

In Treatment III, as in Treatment II, the drugs were administered only after breeding had occurred. Treatment III had one treated group and one control group referred to as Groups I and J, respectively. Between the hours of 1200 and 1600 hours, D-glucose and 5-TDG were given by intubation at a dosage of 45 mg/kg/day. These two groups were sacrificed on day fourteen of gestation.

Experimental Procedures

Vaginal Lavages

Vaginal lavages were taken on all animals to determine the stages of the reproductive cycles. A small pipette containing $\frac{1}{4}$ cc of warm tap water was inserted into the vagina and aspirated three to four times. This obtained an adequate quantity of sloughed cells. The lavages from the mice were placed on a microscope slide and examined at 100X. These data were recorded daily during the various experimental periods. See Appendix B for this information.

During proestrous and estrous, the female ICR mice were placed in breeding cages with sexually mature male ICR mice. When it was possible, there was a one-to-one ratio between males and females. The presence of a vaginal copulatory plug in the vaginal lavages established day one of pregnancy.

Drug Administration

The drug dosage was determined by converting the gram weight of the mice into kilograms and multiplying this by 33 mg or 45 mg depending on the experimental category. The drug dosage was multiplied by seven to give a weekly dosage. The dosage was readjusted at intervals to compensate for any body weight changes. All animals were given their daily drug dosage in aqueous solution by stomach intubation.

The treated animals were given 5-TDG (Pfanstiehl Laboratories, Waukegan, Illinois). The control animals were given D-glucose (Sigma).

Intubation

The D-glucose and 5-TDG were given by stomach intubation using a syringe and tubing. The drug was aspirated into a 0.5 cc glass syringe with 0.025 cc graduations. A blunted 20 gauge needle was attached to the syringe and a 25 mm to 30 mm length of polyethylene tubing of appropriate inner diameter was slipped over the blunt end.

Prior to intubating, the mice were etherized to semiconsciousness in a large specimen jar lined with ether-saturated cotton.

The tubing attached to the syringe was inserted behind the central incisors on the premaxillary portion of the upper jaw, along the midline of the hard and soft palate of the oral cavity, on into the posterior portion of the pharynx and then down the esophagus. Once the tubing was inserted into the esophagus, the drug was released into the stomach. The average weight was 33 grams; the average volume of the drug was a $\frac{1}{4}$ cc with the concentration adjusted to a constant dosage of 33 mg/kg.

Weights

Throughout this investigation, animal weights were obtained using a Ohaus Dial-a Gram Balance. In Treatment I weighing was done at the start of the experimental category, the beginning of the breeding period, when the animal bred, then approximately every four or five days during gestation and finally on the day of sacrifice. In Treatment II, weights were taken at the start of this experimental category, when breeding occurred, then every three to four days gestation and finally on the day of sacrifice. Due to the increased dosage of Treatment III, the weights were taken at the start, when breeding occurred, then on alternate days during gestation and finally on the day of sacrifice. From the weights that were recorded for

each treatment, the average maternal weight gain was obtained. Also, by watching the change in weights, sick or pseudopregnant animals were eliminated from the investigation. Only two sick animals had to be eliminated.

Sacrifice of Mice

At designated times, the mice were weighed and sacrificed by cervical dislocation. Dextrostixes were used to approximate blood glucose values in the mice of Treatment III immediately following cervical dislocation. All sacrificed animals were opened by a midventral laporatomy extending from the sternum to the pelvis. The entire uterus with attached oviducts and ovaries were removed, stripped of fat, and examined. A longitudinal incision was made on the medial surface of each uterine horn and its endometrial lining examined for gross anomalies and resorption sites. All fetuses, living and dead, were removed, stripped of fetal membranes, and the umbilical cord was severed near the abdomen. Each fetal crown-rump length was recorded and three to five fetuses from each uterus were weighed. After removal of the oviducts, the ovaries were weighed and checked for any gross abnormalities. The fetuses and ovaries were weighed on a Torsion Balance. All reproductive organs (ovaries and uteri) and fetuses were preserved in 10% formalin.

Glucose Test

The Dextrostix kit by the Ames Company was used to obtain a semi-quantitative value for glucose in whole blood. Blood from the clipped tail of the mouse was applied directly to the reagent area of the Dextrostix strip printed side. At 60 seconds, the blood was washed off the strip using a wash bottle for one to two seconds. The results were read within

one to two seconds after washing. The strip was held close to the color chart, and the value was determined and then recorded. Results were interpolated if the color produced fell between two color blocks on the color chart.

RESULTS AND DISCUSSION

Mating and Pregnancies

5-TDG had no effect on the mating behavior of the experimental mice. Of the three categories (Treatment I, II, and III), only the female mice in Treatment I received any pre-treatment. Those in Treatment I were pre-treated for fourteen days with 5-TDG or D-glucose at a daily dosage of 33 mg/kg and then mated.

Of the experimental animals in Treatment I, 91.67% of the female mice mated and 68.42% of the mated mice became pregnant (Table 2). Of the control animals in this treatment which received D-glucose by intubation instead of 5-TDG, 84.21% of the female mice mated and 68.75% of the mated mice became pregnant. However, there was no significant difference between the treated and control groups.

In Treatment II which had no pre-treatment with either sugar but the same dosage, 52.00% of the treated mice mated and 50.00% of the controls mated. Of the mated mice, 69.23% of the treated became pregnant and 100.00% of the controls became pregnant.

Treatment III, like Treatment II, had no pre-treatment but received a higher dosage--45 mg/kg. Due to fewer available mice, certain mice couldn't be designated as controls or treated mice before mating. Designations had to be made after mating occurred. Mating occurred among 53.49% of the mice and 95.65% of the mated mice became pregnant.

Of the three treatments, the pre-treated groups of Treatment I had higher mating percentages. No adverse effect on mating behavior was expected, and none was observed. Overall, Treatment I and Treatment II's treated groups had the lowest percentage of pregnancies (68.42, 68.75, and

69.23). These percentages compared unfavorably with Rugh's (1968) studies in which 92.4% of his mated mice became pregnant. Neither the time that treatment began nor the dosage used had any significant influence on the rate of pregnancy. These results indicate the need for additional testing to help resolve the conflicting data.

Table 2. Summary of mating and pregnancy percentages from Treatment I, Treatment II and Treatment III.

Treatment		No. Mice	% Plugs	% Pregnant
I	Treated	24	91.67	68.42
	Control	19	84.21	68.75
II	Treated	25	52.00	69.23
	Control	20	50.00	100.00
III		43	53.49	95.65

Maternal Weight

The average maternal weight gains of this study are seen in Table 3. In Treatment I the treated groups (Group A and C) were pretreated for fourteen days prior to breeding and for the subsequent fourteen and eighteen days of gestation, respectively. The control mice (Group B and D) were given D-glucose for a comparable time as their corresponding treated groups. The weight gains of those females sacrificed at day fourteen of gestation were similar in both the control and treated groups; however, the average maternal weight gains of the treated mice (Group C) sacrificed at day eighteen of gestation were approximately one-half that seen in their

Table 3. Summary of maternal weight gain (grams) averages for Treatment I, Treatment II and Treatment III.

Treatment	Group	Designation	Gestation	Average
I	A	Treated	14 Days	9.6
	B	Control	14 Days	10.7
	C	Treated	18 Days	12.4
	D	Control	18 Days	23.1
II	E	Treated	14 Days	7.7
	F	Control	14 Days	8.9
	G	Treated	18 Days	23.5
	H	Control	18 Days	25.4
III	I	Treated	14 Days	7.7
	J	Control	14 Days	5.8

respective control group (Group D). This difference in weight gains is a reflection of fetal losses between day fourteen and eighteen of gestation. When treatment began immediately following breeding, as in Treatment II, there was no significant difference in maternal weight gains either by sugar treatment or by length of gestation. A higher dosage of 5-TDG (45 mg/kg in Treatment III), after breeding had occurred, appeared to have no effect on the average maternal weight gains at sacrifice on day fourteen of gestation. Maternal weight gains were similar in the three treatment groups at fourteen days of gestation (Groups A, E, I). The average weight gains of the control groups at fourteen days (Groups B, F, and J) were 10.7, 8.9 and 5.8 grams, respectively.

Upon one-way ANOV, Group C's maternal weight gain was significantly lower at the 5% level than its control Group D (Appendix C). Since no other groups varied significantly, it is assumed that the extended period of time exposed to 5-TDG accounted for this significance seen in Group C.

Ovarian Weight

Ovarian weights were compared by both the length of gestation and the possible effects of 5-TDG versus D-glucose. A summary of the average ovarian weights is seen in Table 4. The average ovarian weight of those control animals sacrificed on day fourteen of gestation was 17.3, 18.5, and 17.1 milligrams versus 15.0, 16.3, and 14.9 milligrams for those receiving 5-TDG through day fourteen of gestation. Although the ovaries of the latter groups were slightly lighter in weight, these differences were not significant either between the controls and their respective treated groups or between treatment groups at fourteen days of gestation.

Table 4. Summary of averages for ovarian weights (mgs.) from Treatment I, Treatment II and Treatment III.

Treatment	Group	Designation	Gestation	Average
I	A	Treated	14 Days	15.0
	B	Control	14 Days	17.3
	C	Treated	18 Days	15.0
	D	Control	18 Days	19.5
II	E	Treated	14 Days	16.3
	F	Control	14 Days	18.5
	G	Treated	18 Days	20.5
	H	Control	18 Days	24.8
III	I	Treated	14 Days	14.9
	J	Control	14 Days	17.1

Using two-way ANOV, there was no significant difference in the ovarian weights of Treatment I's animals either between the fourteen and eighteen day gestation groups or in the interaction of the sugars and days of gestation (Appendix D). There was a significant difference in the ovarian weights between the 5-TDG and D-glucose at the 5% level. The ANOV testing showed no significant difference between the groups in Treatment II and III.

Fetuses

Fick (1978) reported the average range in litter size for ICR mice to be 10 to 15 young. The control groups of Treatments I and II were within the range; however, the mice in the control group of Treatment III had an average of 4.6 fetuses per litter. There is no explanation for this discrepancy. The effect of 5-TDG is most evident when comparing the average number of fetuses seen in the treated and control groups of Treatment I (Table 5). Testing by two-way ANOV showed a significant difference at the 5% level in the number of fetuses between the 5-TDG and D-glucose groups for Treatment I but not for those of Treatment II (Appendix E). This indicates an adverse effect of 5-TDG when administered fourteen days prior to and during gestation.

A significant difference at the 5% level between the number of fetuses in the treated and control animals of Treatment III came as a surprise. Originally it was thought that increasing the dosage of 5-TDG to 45 mg/kg would possibly have an adverse effect on early embryonic survival. It did not. More surprisingly the small litter sizes of those given 45 mg/kg of D-glucose was unexpected. This level of D-glucose was not considered to be diabetogenic nor was such a condition indicated when checked by the

Table 5. Summary of averages for the number of fetuses from Treatment I, Treatment II and Treatment III.

Treatment	Group	Designation	Gestation	Average
I	A	Treated	14 Days	7.4
	B	Control	14 Days	9.8
	C	Treated	18 Days	2.8
	D	Control	18 Days	14.2
II	E	Treated	14 Days	10.8
	F	Control	14 Days	9.0
	G	Treated	18 Days	11.6
	H	Control	18 Days	13.4
III	I	Treated	14 Days	10.1
	J	Control	14 Days	4.6

semi-quantitative method used for the glucose test.

Resorptions

The number of resorptions is used as one of the criteria for the effects of drugs on embryonic development. In general the animals given D-glucose had fewer resorptions (Table 6). The average number of resorptions for four of the five control groups lie well within the expected range for resorptions at fourteen and eighteen days of gestation. Two-way ANOV was used to determine that there was a significant difference at the 5% level in the number of resorptions between 5-TDG and D-glucose groups of Treatment I (Appendix F).

When this study was initiated, it was thought that 5-TDG would signficiantly interfere with endogenous D-glucose needed for life support functions of the fetuses. This presumed interference would result in an

Table 6. Summary of averages for the number of resorptions from Treatment I, Treatment II and Treatment III.

Treatment	Group	Designation	Gestation	Average
I	A	Treated	14 Days	2.6
	B	Control	14 Days	0.8
	C	Treated	18 Days	3.4
	D	Control	18 Days	0.2
II	E	Treated	14 Days	2.4
	F	Control	14 Days	1.8
	G	Treated	18 Days	1.6
	H	Control	18 Days	0.8
III	I	Treated	14 Days	1.4
	J	Control	14 Days	3.0

increase in fetal resorptions. This assumption was not supported by the above data.

Crown-Rump Length

One-way ANOV testing showed no significant difference in the fetal crown-rump length for all three treatments (Appendix G). All the fetal crown-rump lengths (Table 7) were well within the normal range of 10.0-11.5 mm for fourteen days of gestation and 16.0-26.0 mm for eighteen days of gestation as described by Rugh (1968). 5-TDG had no significant effect on the crown-rump lengths of the developing fetus.

Table 7. Summary of fetal crown-rump length (mm) averages for Treatment I, Treatment II and Treatment III.

Treatment	Group	Designation	Gestation	Average
I	A	Treated	14 Days	9.94
	B	Control	14 Days	10.02
	C	Treated	18 Days	NA
	D	Control	18 Days	19.86
II	E	Treated	14 Days	10.54
	F	Control	14 Days	9.18
	G	Treated	18 Days	22.54
	H	Control	18 Days	25.52
III	I	Treated	14 Days	10.06
	J	Control	14 Days	10.38

Fetal Weight

No significant difference in the fetal weights for all three treatments was demonstrated using one-way ANOV (Appendix H). The weights were well within the normal range of 99-273 mg for fourteen days of gestation and 707-1860 mg for eighteen days of gestation (Rugh, 1968). In fact many of the control and treated groups had averages within several milligrams of each other (Table 8). Treatment III had the highest weights overall, but these weights were within the upper portion of the range.

Several factors are associated with a successful pregnancy. Changes must take place within the uterus. Many of these are under hormonal control--mainly steroids. One of the major changes is the proliferation of cells in the endometrium. Thus, mitosis is heightened, vascularity is increased, and this is documented in an increased metabolic activity of the

Table 8. Summary of averages for fetal weights (mg.) from Treatment I, Treatment II and Treatment III.

Treatment	Group	Designation	Gestation	Average
I	A	Treated	14 Days	NA
	B	Control	14 Days	202.5
	C	Treated	18 Days	NA
	D	Control	18 Days	1169.3
II	E	Treated	14 Days	162.6
	F	Control	14 Days	190.3
	G	Treated	18 Days	1141.9
	H	Control	18 Days	1160.3
III	I	Treated	14 Days	271.9
	J	Control	14 Days	282.7

female reproductive tract. Immediately following conception, additional metabolic activity is rapidly taking place during the three to five days the egg is unattached. When the embryo attaches (implants) at or near the end of the blastula stage, the uterine mucosa undergoes rapid proliferation particularly at the site of attachment. The maternal portion of the placenta begins forming at this time. Meanwhile, the zygote has undergone several generations of mitosis and is at the stage of gastrulation at the time placentation begins. Rapid fetal cell differentiation and growth follows. By day fourteen of gestation in the mouse, the tissue has become organs and organ systems.

It can be seen from the above facts that any substance that has a suppressive effect on mitosis, metabolism and glucose would result in lowered reproductive performance. Since it has been reported that 5-TDG

inhibits cancer cell growth and it is known to stop spermatogenesis, this substance may lend itself to use as a non-toxic, non-hormonal contraceptive.

The mouse was the animal model chosen to test the above concept. It has been well studied, has a short estrous cycle, and a gestation period of twenty-one days. An additional advantage is its litter size. The gradient effect of any experimental drug on reproductive performance can best be determined in those animals with large litters. The DUB (ICR) strain of mice satisfies these criteria.

Pre-treatment with 5-TDG prior to breeding causes a lowered reproductive performance (See Treatment I in Appendix C, D, E, and F). When single factor ANOV was used to test maternal weight gain, Treatment I was significantly different at day eighteen of gestation. When two factor ANOV was used, to test maternal weight gain, number of fetuses and resorptions, the significant difference occurred between the sugars. There was no significant difference between the gestation periods or interaction of the sugars and gestation periods. In the statistical analyses, the groups of Treatment I differed significantly, at the 5% level for all parameters studied except fetal crown-rump length and fetal weight. The lack of significance of crown-rump length and fetal weight would seem to indicate that where the number of fetuses are reduced the nutrients available is shared by fewer but healthier animals than if all conceived had survived. Obviously, the maternal weight gains are less in those females with fewer fetuses and more resorptions (Group C). The fourteen day pretreatment period appears to be the most significant factor affecting embryonic survival, and

this effect becomes more apparent in the latter part of the gestation period (See Groups A and C, Appendix E).

The ovarian weights were remarkably uniform in the control groups (Groups B, F, J); in fact, the major change in ovarian weights was in Group C that had been pretreated fourteen day and continued on 5-TDG until day eighteen of gestation. The ovaries are indispensable for the maintenance of pregnancy in the mouse. The reduction in ovarian weight in Group C may indicate a decline in the number of ovarian cells or corpora lutea and inadequate hormonal activity needed by this species during pregnancy. Further study is desirable. Reduced ovarian weight in females treated with 5-TDG parallels reduced testicular weight in males treated with 5-TDG. In males, a testicular weight drop has been observed due to a decrease in the number of testicular cells (Hoffman and Whistler, 1968).

Numerous possibilities exist as to the reduced number of fetuses and increased resorptions as seen in Group C. 5-TDG may have inhibited both active and facilitated diffusion transport of D-glucose as well as D-xylose (Whistler and Lake, 1972); therefore, it would decrease the amount of glucose available for development. Also, another factor contributing to these results could be 5-TDG's interference with glycogen utilization by non-competitive inhibition of phosphorylase a and b and by inactivation of phosphorylase a (Chen and Whistler, 1977). Thus, 5-TDG could have prevented the utilization of glycogen as an alternate energy source. Even though 5-TDG's effect is short termed--probably 3 to 6 hours--its effect was long enough to interfere with glucose utilization and caused these results. More dramatic results might be attainable with 5-TDG by giving

it in a slow time-released form; therefore, 5-TDG's short-term effect would be counteracted, and 5-TDG's effect would be more continuous and constant.

SUMMARY

5-TDG was administered by gastric intubation to female mice in three categories referred to as Treatment I, Treatment II and Treatment III. The controls of each category received D-glucose. Within the treated and control groups of each category, there were sub-divisions based on the length of gestation. The length of gestation was either fourteen or eighteen days.

Treatment I consisted of the daily administration of 5-TDG and D-glucose during a fourteen day pre-treatment period prior to breeding, continuing with a breeding period and ending with fourteen or eighteen days of gestation. Of the four groups in Treatment I, there were two treated (Group A and C) and two control groups (Group B and D). Group A and B were sacrificed on day fourteen of gestation. Group C and D were sacrificed on day eighteen. The daily dosage was 33 mg/kg.

Treatment II was like Treatment I except for the lack of a treatment period. The sugars were given only after breeding had occurred. Its treated groups were Group E and G, and the controls were Group F and H. Group E and F had a gestation length of fourteen days. Group G and H gestated for eighteen days.

Treatment III, like Treatment II, did not have any pre-treatment period, but were given a dose of 45 mg/kg. Treatment III had only one treated (Group I) and one control group (Group J). Group I and J were sacrificed on day fourteen of gestation.

The results showed that pre-treatment with 5-TDG caused changes in the majority of the experimental parameters. The administration of 33 mg/kg of 5-TDG caused a significant reduction in maternal weight gains

and a reduction in ovarian weights. It also significantly reduced the number of fetuses and increased the number of resorptions. It was not significant for fetal crown-rump length and fetal weight. Treatment II was not significantly different at any level for any of the experimental parameters. In Treatment III, only the number of fetuses in Group J was significant at the 5% level.

The decreased maternal weight gain of Treatment I was significant at the eighteen day gestation period. This difference in weight gains is a reflection of fetal losses between day fourteen and eighteen of gestation. It is assumed that the extended exposure to 5-TDG accounted for this significance seen in Group C. The significant difference seen in ovarian weights, the number of fetuses and the number of resorptions in Treatment I occurred between the sugars not between the gestation periods or between the interaction of the sugars and gestation periods. The decreased ovarian weight and number of fetuses and increased resorptions indicate other adverse effects of 5-TDG when administered fourteen days prior to and during gestation. These adverse effects could be the result of decreased hormonal activity, fewer ovarian cells, decreased glucose and/or glycogen caused by 5-TDG. The decreased maternal weight and ovarian weight parallel the results obtained with male mice and rats.

Treatment III had a significant decrease in the number of fetuses in control Group J. Since glucose testing demonstrated that the mice in this treatment weren't diabetic, a reasonable explanation cannot be given for these results.

APPENDIX A

Materials for Maintenance and Treatment of Animals

Half and Half Mixture of the following two feeds:

Wayne Lab-Blox

Crude protein	(minimum)	24.0%
Crude fat	(minimum)	4.0%
Crude fiber	(maximum)	4.5%

The ingredients of the food included animal liver meal, fish meal, dried whey, corn and wheat flakes, ground yellow corn, ground oat groats, soybean meal, brewers dried yeast, and vitamin supplements.

Wayne Mouse Breeder Blox

Crude protein	(minimum)	20.0%
Crude fat	(minimum)	10.0%
Crude fiber	(maximum)	2.0%

The ingredients of the food included ground wheat, dried skim milk, fish meal, soybean oil, brewers dried yeast, animal fat preserved with BHA and vitamin supplements.

Both obtained from: Gransville Milling Company
Creedmore, North Carolina

APPENDIX B

Stages in the rat estrous cycle and corresponding cell types in the vaginal lavage (Rugh, 1968).

STAGES OF CYCLE	DESCRIPTION
Diestrus	Lavage is dominated by leukocytes. Functional regression of corpora lutea is occurring 60-70 hours.
Proestrus	Lavage contains nucleated epithelial cells. Involution of corpea lutea occurring.
Estrus	Lavage is filled with squamous and cornified epithelial cells with degenerate nuclei. Follicles grow rapidly. Ovulation occurs. Copulation permitted 9 to 15 hours.
Metestrus	Lavage is a mixture of many leukocytes with a few cornified epithelial cells. It is after ovulation occurs. Follicles are small. No mating occurs. 10 to 14 hours.

APPENDIX C

Summary of single factor ANOV F-values for maternal weight gains obtained from Treatment I, Treatment II and Treatment III.

Treatment	Gestation Length	
	18 Days	14 Days
I (a)	5.75*	0.72
II (a)	0.27	0.38
III (b)		1.57

(a) $F_{0.05\ (1)\ 1,\ 8} = 5.32$

(b) $F_{0.05\ (1)\ 1,\ 17} = 4.45$

*Significantly different at the 5% level.

APPENDIX D

Summary of F-values for ovarian weights obtained from Treatment I, Treatment II and Treatment III.

Factors	Two-Factor Analysis of Variance Treatment I [a]
Sugars: Treated vs. Control	7.30*
Gestation: 18 Days vs. 14 Days	2.70
Interaction: Sugars vs. Gestation	0.39

Factors	Single Factor Analysis of Variance		
	Treatment II		Treatment III
	18 Days [b]	14 Days [c]	14 Days [d]
Sugars: Treated vs. Control	2.07	5.02	1.31

(a) $F_{0.05\ (1)\ 1,\ 16} = 4.49$

(b) $F_{0.05\ (1)\ 1,\ 8} = 5.32$

(c) $F_{0.05\ (1)\ 1,\ 7} = 5.59$

(d) $F_{0.05\ (1)\ 1,\ 15} = 4.45$

*Significantly different at the 5% level.

APPENDIX E

Summary of F-values for the number of fetuses obtained from Treatment I, Treatment II and Treatment III.

Factors	Two-Factor Analysis of Variance	
	Treatment I[(a)]	Treatment II[(a)]
Sugars: Treated vs. Control	17.35*	0.01
Gestation: 18 Days vs. 14 Days	4.49	3.14
Interaction: Sugars vs. Gestation	6.33	1.81

Factors	Single Factor Analysis of Variance
	Treatment III[(b)]
Sugars: Treated vs. Control	4.95*

(a) $F_{0.05\ (1)\ 1,\ 16} = 4.49$

(b) $F_{0.05\ (1)\ 1,\ 15} = 4.45$

*Significantly different at the 5% level.

APPENDIX F

Summary of F-values for the number of resorptions obtained from Treatment I, Treatment II and Treatment III.

Factors	Two Factor Analysis of Variance	
	Treatment I[(a)]	Treatment II[(a)]
Sugars:		
Treated vs. Control	5.30*	0.85
Gestation:		
18 Days vs. 14 Days	0.008	1.41
Interaction:		
Sugars vs. Gestation	0.42	0.02

Factors	Single Factor Analysis of Variance
	Treatment III[(b)]
Sugars:	
Treated vs. Control	0.99

(a) $F_{0.05\ (1)\ 1,\ 16} = 4.49$

(b) $F_{0.05\ (1)\ 1,\ 15} = 4.45$

*Significantly different at the 5% level.

APPENDIX G

Summary of single factor ANOV F-values for crown-rump lengths obtained from Treatment I, Treatment II and Treatment III.

Treatment	Gestation Length	
	18 Days	14 Days
I (a)	(b)	0.09
II (a)	0.10	0.47
III (a)		0.46

(a) $F_{0.05\ (1)\ 1,\ 8} = 5.32$
(b) Not adequate data

APPENDIX H

Summary of single factor ANOV F-values for fetal weights obtained from Treatment I, Treatment II and Treatment III.

Treatment	Gestation Length	
	18 Days	14 Days
I(a)	(b)	0.00028
II (a)	0.01	0.47
III (a)		0.48

(a) $F_{0.05\ (1)\ 1,\ 8} = 5.32$
(b) Not adequate data

BIBLIOGRAPHY

Babbott, D., A. Rubin, and S. J. Ginsburg. 1958. The reproductive characteristics of diabetic men. Diabetes 7:33-35.

Banks, P., W. Bartley, and L. M. Birt. 1976. Biochemistry of the Tissues. John Wiley & Sons, New York.

Barnett, J. E. G., A. Ralph and K. A. Munday. 1970. Structural requirements for active intestinal transport. Biochem. J. 118:843-850.

Biggers, J. D. 1970. Metabolism of mouse embryos. J. Reprod. Fert. 14:41-55.

Biggers, J. D., D. G. Whittingham and R. P. Donahue. 1967. The pattern of energy metabolism in the mouse oocyte and zygote. Proc. Natn. Acad. Sci. 58:560-567.

Boell, E. J. and J. S. Nicholas. 1948. Respiratory metabolism of the mammalian egg. J. Exptl. Zool. 109:267-281.

Brinster, R. L. and J. L. Thomson. 1966. Development of the eight cell mouse embryos in vitro. Exptl. Cell Res. 42:303-315.

Bushway, A. A. and R. L. Whistler. 1975. Repression of cancer cell growth 5-thio-D-glucose. J. Carbohydrate Nucleosides Nucleotides. 2:339-405.

Bushway, A. A., R. L. Whistler, and R. D. Myers. 1977. Effect of 5-thio-D-glucose on food and water intakes and on acquisition and performance of maze tasks in the rat. Physiol. Ben. 19:249-253.

Chen, M. and R. L. Whistler. 1975. Action of 5-thio-D-glucose and its 1-phosphate with hexokinase and phosphoglucomutase. Arch. Biochim. Biophys. 169:392-396.

Chen, M. and R. L. Whistler. 1977. Action of 5-thio-D-glucose in the control of glycogen depolymerization. Biochim. Biophys. Res. Comm. 74:1642-1646.

Critchley, D. R., A. Eichholy and R. K. Crane. 1970. Transport of 5-thio-D-glucose in hamster small intestine. Biochim. Biophys. Acta. 211: 244-254.

Daniel, J. C. 1967. The pattern of utilization of respiratory metabolism intermediates by pre-implantation rabbit embryos in vitro. Exptl. Cell Res. 47:619-623.

Davis, J. R. 1969. Metabolic aspects of spermatogenesis. Biol. Reprod. 1: 93-118.

Feather, M. S. and R. L. Whistler. 1962. Derivatives of 5-deoxy-5-mercapto-D-glucose. Tetrahedron Letters. 15:667.

Fick, Ken. 1978. The Effect of 5-TDG on Reproduction in Male Mice: Histological, Histochemical, and Fertility Studies. Master's Thesis. East Carolina University, Greenville, N.C.

Folgia, V. G., R. F. Borghelli, R. A. Chieri, L. L. Fernandez-Collazo; I. Spindler, and O. Wesely. 1963. Sexual disturbances in the diabetic rat. Diabetes. 12:231-237.

Hellman, B., A. Lernmark, J. Schlin, I. Taljedal, and R. L. Whistler. 1972. The pancreatic beta-cell recognition of insulin scretagogues. Biochim. Pharmacol. 22:29-35.

Hoffman, D. J. and R. L. Whistler. 1968. Diabetogenic action of 5-thio-D-glucopyranose in rats. Biochemistry. 7:4479-4483.

Homm, R. E., C. Rusticus, and D. W. Hahn. 1977. The antispermatogenic effects of 5-thio-D-glucose in male rats. J. Reprod. 17:697-700.

Klebanow, D. and J. Mac Cleod. 1960. Semen quality and certain disturbances of reproduction in diabetic man. Fert. Steril. 11:255-261.

Kleinzeller, A. 1970. The specificity of the active sugar transport in kidney cortex cells. Biochim. Biophys. Acta. 211:264-276.

Kleinzeller, A., J. Kolenska and I. Benes. 1967a. Transport of glucose and galactose in kidney-cortex cells. Biochem. J. 104:843-851.

Lobl, T. J. and S. E. Porteus. 1978. Antifertility activities of 5-thio-D-glucose in mice and rats. Contraception. 17:123-130.

Maugh, T. H. 1974. 5-Thio-D-glucose: A unique male contraceptive. Science. 186-431.

Needham, J. 1931. Chemical Embryology. Cambridge University Press, New York.

Paranjpe, S. V. and V. Jagannathan. 1971. 5-Thio-D-glucose inhibition of hexokinase from ox heart. Indian J. Biochem. 8:227-236.

Rugh, R. 1968. The Mouse, Its Reproduction, and Development. Burgess Publishing Co., Minneapolis, Minnesota.

Schoffling, I., K. Federlin, H. Ditschuneit, and E. F. Pfeiffer. 1963. Disorders of sexual function male diabetics. Diabetes. 12:519-527.

Segal, S., S. Thier, M. Fox and L. Rosenberg. 1962. Inhibitory effect of sugars on amino acid accumulation in rat kidney cortex. Biochim. Biophys. Acta. 65:567-568.

Shankland, D., J. H. Stark, and R. L. Whistler. 1968. The effect of 5-thio-D-glucose on insect development. J. Insect Physiol. 14:63-72.

Sugawara, S. and M. Umezu. 1961. Studies on metabolism of the mammalian ova. II. Oxygen consumption of the cleaved ova of the rat. Tokoku J. Agricultural Res. 12:17-28.

Suzuki, M., R. L. Whistler. 1972. 1, 2, 3, 4, 6-Penta-O-acetye-t-thio-D-glucopyranose. Carbohyd. Res. 22:473-476.

Thier, S., M. Fox, L. Rosenberg and S. Segal. 1964. Hexose inhibition of amino acid uptake in the rat kidney cortex. Biochim. Biophys. Acta. 93:106-115.

Wales, R. G. 1969. Accumulation of carboxylic acids from glucose by the pre-implantation mouse embryo. Aust. J. Biol. Sci. 22:701-707.

Wales, R. G. and R. L. Brinster. 1968. The uptake of hexoses by pre-implantation mouse embryos in vitro. J. Reprod. Fert. 15:415-422.

Wales, R. G. and D. G. Whittingham. 1967. A comparison of uptake and utilization of lactate and pyruvate by one and two-cell mouse embryos. Biochim. Biophysiol. Acta. 148:703-712.

Whistler, R. L. and D. J. Hoffman. 1968. Diabetogenic action of 5-thio-glucopyranose in rats. Biochemistry. 7:4479-4483.

Whistler, R. L. and W. C. Lake. 1972. Inhibition of cellular transport processes by 5-thio-D-glucopyranose. Biochem. J. 130:919-925.

Whitten, W. K. 1956. Culture of tubal mouse ova. Nature. 177:96.

Whitten, W. K. 1957. Culture of tubal mouse ova. Nature. 179:1081.

Zysk, J. R., A. A. Bushway, R. L. Whistler, and W. W. Carlton. 1975. Temporary sterility produced in male mice by 5-thio-D-glucose. J. Reprod. Fert. 45:69-72.