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REPRODUCTIVE AND MUTAGENIC EFFECTS OF 5-THIO-D-GLUCOSE INJECTIONS IN MALE MICE, *MUS MUSCULUS*, ICR STRAIN

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REPRODUCTIVE AND MUTAGENIC EFFECTS OF 5-THIO-D-GLUCOSE INJECTIONS IN MALE MICE, *MUS MUSCULUS*, ICR STRAIN

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TABLE OF CONTENTS

ABSTRACT

Adult male ICR mice of proven fertility were intraperitoneally injected daily with 60 mg/kg of 5-thio-D-glucose for 28 days. Body weights of treated male mice were unaffected during the experimental period. Relative testicular weights were significantly reduced at 121 days after initial injections in 5-thio treated males, but no significant decrease was observed at 28 days.

Onset of sterility was estimated to occur at approximately 4 weeks and persisted for 5 to 6 weeks after cessation of treatment at which time fertility was restored.

Beginning with the first injections and continuing for 121 days, the males were mated with untreated ICR female mice of proven fertility. Gross examinations of mouse embryos and fetuses were made at 18 days of gestation. It was repeatedly shown that there was a significant decrease in mean litter size in both pre- and post-sterility periods. Only minor fetal anomalies were found in treated and control groups. No significant difference was observed in frequencies of malformations or resorptions. However, in the post-sterility period, a significant decrease in normals and an increase in dead fetuses were observed.

Histological examination of 5-thio treated and control testes was made at 28 and 121 days. Testes of

treated mice at 28 days revealed giant multinucleated cells and a number of seminiferous tubules with no spermatids or mature sperm. At 121 days, testes of treated males had only partially recovered. However, many normal tubules with mature sperm were evident.

Results from this study indicate that 5-thio-D-glucose does cause temporary sterility in male ICR mice and that the compound may be mutagenic.

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INTRODUCTION

Unique responses are induced by 5-thio-D-glucose (5-thio) in *in vivo* and in *in vitro* systems. It was synthesized by Feather & Whistler (1962) and is structurally similar to its nearest analog D-glucose (Fig. 1). Because of this similarity, it fits in the active transport system and competitively inhibits transport of D-glucose across cellular membranes (Whistler & Lake, 1972). However, only a minute amount of 5-thio is metabolized and over 90% is rapidly excreted unchanged through the renal system (Pitts *et al.*, 1975). This drug is considered as a potential male contraceptive and carcinostatic agent (Bushway & Whistler, 1975; Song *et al.*, 1976, 1977; Kim *et al.*, 1978).

Pharmacokinetics and biodistribution studies show a remarkable uptake of 5-thio into organs throughout the test animal within 5 minutes, with highest levels in the kidney, liver and blood (Markoe *et al.*, 1979). In spite of the low level of concentration, the only organ apparently affected by this drug is the testis (Risch, 1979). Histopathological changes in the testes were first observed by Zysk *et al.* (1975) when sterility was induced in mice. After discontinuation of treatment, fertility was restored within 5 to 8 weeks .

All research involving progeny of 5-thio treated male mice inclusively have indicated that average litter size

was unaffected, gross anatomy of fetuses was normal and lethalities were not elevated in treated groups (Zysk et al., 1975; Lobl & Porteus, 1978; Majumdar et al., 1979a, c). Conflicting results were presented by Homm et al. (1977) in a rat study; average litter size was reduced 65% after cessation of treatment. However, the authors made no explanation concerning the reduced litter size.

Because of the possibility of this compound being utilized as a reversible male contraceptive, the question arises as to whether 5-thio is mutagenic either during the period of arrest of spermatogenesis or after recovery of fertility. To date, only two mutagenicity studies have been presented (Majumdar et al., 1979b, c) with the conclusion that 5-thio is non-mutagenic. Nevertheless, negative results should not be considered final in mutagen testing (Auerbach, 1967). Positive results indicating dominant lethal mutations induced in the male germ cell by a steroidal oral contraceptive were published recently (Hemsworth, 1979). Further, Evans & Harbison (1977) showed the mutagenicity of a compound, rubratoxin B, by an increased number of early fetal deaths.

The purpose of the present investigation was to further study the possibility of mutagenicity of 5-thio in the germ cells of the male ICR mouse. Design of the experiment enhanced the opportunity for detection of mutations in the germ cells. All the reproductive data of

previous mice studies were derived only from matings after the male mice had recovered fertility. In the present study, female mice were continuously provided for mating with each male mouse during the pre-sterility period as well as the post-sterility period. Therefore, implantation data were obtained from both periods.

The present study also involved a different strain of mice, not previously tested, and an evaluation of the testis was made, histologically, to determine the effect of 5-thio on the ICR mouse testis.

MATERIALS AND METHODS

Eighteen male and 88 female mice (Mus musculus, ICR strain), at 6 weeks of age, were obtained from Flow Laboratories, Dublin, Virginia. The weight range for the male and female mice was between 37.2-41.6 g and 26.8-34.5 g, respectively. The mice were caged and received food (Rodent Laboratory Chow, Ralston Purina Co., St. Louis, Missouri) and water ad libitum. The temperature ranged from 22 to 24 C. Six fluorescent light tubes, positioned on a rack approximately 5 feet from the animal cages, were used to provide lighting which was automatically timed for 14 h light and 10 h dark.

As this experiment involved the impregnation of female mice and fetal and embryo examination, both male and female mice had to be of proven fertility. Therefore, these mice were allowed to mate and in the process, both males and females were simultaneously proven as the females became impregnated and dropped one litter. Each male mouse was able to successfully impregnate 4 females. The length of the fertility test period was 5 weeks.

Twelve of the 18 male mice, now at 11 weeks of age, received daily intraperitoneal injections for 28 days of 60 mg/kg of body weight of 5-thio-D-glucose (5-thio) (Pfanstiehl Laboratories, Waukegan, Illinois) in Dulbecco's phosphate buffered saline (PBS) (Altman & Dittmer, 1972). Six control male mice were intraperitoneally injected with

a similar volume of PBS for 28 days. Eight of the 5-thio treated males and 4 of the PBS treated males were used in the reproduction study and for testicular histology; whereas, the remaining four 5-thio treated males and 2 PBS treated males were only used for testicular histology at 28 days after initial injections. The former groups were used for testicular histology at 121 days after the initial injections. Therefore, testes of male mice were examined at 28 and 121 days after first injections in order to histologically determine the state of the testis.

The 5-thio dosage and treatment schedule used in this study was chosen because it was shown by Majumdar et al. (1979a) that this regimen induced total sterility in male STS mice. After the injections were terminated, for the remaining recovery period of 93 days, body weights of the male mice were taken twice each week in the present study. The entire experimental period was 121 days.

At the commencement of injections, 3 female mice, 11 weeks of age, were placed in each cage with a male for mating. When it appeared that a female was approximately at 18 days of gestation, it was removed from the cage and another proven female mouse replaced the gravid female so that a consistent number of females in each cage could be maintained. The gravid female was killed by cervical dislocation and an abdominal incision was made to expose the

uteri which were surgically excised and placed in a petri dish filled with water for examination. Evaluation of the litters prenatally rather than postnatally was decidedly more accurate as female mice tend to destroy any defective or dead embryos or fetuses at birth. In certain cases, the litters were delivered by the female mouse. Implantation data from these particular females were used only if the number of neonatals correlated with the number of metrial glands. These glands which appeared as highly vascular yellowish nodules at the base of the placental attachment, were indicative of all original implantation sites, whether the embryos survived or not.

A consistent system was utilized in which all fetuses were numbered and position in the uteri was recorded. Each time, examination of the fetuses began at the right ovary and proceeded to the left. Each fetus was listed serially and a line indicated where the cervix appeared and therefore, numbers of implantations for each uterine horn were determined (Fig. 2). The table and figure involving the frequencies of resorptions, deaths and anomalies in fetuses relative to intrauterine position were designed after those in Ward et al. (1977). The uteri were divided into ovarian, upper middle, middle, lower middle and cervical regions and the percentage of resorptions, deaths and anomalies were determined for each region and for all positions of the uteri.

Each fetus was placed on blotting paper to remove excess amniotic fluid and blood, then weighed to the nearest 0.01 g. External examination of each fetus began at the cranial region and ended at the caudal region. The contour of the head and mouth was observed for abnormalities such as hydrocephaly and micrognathia (shortened mandible). Cleft palate was investigated by insertion of forceps into the fetal mouth and forcing apart the jaws. Ocular malformations such as microphthalmia and anophthalmia and external central nervous system defects including exencephaly and spina bifida were investigated. Anomalies of the trunk region, e.g. umbilical hernia in which loops of the small intestine protrude through an abdominal opening and anomalies of the caudal vertebrae, such as a shortened or curly tail were investigated. Forelimbs and hindlimbs were examined for shortened or malformed appearance and digits were routinely counted to determine such digital malformations as polydactyly, syndactyly and ectrodactyly.

Stunting was identified quantitatively and qualitatively. As not all litters were exactly at 18 days of gestation when examined, average weights of the litters could not be effectively compared. Each litter was handled as a separate entity and each fetal weight was compared with the average fetal weight for that particular litter and any fetus weighing less than 65% of the average was considered to be stunted. Discolorations of

the skin were considered as suggestive of internal hemorrhage. Dead fetuses were easily identified by lack of movement and a whitish coloration. Resorptions were also recorded and these signified dead early conceptuses. Any abnormal fetus was preserved in Bouin's fixative and later photographed or illustrated.

In order to prepare the testes of 5-thio and PBS treated mice for histology at 28 and 121 days, mice were weighed, killed by cervical dislocation and the right testis was removed, weighed and placed in Bouin's fixative for several days. Testes were then placed in a series of dehydrating reagents, from 35% to 100% ethanol and xylene. The tissue was infiltrated and embedded in Paraplast Tissue Embedding Medium (Sherwood Medical Industries, Inc., St. Louis, Missouri). All tissue was sectioned at 8 μm and attached to slides with egg albumin. Slides were allowed to completely dry then the sections were rehydrated and stained with Delafield's hematoxylin for 22 minutes. Acid water containing HCL destained the slides and lithium carbonate was used to counteract the destaining process; dehydration of sections was repeated. Balsam was used to seal the coverslips.

Two slides of each testis was thought to be sufficient to reveal the microanatomical state of that testis. Each slide bore approximately 10 tissue sections, each having ample seminiferous tubules for microscopic examination. Testes from the 5-thio treated mice were compared with

the testes of the PBS treated mice and deviations from the normal were recorded and photographed.

Statistical analysis of body weight, testicular weight and litter size was performed by the two-tailed 'Student's' t-test. The remainder of the data which involved fused placentae, normal, dead, resorbed and anomalous fetuses were statistically analyzed with the binomial test. Confidence levels were at 95%.

RESULTS

While the mean body weights of all male mice gradually increased throughout the experiment, there were no differences in body weight attributed to treatment with 5-thio (Table 1).

Treatment of male mice with 5-thio produced a slight reduction in relative testicular weight below the control value at 28 days and a significant decrease at 121 days (Table 2). When the control ratio of testis weight to body weight was represented as 100%, the treated group values declined to 89.2% at 28 days and 59.2% at 121 days.

Initially, the female mice were impregnated by PBS as well as 5-thio treated mice and the pregnancies were first evident in both groups approximately 18 days after females were placed in the male cages. Mice treated with PBS were able to continually impregnate the females with no interruption, throughout the experimental period. However, sterility in the experimental mice began to appear after 4 weeks of treatment. These mice remained infertile for the subsequent 5 weeks at which time fertility was regained. In one case, however, a male did not recover from sterility within the experimental period and the remaining females in that particular cage were not impregnated.

The results for the implantation examinations for

the entire experimental period are summarized in Table 3. A total of 88 impregnated females (56 from 5-thio treated males and 32 from PBS treated males) were dissected and 1153 implantations were examined. Mating of 5-thio treated male mice to normal female mice resulted in a significant decrease in mean litter size. The mean litter size for the experimentals and controls were 12.27 and 14.56, respectively.

Table 4 compares litter size from pre-sterility and post-sterility periods with litter size of the control group. These data reveal that the mean litter size from pre-sterility males ($\bar{x}$ = 12.68) and post-sterility males ($\bar{x}$ = 11.25) were both significantly smaller than the mean litter size from control males ($\bar{x}$ = 14.56).

Treatment of male mice with 5-thio did not result in significant increases in the numbers of resorbed, dead or anomalous fetuses (Table 3). Even though there were no significant changes in the above parameters, there were suggestions that the litters from 5-thio treated males had more defects than the litters from control males. For example, there were fewer litters from the 5-thio treated males having 100% normal fetuses (Table 5). In addition, the frequency of litters having resorptions was higher in the 5-thio group (Table 6). The frequency of anomalies was higher in the 5-thio group (Tables 3 and 7; Figs. 3 and 4).

Table 4 compares resorptions, deaths and anomalies

in litters from 5-thio treated males in the pre-sterility and post-sterility periods with the same parameters in litters from control males. Significant differences were observed in the post-sterility period. Normal fetuses were decreased and dead fetuses were increased in the 5-thio group. Anomalies tended to be slightly higher in pre- and post-sterility groups when compared with controls.

The effects of intrauterine position on frequencies of resorptions, deaths and anomalies in litters from 5-thio and PBS treated male mice appear in Table 8 and Figure 5. There was no difference between these frequencies when the experimentals and controls were compared. However, the highest numbers of resorptions and deaths occurred at the extremities of each uterine horn (near ovary and near cervix) in the litters from 5-thio treated males and in the litters from control males. There was no noticeable trend with regard to the effect of intrauterine position on number and type of anomalies.

The average number of implantations in the right horn of the female mice mated with males receiving 5-thio was compared with the average number of implantations in the right uterine horn of normal female mice mated with control male mice (Table 9). An identical comparison was made with the left uterine horn. It was found that the average number of implantations in the left uterine horn

from the 5-thio treated group ($\bar{x}$ = 5.96) was significantly lower than the number of implantations in the left uterine horn from the control group ($\bar{x}$ = 7.68). The numbers of implantations in the right uterine horn did not differ significantly when the two groups were compared.

Each of the groups, experimental and control, had 3 litters with fused placentae (Table 10). There was no significant difference when the two groups were compared. However, it was noted that in most cases, the fetuses associated with the fused placentae exhibited a decreased weight in relation with the average fetal weight of that particular litter. Several of these fetuses were also dead. There appeared to be a tendency for the fused placentae to be located at either extremity of the uterine horn, i.e. ovarian and cervical ends.

Histological examination of testes of experimentals revealed marked differences from the control testes at 28 days, when injections were terminated and also at 121 days. The normal testes of the mice treated with PBS appeared as follows: the seminiferous tubules had a densely packed appearance and numerous interstitial cells were present. Spermatogonia, spermatocytes, spermatids and abundant mature sperm were visible. Flagellae of mature spermatozoa frequently filled the lumina of tubules. The microanatomical structure of the germinal

epithelium exhibited normal organization of germ cell types within the Sertoli cells (Fig. 6).

The most obvious and consistent difference in the testes seen in all of the 5-thio treated male mice at 28 days was the occurrence of giant multinucleated cells (Fig. 7a, b). The seminiferous tubules having these abnormal cells tended to be concentrated on the periphery of the testis. This finding suggests that the compound might be absorbed directly through the tunica albuginea affecting the periphery first. The tubules with giant cells usually had few or no mature sperm. The most severely affected tubules showed empty lumina with the appearance of only spermatogonia and primary spermatocytes and also a disorganized appearance (Fig. 8a) in contrast with the orderly array of germ cells found in the control testes.

At 28 days, degeneration of cells was occasionally observed and vacuoles were found in the germinal epithelium of testes of 5-thio treated mice. It was unclear whether the large fenestrations observed in the giant cells were vacuoles or large lipid droplets. It was of interest to note in one instance a section of testis which showed transparent globules in the process of pinching off into the lumina of the tubule. They appeared to stem from the Sertoli cells and this may indicate the origin of the giant cells that result from the administration of 5-thio.

All experimental mice, except one, regained fertility during the experimental period. However, the histology of 5-thio treated mice testes at 121 days showed various degrees of structural recovery. All testes examined at this time displayed normal seminiferous tubules; some testes consisted of a small proportion of normal tubules, but most of the others were largely composed of normal tubules with mature sperm. However, each testis examined also showed tubules composed only of spermatogonia and filled with degenerating giant cells (Fig. 8b). Nuclei were absent in the degenerating giant cells at this time. Some of the tubules contained only spermatogonia (Fig. 9a). In certain areas of the testis, the tubules were generally loosely packed as compared to the tubules of the controls. The tubules had a tendency to be reduced in diameter and, accordingly, the intertubular spaces were enlarged. The interstitial cells appeared to be unaffected by the administration of 5-thio.

The one particular 5-thio treated mouse which showed no recovery during the experimental period revealed the most severely damaged testis when examined histologically (Fig. 9b). There were many abnormal tubules with degenerating giant cells and tubules with absent germ cell types; mature spermatozoa being absent. Nevertheless, a few normal tubules with sperm were noted in the testis of this mouse.

DISCUSSION

The observation that injections of 5-thio-D-glucose did not affect body weights of male ICR mice is in agreement with similar studies with mice (Zysk et al., 1975; Lobl & Porteus, 1978; Majumdar et al., 1979a, b), rats (Homm et al., 1977) and hamsters (Das & Yanagimachi, 1978). Only in a study where higher dosages (i.e. 75 and 165 mg/kg) were administered, were body weights decreased (Zysk et al., 1975).

The reduction in testicular weight caused by 5-thio has also been observed in other rodent studies (Zysk et al., 1975; Homm et al., 1977; Lobl & Porteus, 1978; Majumdar et al., 1979a). Majumdar et al. (1979a), using C3H mice, with a regimen similar to the present study, reported that the reduction in testicular weight occurred earlier than the 121 days found in the ICR mice of the present study. This discrepancy might be related to mouse strain differences. As the testicular weights of ICR mice were reduced to 59.2% of the control value at the end of the experimental period, it appeared there was only a partial recovery. A majority of other authors (Homm et al., 1977; Lobl & Porteus, 1978; Majumdar et al., 1979a) also report only a partial recovery of testicular weights after administration of 5-thio. However, one study reported total recovery even after administration of 100mg/kg 5-thio in mice (Zysk et al., 1975).

Five-thio was demonstrated to produce temporary sterility in the ICR mice and comparable results were found for the STS mice of another study (Majumdar et al., 1979a). Both the ICR and STS strains were observed to recover fertility approximately 5 weeks after sterility was induced. The fact that one mouse in the present study showed a lack of recovery of fertility, within the experimental period, demonstrates differences in response among individual test subjects. However, if given a longer recovery period, this mouse might also have regained fertility.

A significant decrease in mean litter size was repeatedly shown in ICR mice mated with 5-thio treated ICR male mice. Previous studies, however, revealed no decrease in mean litter size of mice (Zysk et al., 1975; Lobl & Porteus, 1978; Majumdar et al., 1979a). The reduced litter size in the present study, involving ICR mice, is indicative but not conclusive evidence of dominant lethal mutations in the germ cells of treated male mice. This conclusion is contradictory to recent studies by Majumdar et al. (1979b, c), which conclude that 5-thio is not mutagenic in STS mice or Drosophila.

A reduced litter size caused by a mutagenic agent would be a measurement of preimplantation losses, as death occurs shortly after fertilization of the egg (Bateman & Epstein, 1971; Bateman & Jackson, 1974). Mutagenicity of 5-thio in ICR mice can only be suggested

in the present study because unless the preimplantation eggs are examined, it cannot be determined whether reduced litter size was due to dominant lethal mutations or to unfertilized eggs (Bateman & Epstein, 1971). In addition, it appears that the agents which produce high mutagenic rates tend to produce high preimplantation losses due to dominant lethal mutations. Five-thio does not appear to be highly mutagenic or this would most probably have been exhibited in the previous mutagenicity studies of Majumdar et al. (1979b, c). There may be other non-genetic factors involved in the decreased litter size (e.g. abnormal sperm) (Bateman & Epstein, 1971). Low sperm production (oligospermy) is another possibility.

The post-sterility period revealed a decreased number of normal and an increased number of dead fetuses in litters from 5-thio treated males, which, again, could indicate dominant lethal mutations as deaths produced by these mutations can occur any time during development, even perinatally (Soares, 1972). However, resorptions and anomalies in litters sired by 5-thio treated males were not significantly different from those found in the control litters. These data were similar to results of previous studies (Zysk et al., 1975; Lobl & Porteus, 1978; Majumdar et al., 1979a). An increase in anomalies due to dominant mutations in the heterozygous state induced by 5-thio were not expected as these occurrences are extremely rare (e.g. achondroplasia) (Bateman & Jackson,

1974).

The relationship of intrauterine position to frequency of resorptions, deaths and anomalies revealed one trend: the middle region appeared to be more favorable for the surival of the developing mouse embryos. Ward et al. (1977) demonstrated similar results when irradiated rat embryos implanted in the cervical and ovarian ends of the uteri were found to have a higher mortality and the reason for this response was unclear.

The fetuses associated with fused placentae displayed a general reduction in weight relative to the average fetal weight of the litter in which they were found. It is conceivable that the reduction in weight and the deaths were due to competition for nutrients and oxygen supply between the fetuses attached to the fused placentae.

Giant multinucleated cells were present in testes of 5-thio treated ICR mice at 28 days, however, other studies showed a higher degree of exfoliation of the germinal epithelium with a similar regimen in C3H and STS mice (Majumdar et al., 1979a) and gerbils (Udelsman & Majumdar, 1976). Differences in response of the testes to 5-thio treatment of ICR mice as compared with the response in other studies may be due to strain or species differences.

It has been postulated that if the germ cells lose contact with the Sertoli cells, which are responsible

for synchronization of the maturation process of the germ cells, the integrity of these cells will be altered (Fawcett, 1975; Neumann & Schenk, 1977) and this may lead to giant cell formation. The spermatids were observed to lose contact with the Sertoli cells before the giant cells were formed (Neumann & Schenk, 1977) and also vacuolization was noted as one of the preliminary histopathological effects in the testis after 5-thio treatment. This may be related to giant cell formation (Majumdar & Udelsman, 1979). It has been hypothesized that the giant cells arise from abnormalities in cytokinesis and karyokinesis (Majumdar & Udelsman, 1979). Because of the large volume of cytoplasm seen in the giant cells of the present study, it appears that these cells consist largely of Sertoli cell cytoplasm and not mainly spermatids and spermatocytes as previously reported (Neumann & Schenk, 1977). As it is known that Sertoli cells are capable of phagocytic activity (Fawcett, 1975), it is thus possible that the germ cells damaged by 5-thio were engulfed by the Sertoli cells which subsequently pinch off into the lumen of the seminiferous tubule.

In the present study, total structural recovery of the testes of 5-thio treated ICR mice was not observed at the end of the 121 day experimental period. These data contrasted the data of Zysk et al. (1975) who reported normal testicular histology within 5 to 8 weeks after induced sterility in mice. Rats appear to be more

sensitive than mice as demonstrated by Homm et al. (1977) who found severe testicular damage after a recovery period of one year.

The importance of glucose for normal testicular morphology was demonstrated by Deb & Chatterjee (1963) and Mancine et al. (1960). Davis (1969) revealed that spermatids were markedly sensitive to the presence of glucose as compared with other body tissues and he suggested the possibility of a glucose analog as a male antifertility agent. It appears that 5-thio competitively inhibits the transport of D-glucose (Critchley et al., 1970), but the exact mechanism of action of 5-thio is not clearly understood even though extensive study has been carried out in this area (Hoffman & Whistler, 1968; Paranjpe & Jagannathan, 1971; Whistler & Lake, 1972; Chen & Whistler, 1975, 1977; Prabhakaran, 1976; Nakamura & Hall, 1976, 1977; Burton & Wells, 1977). It has not been determined which testicular cells are the target for 5-thio and it has not been elucidated why the spermatocytes are also affected as they are not as dependent upon glucose as the spermatids (Davis, 1969; Majumdar & Udelsman, 1979).

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Table 1. The effect of 5-thio-D-glucose injections on the body weights of male ICR mice

Week[a]	Mean body weight ± S.D. (g) PBS (controls)[b] (4)[d]	Mean body weight ± S.D. (g) 60 mg/kg (5-thio)[bc] (8)[d]
1	37.9 ± 3.2	38.9 ± 1.9
2	38.0 ± 2.7	39.3 ± 2.4
3	38.9 ± 2.8	39.6 ± 2.6
4	38.3 ± 3.1	39.5 ± 2.0
5	38.8 ± 4.0	39.7 ± 2.1
6	39.2 ± 3.7	39.9 ± 2.5
7	40.0 ± 3.3	40.2 ± 2.8
8	40.2 ± 3.8	41.3 ± 3.0
9	41.6 ± 3.7	41.7 ± 3.3
10	41.5 ± 3.8	41.9 ± 3.0
11	42.5 ± 3.4	42.0 ± 3.0
12	43.0 ± 2.8	42.3 ± 3.0
13	43.4 ± 3.0	42.8 ± 3.2
14	43.3 ± 3.0	42.9 ± 3.6
15	43.6 ± 4.4	43.1 ± 3.5
16	43.9 ± 5.6	43.7 ± 4.2
17	47.0 ± 1.4	44.9 ± 4.1

a weeks 1-4, weights were taken daily; weeks 5-17, weights were taken semi-weekly

b male mice were intraperitoneally injected daily with 5-thio or PBS for 28 days

c body weights were not found to be significantly different from control weights at any week (95% confidence level)

d number of males

Table 2. The effects of 5-thio-D-glucose on relative testicular weights in ICR mice

Treatment[a]	28 days (%)	121 days (%)
PBS[b] (controls)	100 (2)[c]	100 (3)
60 mg/kg (5-thio)	89.2 (4)	59.2[d] (8)

a mice were intraperitoneally injected daily with 5-thio or PBS for 28 days
b control ratio (testis weight/body weight) = 100%
c numbers in parentheses are number of mice
d significant (95% confidence level)

Table 3. Litter size and the frequencies of resorptions, deaths and anomalies appearing in the litters of ICR mice injected with 5-thio-D-glucose

Treatment[a]	No. Males	No. Females Impregnated	Total Implants	Mean Litter Size (± S.D.)	Mean No. Normals[b] (± S.D.)	Mean No. Resorptions[b] (± S.D.)	Mean No. Deaths[b] (± S.D.)	Mean No. Anomalies[b] (± S.D.)
PBS (controls)	4	32	466	14.56 ± 3.07	13.44 ± 3.18	0.78 ± 0.91	0.25 ± 0.67	0.09 ± 0.30
60 mg/kg (5-thio)	8	56	687	12.27[c] ± 4.07	11.07 ± 3.94	0.77 ± 1.27	0.25 ± 0.51	0.18 ± 0.54

a mice were intraperitoneally injected daily with 5-thio or PBS for 28 days
b number of normals, resorptions, deaths and anomalies for average female
c significant (95% confidence level)

Table 4. A comparison of litter size and the frequencies of resorptions, deaths and anomalies appearing in the litters of ICR mice mated with 5-thio-D-glucose treated ICR male mice during the pre-sterility and post-sterility periods

Treatment[a]	No. Males	No. Females Impregnated	Total Implants	Mean Litter Size (± S.D.)	Mean No. Normal[b] (± S.D.)	Mean No. Resorptions[b] (± S.D.)	Mean No. Deaths[b] (± S.D.)	Mean No. Anomalies[b] (± S.D.)
PBS (controls)	4	32	466	14.56 ± 3.07	13.44 ± 3.18	0.78 ± 0.91	0.25 ± 0.67	0.09 ± 0.30
60 mg/kg (5-thio) Pre-sterility	8	40	507	12.68[c] ± 3.73	11.63 ± 3.48	0.75 ± 0.95	0.15 ± 0.36	0.15 ± 0.53
60 mg/kg (5-thio) Post-sterility	8	16	180	11.25[c] ± 4.81	9.69[c] ± 4.77	0.81 ± 1.87	0.50[c] ± 0.73	0.25 ± 0.58

a mice were intraperitoneally injected daily with 5-thio or PBS for 28 days
b number of normals, resorptions, deaths and anomalies for average female
c significant (95% confidence level)

Table 5. Frequency of litters with 100% normal fetuses derived from gross morphological examination of offspring of 5-thio-D-glucose treated ICR male mice mated with normal ICR female mice

Treatment[a]	Male ID.	No. Litters	Litters with 100% normal fetuses (%)
PBS (controls)	PB1	9	3 (33.3)
	PB2	8	3 (37.5)
	PB3	8	2 (25.0)
	PB4	7	3 (42.9)
Mean % of litters with 100% normal fetuses			(34.4)
60 mg/kg (5-thio)	TG1	9	3 (33.3)
	TG2	7	1 (14.3)
	TG3	8	2 (25.0)
	TG4	7	4 (57.1)
	TG5	5	2 (40.0)
	TG6	6	1 (16.7)
	TG7	7	3 (42.9)
	TG8	7	2 (28.6)
Mean % of litters with 100% normal fetuses			(32.1)[b]

a mice were intraperitoneally injected daily with 5-thio or PBS for 28 days

b not significantly different when compared with control value (95% confidence level)

Table 6. Number of litters containing resorptions in litters of ICR mice mated with male ICR mice treated with 5-thio-D-glucose

Treatment[a]	No. Males	No. Litters	Frequency of Resorptions in litters 1 or more (%)	3 or more (%)	5 or more (%)
PBS (controls)	4	32	17 (53.1)	2 (6.3)	0
60 mg/kg[b] (5-thio)	8	56	27 (48.2)	4 (7.1)	2 (3.6)

a mice were intraperitoneally injected daily with 5-thio or PBS for 28 days

b no significant difference between experimentals and controls (95% confidence level)

Table 7. Anomalies observed in fetal offspring of 5-thio-D-glucose and PBS treated male ICR mice mated with normal female ICR mice

Treatment[a]	Male ID.	Type of Anomaly (no.)
PBS (controls)	PB1	Polydactyly (1)
	PB2	None
	PB3	Stunted fetus[b](2)
	PB4	None
60 mg/kg (5-thio)	TG1	Hemorrhage (1)
	TG2	Stunted fetus (1) Extra digital appendage (1)
	TG3	Stunted fetus (1)
	TG4	None
	TG5	None
	TG6	None
	TG7	Stunted fetus (1)
	TG8	Extra digital appendage (5)

a mice were intraperitoneally injected daily with 5-thio or PBS for 28 days

b fetuses were considered stunted if less than 65% of the average fetal weight of the litter

Table 8. The frequencies of resorptions, deaths and anomalies in fetuses relative to intrauterine position within normal ICR mice mated with male ICR mice injected with 5-thio-D-glucose

Intrauterine Position	PBS (controls)[a] % Resorptions[b]	% Deaths[b]	% Anomalies[b]
Ovarian	(74) 4.00	(58) 1.00	(308) 2.00
Upper middle	(141) 7.59	(220) 3.80	(195) 1.27
Middle	(72) 3.90	(0) 0	(0) 0
Lower middle	(69) 3.74	(162) 2.80	(0) 0
Cervical	(148) 8.00	(58) 1.00	(0) 0
All positions	(100) 5.40	(100) 1.73	(100) 0.65
	60 mg/kg (5-thio)[a]		
Ovarian	(85) 5.41	(191) 4.05	(89) 1.35
Upper middle	(146) 9.26	(131) 2.78	(0) 0
Middle	(53) 3.36	(40) 0.84	(55) 0.84
Lower middle	(115) 7.30	(34) 0.73	(288) 4.38
Cervical	(106) 6.76	(96) 2.03	(45) 0.68
All positions	(100) 6.36	(100) 2.12	(100) 1.52

a mice were intraperitoneally injected daily with 5-thio or PBS for 28 days

b % is the resorptions, deaths or anomalies divided by implantations in the particular position x 100. Numbers in parentheses indicate resorptions, deaths and anomalies as a percent of the average of these found in all positions of the uteri

Table 9. A comparison of the numbers of implantations in the right and left uterine horns of ICR mice mated with 5-thio-D-glucose treated ICR male mice

Treatment[a]	No. Females	Mean no. implantations ± S.D. Right horn[b]	Left horn[b]
PBS (controls)	31	7.26 ± 1.93	7.68 ± 1.97
60 mg/kg (5-thio)	53	6.49 ± 2.76	5.96 ± 2.64[c]

a mice were intraperitoneally injected daily with 5-thio or PBS for 28 days
b implantation numbers of the right and left uterine horns of mice mated with 5-thio treated male mice were compared with implantation numbers of right and left uterine horns, respectively, of mice mated with PBS treated male mice
c significant (95% confidence level)

Table 10. Occurrence of fused placentae in the uteri of ICR mice mated with 5-thio-D-glucose treated male ICR mice

Treatment[a]	Male ID.	No. Litters	No. Litters With Fused Placentae	Av. Fetal Weight (g)	Fetal weights Within Fused Placentae (g)
PBS (controls)	PB1	9	1	0.18	0.15; 0.15
	PB2	8	0		
	PB3	8	1	0.71	0.54; 0.63
	PB4	7	1	0.72	0.69; 0.73
60 mg/kg[b] (5-thio)	TG1	9	1	0.96	0.80; 0.20
	TG2	7	0		
	TG3	8	1	0.64	0.53; 0.59
	TG4	7	0		
	TG5	5	0		
	TG6	6	0		
	TG7	7	0		
	TG8	7	1	0.94	0.78; 0.76

a mice were intraperitoneally injected daily with 5-thio or PBS for 28 days

b no significant difference between experimentals and controls (95% confidence level)

Fig. 1. Structure of 5-thio-D-glucose.

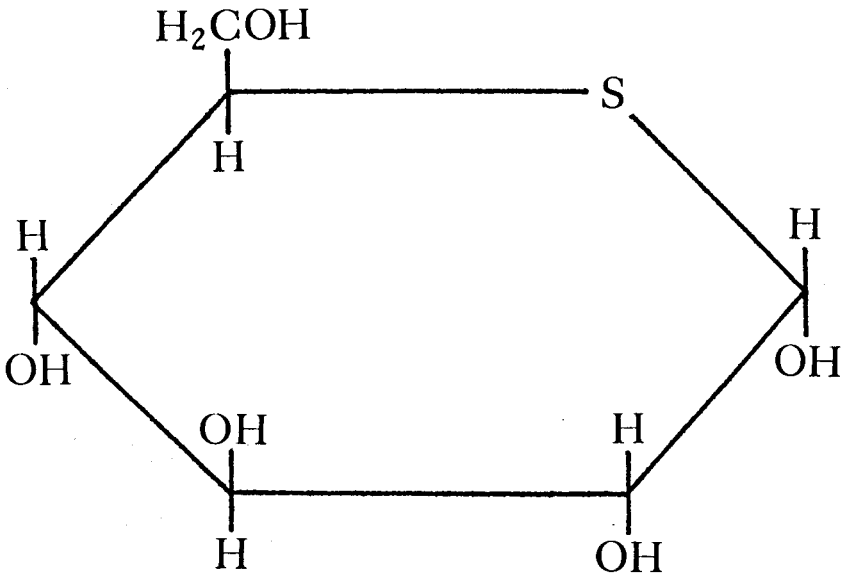

H2COH
S
H
H
H
OH
OH
OH
H
H
OH

Fig. 2. A diagram representative of the system used to examine implantations within the mouse uteri. (R) right ovary; (L) left ovary; (C) cervix; (A-J) fetuses.

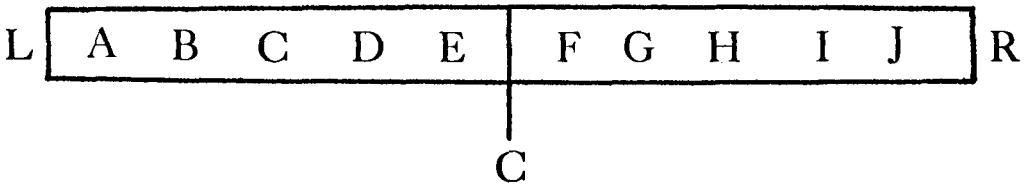

L
A B C D E
F G H I J
R
C

Fig. 3. Anomalies found in litters of ICR mice mated with ICR male mice intraperitoneally injected daily with 5-thio-D-glucose or PBS for 28 days.

a. control mouse fetus exhibiting polydactyly (arrow points to extra digit). X18.

b. stunted mouse fetus shown beside a fetus of normal size. Malformations of this type were found in experimental and control groups. X4.

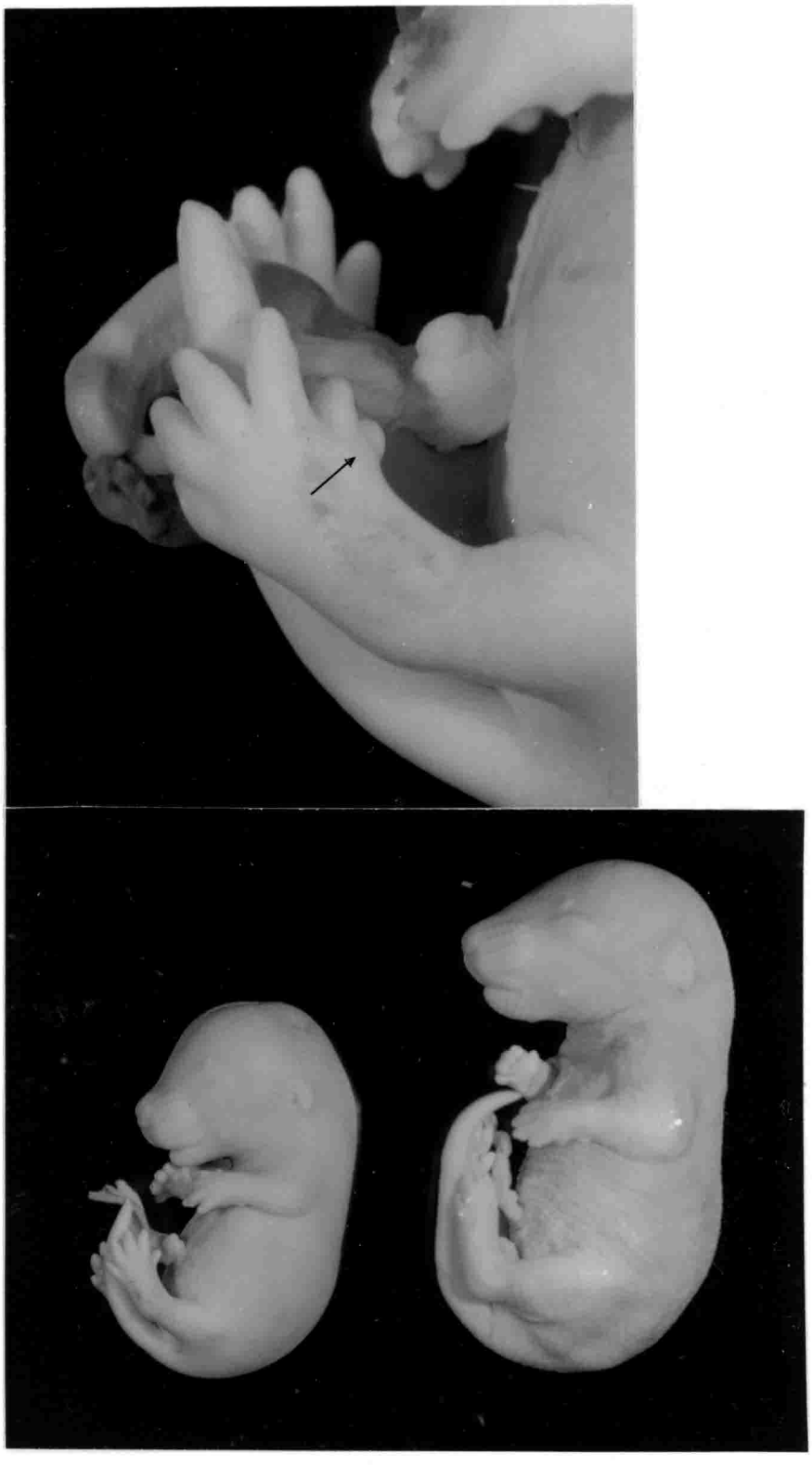

a
b

Fig. 4. Anomalies found in litters of ICR mice mated with ICR male mice intraperitoneally injected daily with 5-thio-D-glucose for 28 days.

a. mouse fetus with hemorrhagic area (arrow). X6.

b. extra digital appendage (arrow) shown on forelimb of fetus. X7.

a

b

Fig. 5. The frequencies of resorptions, deaths and anomalies in fetuses relative to intrauterine position within normal ICR mice mated with ICR male mice injected with 5-thio-D-glucose or PBS for 28 days[1].

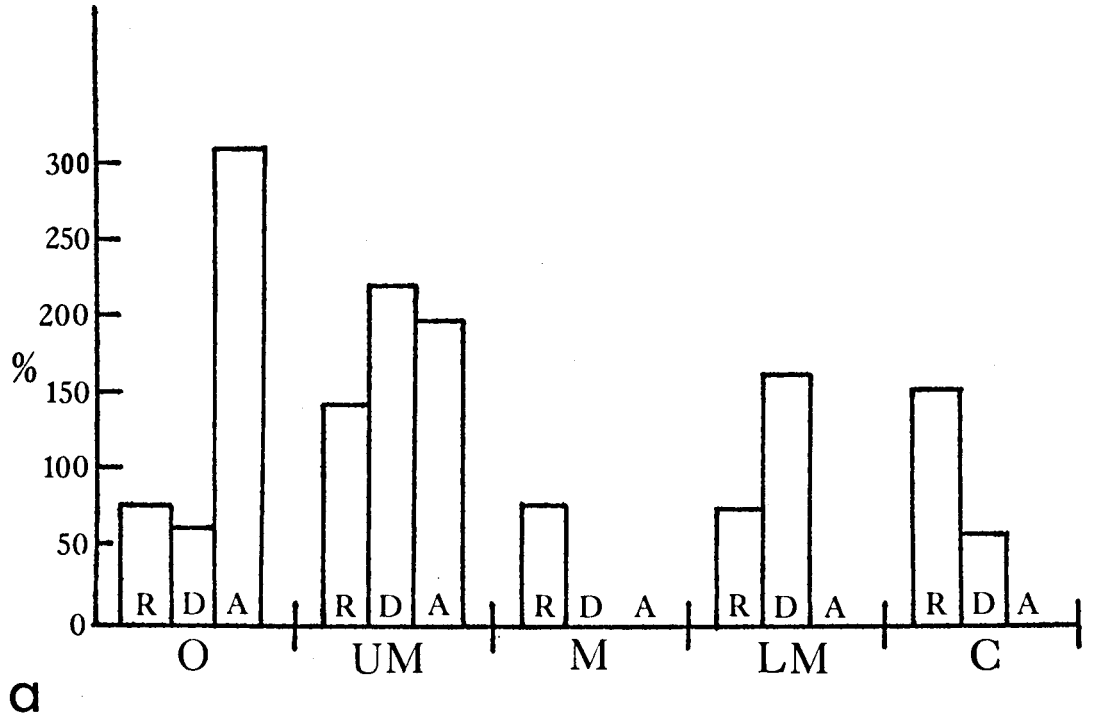
a. PBS (controls)[2]

b. 60 mg/kg (5-thio)[2]

(R) resorptions
(D) deaths
(A) anomalies
(O) ovarian
(UM) upper middle
(M) middle
(LM) lower middle
(C) cervical

1 resorptions, deaths and anomalies are relative to those found in all positions of the uteri

2 mice were intraperitoneally injected daily with 5-thio or PBS for 28 days


300
250
200
%
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100
50
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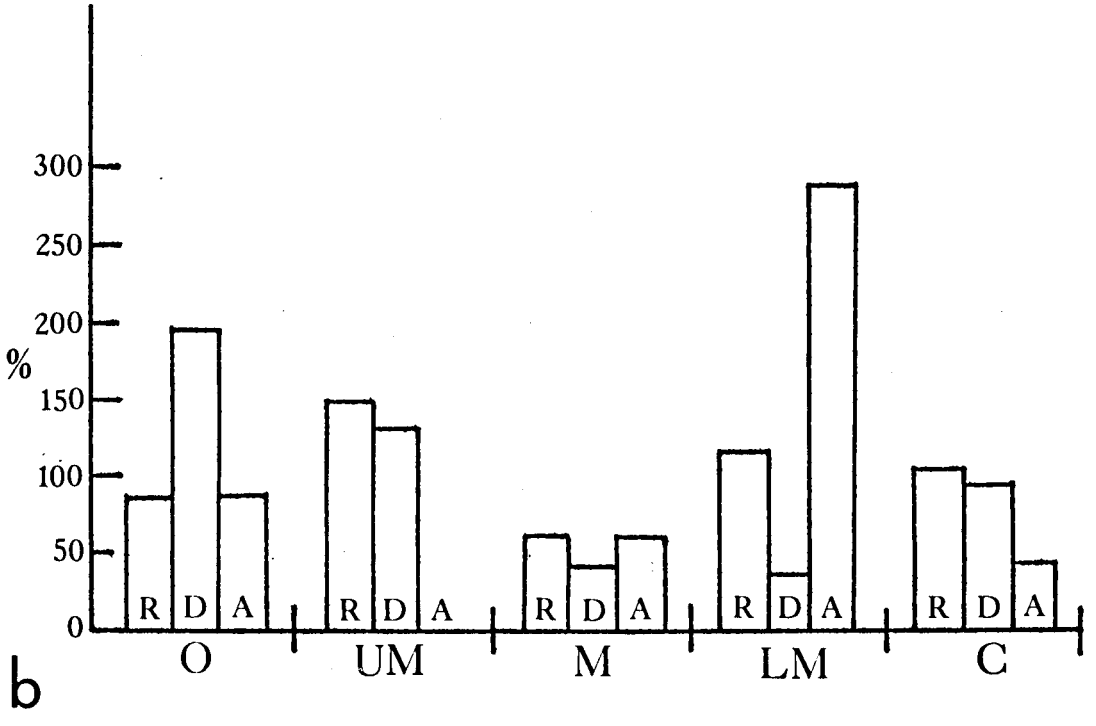
R D A
R D A
R D A
R D A
R D A
O
UM
M
LM
C

a


300
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R D A
R D A
R D A
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UM
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LM
C

b

Fig. 6. Cross section of a normal seminiferous tubule of an ICR mouse intraperitoneally injected daily with PBS for 28 days. (S) spermatogonium; (Sp) spermatocyte; (sp) spermatid; (M) mature sperm. X890.

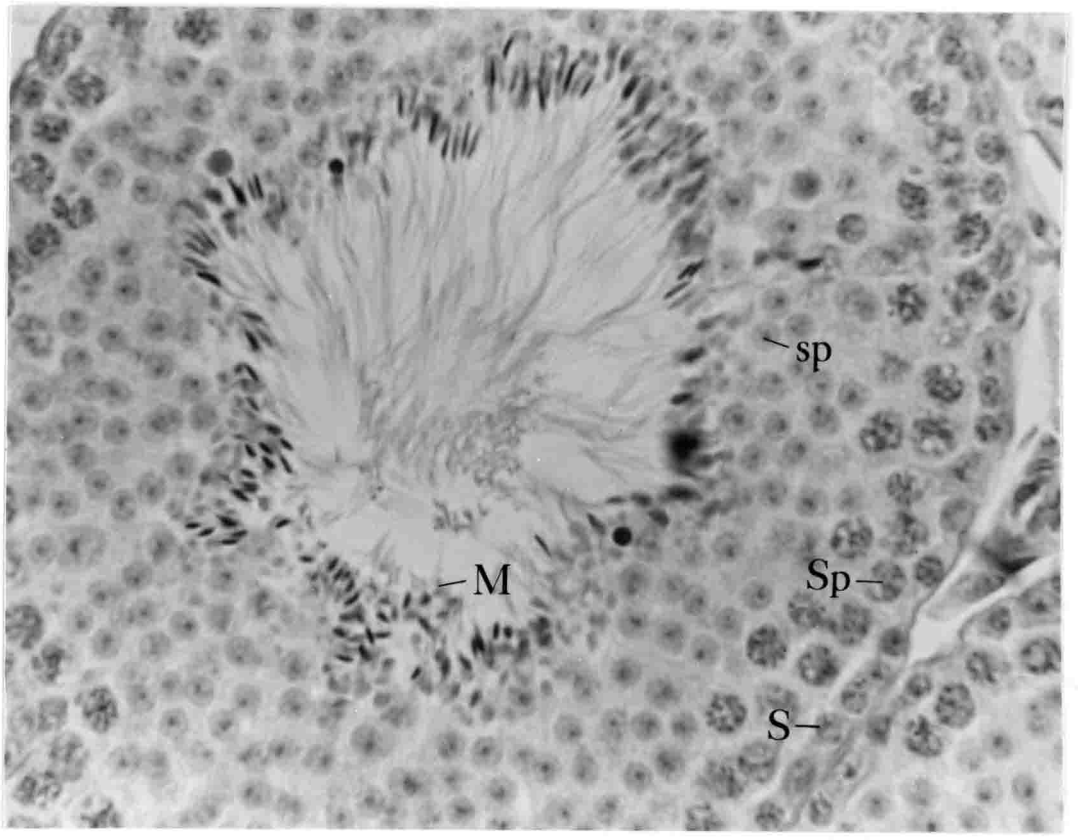

sp
M
Sp
S

Fig. 7. Cross sections of seminiferous tubules of an ICR mouse intraperitoneally injected daily with 5-thio-D-glucose for 28 days.

a. peripheral region of the testis at 28 days after initial injections. Many giant multinucleated cells (G) are present. X230.

b. higher magnification of Fig. 7a showing giant cells (G) with nuclei (n) centrally or peripherally located. No mature sperm visible. IC, interstitial cells. X780.

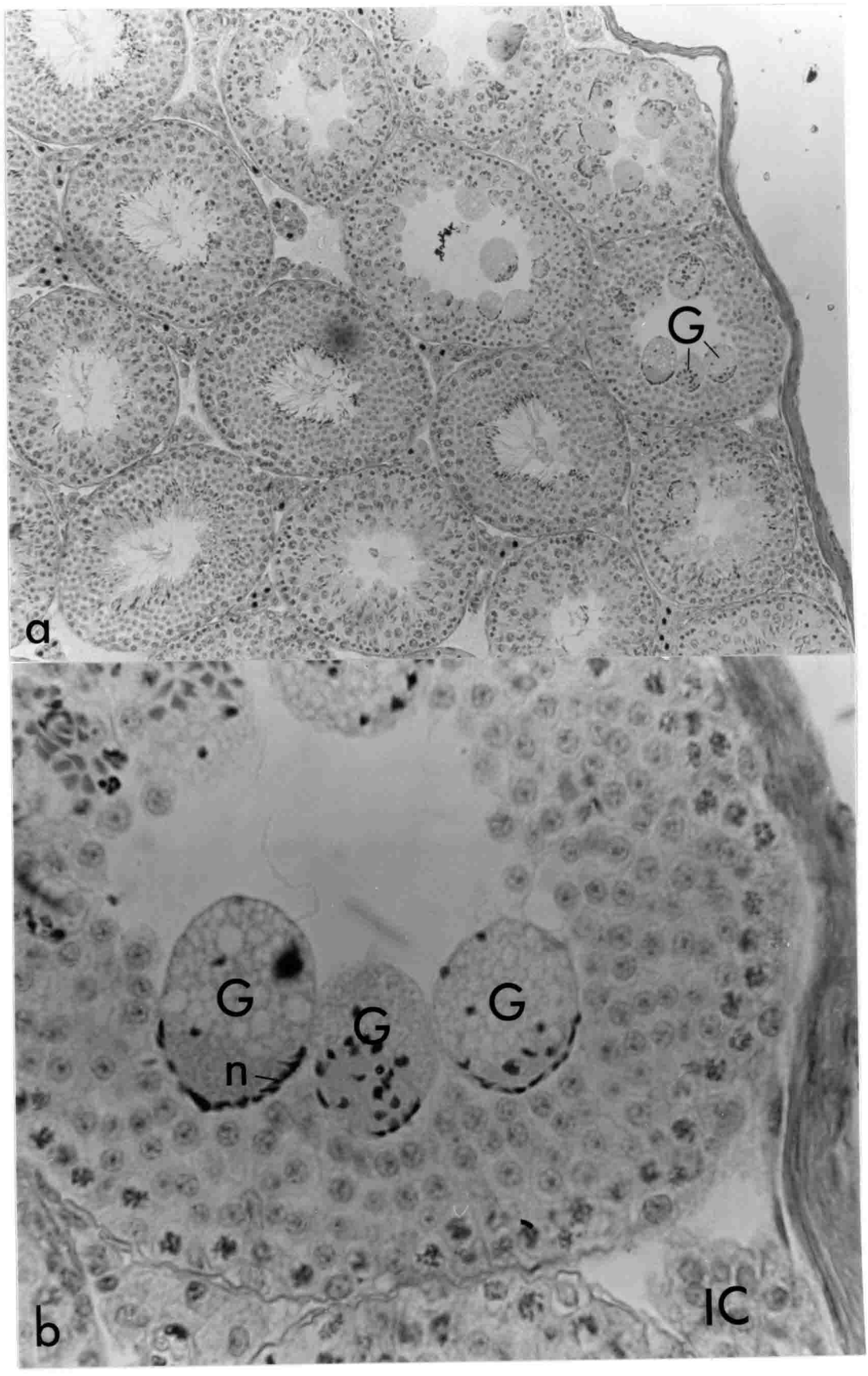

G
a
G
G
G
n
b
IC

Fig. 8. Cross sections of seminiferous tubules of ICR mice intraperitoneally injected daily with 5-thio-D-glucose for 28 days.

a. seminiferous tubule exhibiting a disorganized appearance of the germinal epithelium and giant cell formation (G) at 28 days after initial injections. X570.

b. seminiferous tubule at 121 days after initial injections. The shape of the tubule is distorted; the only germ cells evident are the spermatogonia; lumina is filled with degenerating giant cells (G) and debris. X630.

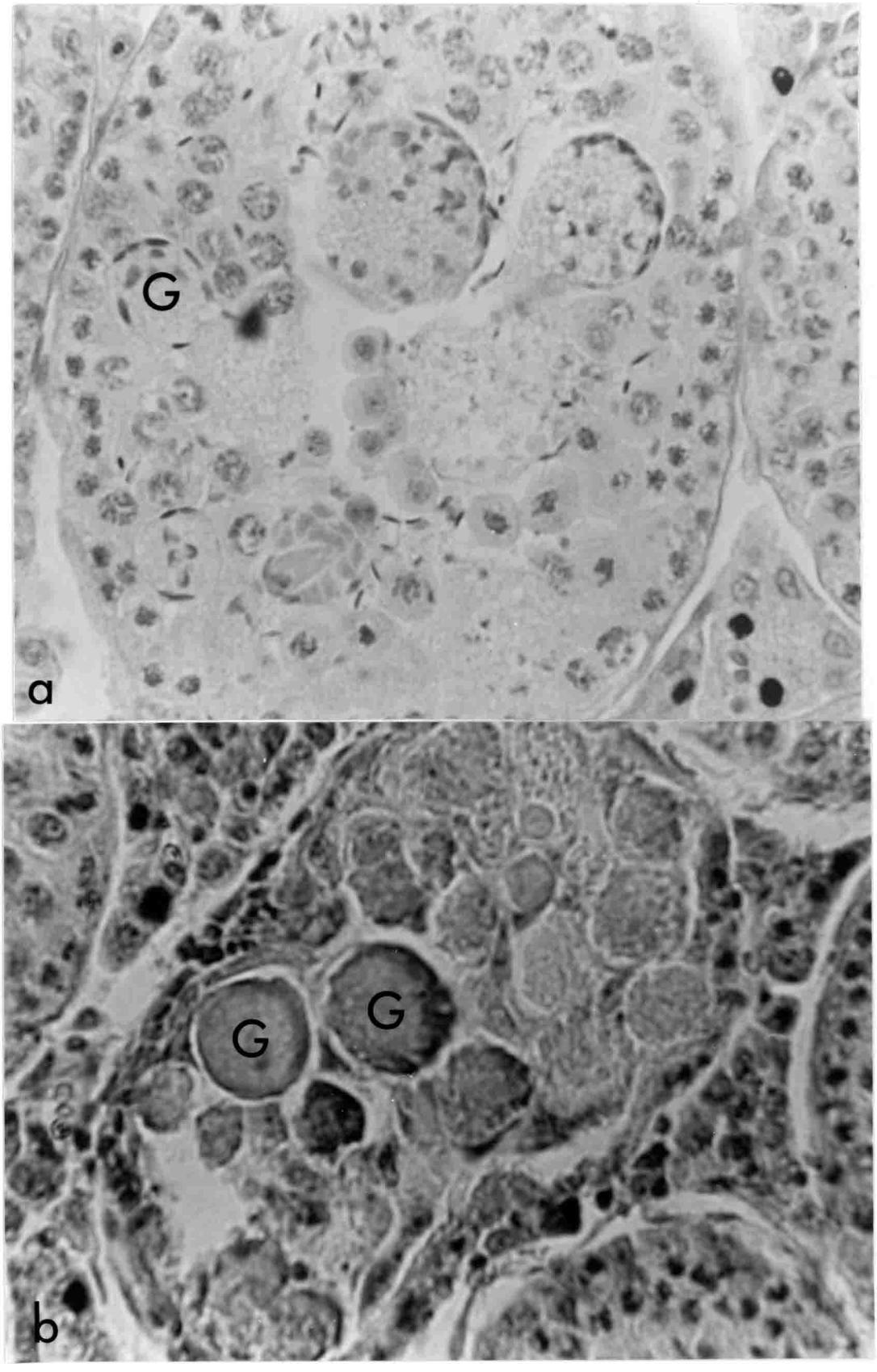
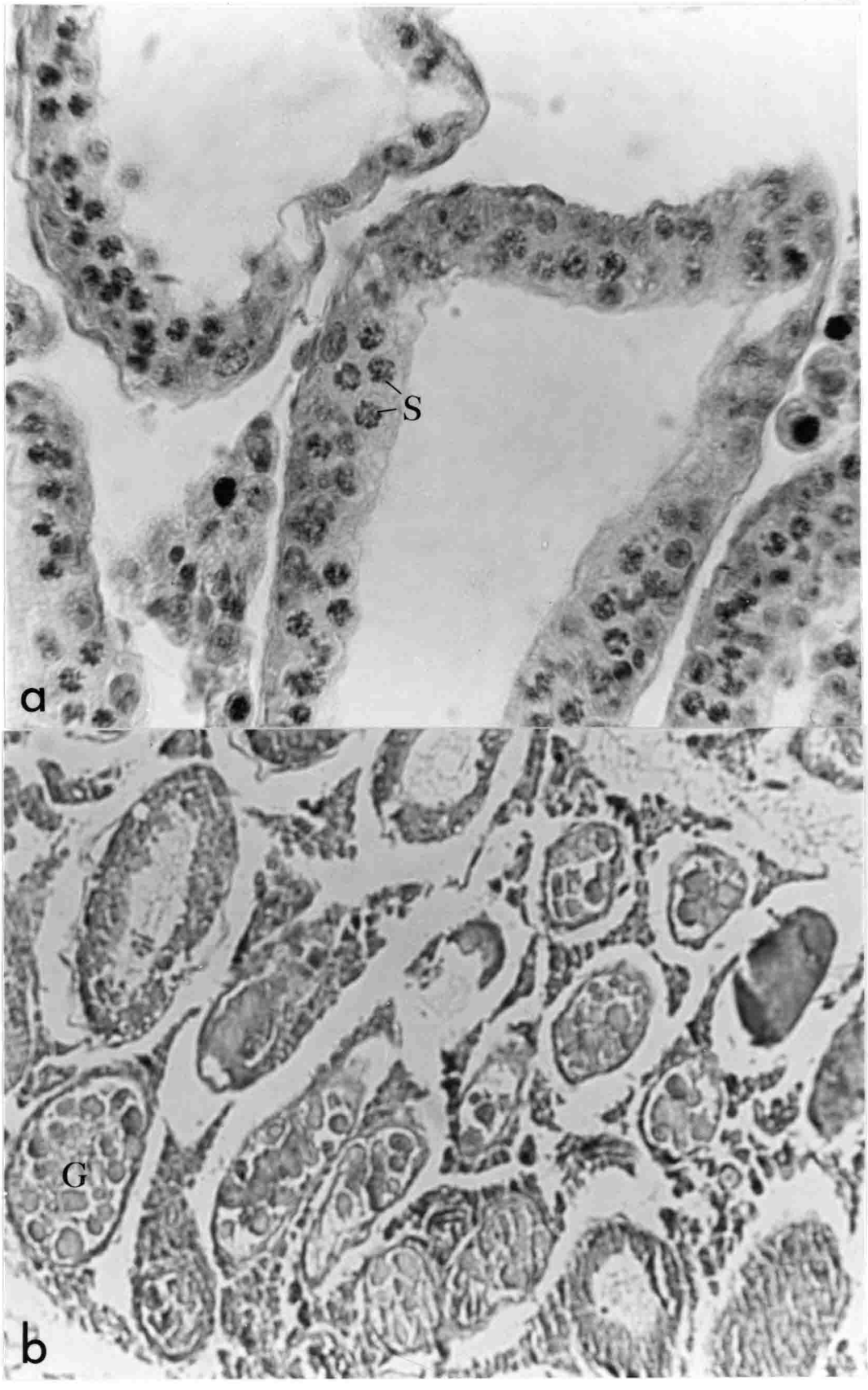

G
a
G
G
b

Fig. 9. Cross sections of seminiferous tubules of ICR mice intraperitoneally injected daily with 5-thio-D-glucose for 28 days.

a. seminiferous tubules at 121 days after initial injections. Tubules are reduced, distorted and composed only of spermatogonia (S). No spermatids or spermatozoa are visible. X625.

b. severly damaged tubules at 121 days after initial injections. Tubules are reduced and filled with degenerating giant cells (G). X145.


S
a
G
b

VITA

Mary Ellen McManaway was born June 20, 1950 in Roanoke, Virginia. Secondary education was completed in 1968 at William Fleming High School in Roanoke. An A.A. degree in liberal arts was received in 1970 from Ferrum College in Ferrum, Virginia. She also received her B.S. degree in biology, with an art minor, in 1973 from Virginia Polytechnic Institute and State University in Blacksburg, Virginia. After graduation, she attended the Corcoran School of Art in Washington, D. C. as a part-time student for one year and for four years was employed by the Bureau of Radiological Health of the Food and Drug Administration in Rockville, Maryland. This work involved radiation effects on the developing mouse embryo. In 1978, she enrolled in the University of Richmond graduate program in biology in order to receive the degree of Master of Science.