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The Induced Histopathology of the Testes and Epididymides of Cf-1 Mice by Known or Suspected Teratogens, Mutagens, and Carcinogens

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THE INDUCED HISTOPATHOLOGY OF THE TESTES AND EPIDIDYMIDES OF CF-1 MICE BY KNOWN OR SUSPECTED TERATOGENS, MUTAGENS, AND CARCINOGENS

by

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THE INDUCED HISTOPATHOLOGY OF THE TESTES AND EPIDIDYMIDES OF CF-1 MICE BY KNOWN OR SUSPECTED TERATOGENS, MUTAGENS, AND CARCINOGENS

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With each passing year, humans are exposed to ever increasing numbers of chemical agents, many of which may have mutagenic, carcinogenic, or teratogenic effects on the human species. Animal studies must be performed on any chemical substance that is suspected of being acutely dangerous to humans by way of cancer induction or induced malformations to infants. Seven drugs of known or suspected mutagenic, carcinogenic, or teratogenic potential were examined for their ability to induce histopathology in the testes and epididymides of CF-1 mice. Mitomycin-C, hydroxyurea, and adriamycin induced significant histopathology whereas prednisolone, thalidomide, streptomycin sulfate, and polybrominated biphenyl induced minimal or no histopathology to the testes and epididymides. The results of this project will provide additional information on the toxicity and teratogenic potentials of these chemical agents.

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INTRODUCTION

With each passing year, humans are exposed to ever increasing number of chemical agents, many of which may have detrimental mutagenic, carcinogenic, and teratogenic effects on the species. It is because of this increasing exposure to these chemical agents and because of the ignorance of the effects of these drugs on humans, that animal studies be performed on any chemical substance that is suspect of being acutely dangerous to humans by way of cancer induction or induced malformations to infants. The present project addressed itself to surveying the potential of a number of known or suspected mutagens, carcinogens, and teratogens, for inducing histopathology in the testes and epididymides of CF-1 mice. It is believed that the results of this project will provide additional information on the toxicity and teratogenic potential of these chemical agents.

Experiment 226-5B: Polybrominated Biphenyl (PBB)

In July of 1973, polybrominated biphenyl (PBB) of the commercial name Firemaster BP-6, was mistakenly mixed into commercial livestock feed and then sold to farmers in central and southern lower Michigan. As a result of this mistake, thousands of Michigan cattle ingested large quantities of PBB and several Michigan families were exposed to high levels of PBB by eating the meat of the PBB contaminated cattle. In addition, several million Michigan residents were exposed to PBB as a result of

consuming dairy products from PBB contaminated cattle. Public concern mounted about the possible toxic and teratogenic effects that the PBB exposure would have upon Michigan residents.

Jackson and Halbert (1974) discussed the toxic effects that were induced in a Michigan dairy herd exposed to the PBB. One of many clinical symptoms displayed by the contaminated cows was severe calving problems which resulted in the calf being born dead or dying soon after birth. Corbett, Beaudoin, Cornell, Anver, Schumacher, Endres and Szwabowska (1975) administered PBB to pregnant rats and pregnant mice which resulted in lower mean weights of the rat and mouse fetuses as compared to control fetuses. At the higher dosages of 100 and 1,000 parts per million (PPM) PBB, the mouse fetuses also displayed exencephaly and cleft palate. Beaudoin (1977) reported that a single oral dose of PBB at 200 or 400 mg of PBB per kg of body weight (mg/kg) resulted in no malformations in the fetuses born to female rats exposed to PBB on any single day, from day 6 to day 14, of their pregnancy period. Ficsor and Wertz (1976) reported similar results when pregnant rats were force fed 100 mg PBB/kg, six times in 2 day intervals beginning on day 6 of pregnancy. At the higher dosages of 400 or 800 mg/kg, Beaudoin (1977) reported that a single oral dose of PBB administered to pregnant rats resulted in a significant increase in the number of resorbed rat fetuses and at 800 mg PBB/kg, large percentages of the surviving fetuses

were malformed with cleft palate and diaphragmatic hernia. Fries, Marrow and Cook (1978) reported that PBB is "readily transferred across placental membranes" (p. 44) in pregnant cows as evidenced by the concentration of PBB appearing in the fat and blood of the calves and fetuses of those cows exposed to PBB during their pregnancy. Moorehead, Willet and Schanbacher (1978) administered daily oral doses of 25 grams of PBB to 6 pregnant heifers until the animals died. Necropsy of the 6 pregnant heifers revealed placentas with hemorrhagic and necrotic cotyledons and dead hemorrhagic fetuses in their uteri. Lambrecht, Barsotti and Allen (1978) reported that female rhesus monkeys exposed to low levels of PBB in their diets for several months and then mated, resulted in offspring that "were consistently smaller than control infants" (p. 144), although no malformations were observed in the offspring born to the treated females.

Cook, Helland, VanderWeele and Dejong (1978) collected tissue samples from the testes of bulls that had ingested PBB contaminated feed as a result of the original feed contamination in July of 1973. Histological examination of the testes revealed that the population of mature spermatazoa was greatly reduced, the germinal cells of the seminiferous tubules had been lost and only the Sertoli cells remained in the seminiferous tubules. Jackson and Halbert (1974) reported that one 18 month old bull that had consumed the PBB contaminated livestock feed, was found to have atrophied

testes when the animal was operated on. Semen samples taken from this 18 month old bull showed a loss of sperm motility as well as spermatazoa lacking either heads or tails.

In reference to the mutagenic potential of PBB, Wertz and Ficsor (1978) reported a "dose of 50 or 500 mg PBB/kg failed to increase significantly chromosome or chromatid breaks" in the bone marrow cells of treated mice.

The present experiment is concerned with the potential for PBB to induce histopathological changes in the testes and epididymides of male mice which will be treated at various dosages and then sacrificed at various post treatment intervals. Histopathological assessment of the testes and epididymides of PBB treated mice will provide information about the possible toxicity and/or teratogenicity of PBB on spermatazoa.

Experiment 226-5C, 226-5K: Hydroxyurea

Hydroxyurea, which has the formula $H_2NCONHOH$, was first synthesized in 1869, but was not thought of as an antineoplastic drug until 1960. Hydroxyurea is now used as an anti-leukemia and anti-tumor drug in the clinical treatment of cancer patients. Hydroxyurea is a strong inhibitor of deoxyribonucleic acid (DNA) synthesis and it is for this reason that hydroxyurea is effective against tumor cells that are undergoing rapid DNA synthesis (Timpson, 1975).

In reference to the inhibition of DNA synthesis by hydroxyurea,

Young and Hodas (1964) reported that exposing cultured Hela cells to hydroxyurea inhibited the incorporation of thymidine during DNA synthesis. Sinclair (1965) reported that hydroxyurea had a differential lethal effect on cultured Chinese hamster ovary cells. Cells that were in the synthetic phase (S) were lethally damaged by exposure to hydroxyurea whereas Chinese hamster ovary cells that are in the first growth phase (G_1), were not killed but were unable to begin the S phase of the cell cycle. Cells in G_2 also survived but were also unable to begin the S phase. Sinclair (1965) also reported that hydroxyurea completely inhibited Chinese hamster cells from incorporating thymidine essential for DNA synthesis. Schwartz, Garafalo, Sternberg, & Philips (1965) reported that hydroxyurea inhibited incorporation of thymidine into the DNA of regenerating rat livers. Brachet (1967) reported variable effects of hydroxyurea on Escherichia Coli, depending on the concentration of hydroxyurea that the bacteria were exposed to. Brachet (1967) suggested that hydroxyurea, at low concentrations, may inhibit the reduction of ribonucleotides whereas at higher concentrations, hydroxyurea might affect the template activity of DNA making the DNA incapable of both transcription and replication. Timpson (1975) reported that the general concensus for the mode of action whereby hydroxyurea inhibits DNA synthesis, is that hydroxyurea causes a "blocking of the conversion of ribonucleotides to deoxyribonucleotides by means of the inhibition of the enzyme ribonucleotide reductose" (p. 119).

Rajewsky, Fabricus and Hulser (1971) reported that hydroxyurea administered to pregnant rats, crossed the placental barrier and caused inhibition of DNA synthesis in the rat embryos. No toxic effects on the embryos were found after administering a dose of 250 mg/kg of hydroxyurea to the pregnant rats. Scott, Ritter and Wilson (1971) reported that hydroxyurea crosses the placental barrier in rats. Rapid cell death in the limb buds and neural tube of the rat embryos as well as severe inhibition of DNA synthesis in the embryo was found when a 500 or 1,000 mg/kg dose of hydroxyurea was administered to pregnant rats. Murphy and Chaube (1964) reported that administering hydroxyurea in doses above 250 mg/kg to pregnant rats increased fetal mortality as well as causing cleft palate, encephaly, harelip, deformed limbs and skeletal deformities. Treating pregnant gold hamsters on the eighth day of their pregnancy with a 5-10 mg/kg dose of hydroxyurea resulted in embryo malformations which included spina bifida, exencephaly, cardiac anomalies and a failure of neural closures (Ferm, 1966). Khera (1979) reported that administering a 100 mg/kg dose of hydroxyurea to cats, resulted in increased resorption sites and few live fetuses. A single 1,000 mg/kg dose of hydroxyurea administered to pregnant monkeys resulted in some of the fetuses showing skeletal and organ malformations which included missing and fused ribs, defects of vertebral number and shape, large kidneys, interrupted aortic arch and absent left pulmonary artery.

Long exposure (24-72 hours) of cultured Chinese hamster cells to hydroxyurea resulted in chromatid and chromosome breaks and chromosome translocations (Borenfreund, Krim & Bendich, 1964). Oppenheim and Fishbein (1965) reported that cultured human lymphocytes exposed to hydroxyurea at clinical doses, resulted in a large number of chromosome breaks.

Hydroxyurea has also been shown to have a number of effects on the mammalian testes. Seiler (1977) reported that hydroxyurea caused inhibition of DNA synthesis in the testes of mice. Lambert and Eriksson (1979) reported that hydroxyurea inhibited testicular DNA synthesis in rats. Wyrobek and Bruce (1975) reported that hydroxyurea induces abnormally shaped spermatazoa in mice. Ficsor and Ginsberg (1980) reported that hydroxyurea decreased sperm motility in a dose dependent manner. Mecklenburg, Hetzel, Gulyas and Lipsett (1975) reported that hydroxyurea caused multinucleated giant cells in the seminiferous tubules as well as sterile seminiferous tubules in rats that were administered the drug. There was also a significant decrease in testes weight as a result of the hydroxyurea treatments.

The histopathological results of the present experiments will provide further information about the toxicity and teratogenic capabilities of hydroxyurea.

Experiment 226-5D: Thalidomide

According to the accounts of Taussig (1962) and Lenz (1966) the synthetic drug thalidomide was first developed by Ciba

Pharmaceutical in 1956, when it was thought that it may be effective against influenza. However, Ciba dropped the drug when it appeared to have no effects on animals. In 1958, Gruenthal Pharmaceutical marketed thalidomide under the commercial name of Contergan after it was demonstrated to be an effective sleep inducer in man without any apparent signs of toxicity even at high doses. As Taussig (1962) relates:

> [Thalidomide] had a prompt action, gave a natural deep sleep and had no hangover. It appeared innocent and safe. Man could not commit suicide with it. [Thalidomide] became West Germany's most popular sleeping tablet and was widely used in hospitals and in mental institutions. It was used for grippe, neuralgia, asthma and as a cough medicine. It was also found useful as an antiemetic in pregnancy. The drug was manufactured "by the ton" and sold without prescription. (p. 1109)

Taussig (1962) reported that in 1959, in both Germany and Great Britain, there were suddenly more reported cases of a rare congenital limb malformation called phocomelia than had been reported in the whole 10 year period from 1949 to 1959. The incidence of phocomelia and other congenital limb and internal disorders increased successively in the years 1960 and 1961. On November 15, 1961, Dr. W. Lenz warned Gruenthal Pharmaceutical that thalidomide (Contergan) was probably responsible for the recent increase in infant limb malformations and congenital disorders seen in European hospitals and pediatric clinics since 1959. According to Taussig (1966), on November 26, 1961, Gruenthal Pharmaceutical withdrew thalidomide from the German market and a few days later, Distillers,

the British pharmaceutical company responsible for producing thalidomide in Great Britain, also withdrew the drug from further production. As Lenz (1966) relates:

> In the wake of [thalidomide's] triumph, thousands of despaired and mourning parents were left with their dead or crippled children. The incidence of certain types of malformations of the limbs and ears followed the sales figures of the drug by a distance of about three quarters of a year, as is expected on biological grounds. The hypothesis of a causative role of thalidomide has proved its predictive value when, by the end of July, 1962, the wave of malformations abruptly declined all over the Federal Republic of Germany and some what later in those countries which reacted less promptly to the warning. (p. 102) It is difficult to express any definite opinion about the total number of thalidomide victims. I think it is unlikely that less than 6,000 or more than 8,000 children have been affected. (p. 103)

There are numerous scientific and medical journal articles and letters reporting on the various types of congenital malformations associated with the mother's intake of thalidomide during the first trimester of her pregnancy. Several of these articles (McBride, 1961; Lenz, 1962; Pliess, 1962; Taussig, 1962; Lenz, 1966; Smithells, 1973) describe clearly the types of congenital limb and organ malformations induced by thalidomide, attesting to the teratogenic potential of thalidomide in humans.

In researching the teratogenic effects of thalidomide on laboratory animals (Somers, 1962; Spencer, 1962; Giroud, Tuchmann-Duplessis, & Mercier-Parot, 1962), pregnant rabbits were orally administered thalidomide during early periods of their pregnancies which corresponded to the critical period for thalidomide intake

in human pregnancy. The thalidomide treated rabbits showed increased numbers of resorption site, and their offspring contained increased numbers of stillborns and young with numerous limb and skeletal deformities. Increased numbers of fetal resorptions, stillborns and offspring with limb and skeletal malformations were also found in the litters of pregnant mice that had been administered thalidomide during early periods of their pregnancies (Giroud et al., 1962; DiPaolo, Gotzek & Pickren, 1964). Hamilton and Poswillo (1972) administered thalidomide to pregnant marmosets and found that the embryos and fetuses obtained after hysterotomy of the pregnant marmosets, revealed many limb and skeletal deformities. Delahunt and Lassen (1964) treated pregnant monkeys with thalidomide and found that the fetuses of two of the four treated monkeys, showed malformations that "were anatomically identical to the deformities reported in children whose mothers had taken thalidomide during pregnancy" (p. 1305). The effects of thalidomide on the offspring of rats that were treated with the drug during their early pregnancy, (Bignami, Bovet, Bovet-Nitte & Rosnati, 1962; Pliess, 1962; Giroud et al., 1962) were not as severe as those effects found in thalidomide treated mice and rabbits. Though the drug did increase the number of fetal resorptions in the treated pregnant rats, there were only a few malformations found in the litters of offspring. Bignami et al. (1962) reported finding two pups in the litters of treated rats that showed a rear limb and tail malformation. However,

Pliess (1962) and Giroud, et al., (1962) reported finding no gross malformations in the offspring of the thalidomide treated rats. The results of the rat experiments make clear that several species should be tested when screening a drug for teratogenic effects.

In reference to the mutagenic effects of thalidomide, Pfeiffer and Kosenow (1962) reported that after looking at tissues of 14 infants who were afflicted with phocomelia as a result of their mothers' intakes of thalidomide, no chromosome abnormalities were discovered in the tissues of these infants. Hughes, Delhanty, Chitham, Playfair and Hopper (1962) reported finding no chromosomal aberrations in primary tissue cultures of skin, lung, kidney and testes made from the aborted foetuses of mothers who had taken thalidomide during the critical period of their pregnancy. Luers (1962) found no evidence of mutagenesis in the spermatazoa or earlier germ cells of drosophila that had been fed a thalidomide solution. Roux, Emerit and Taillemite (1971) reported finding increased numbers of chromatid and chromosome breaks and chromatid exchanges in human blood cultures treated with thalidomide. Roux et al., (1971) also reported finding an increased number of chromatid gaps and breaks in tissues of rabbit embryos whose mothers had been treated with thalidomide.

The present experiment is concerned with the effectiveness of thalidomide, at various dosages, to induce histopathological changes in testes and/or epididymides of male CF-1 mice. The

results of this experiment will provide further information about the toxicity and teratogenic capabilities of the drug.

Experiment 226-5E, 226-5F, 226-5G: Presnisolone

The synthetic glucocorticoid prednisolone (1-dehydro hydrocortisone), like its natural analogue cortisone, is therapeutically administered for its antiinflamatory effects. One negative effect of prednisolone, like cortisone, is that prednisolone has been shown to have teratogenic potential in several species of animals. Pinsky and DiGeorge (1965) injected pregnant A/Jax mice with 1 mg of prednisolone per day on days 11, 12, 13, and 14 of their pregnancies. Of the viable embryos, 77% showed a cleft palate. When prednisolone was applied ocularly to mice (Ballard, Hearney & Smith, 1971) there was an increased incidence of cleft palate in the embryos of the treated female mice. Ohtori (1971) reported an increased incidence of fetal resorptions and increased incidence of cleft palate occurring in the embryos of mice treated with prednisolone. Walker (1967) reported that prednisolone induced cleft palate in the embryos of pregnant rabbits. The incidence of cleft palate increased with the dosage of prednisolone administered to the pregnant rabbits, until there was complete litter resorption at 8.0 mg of prednisolone per day. Shah and Kilistoff (1976) reported increased fetal resorptions and an increased incidence of cleft palate in the embryos of Syrian hamsters as the administered dosage of prednisolone was increased. When prednisolone acetate was administered to pregnant

rats (Kalter, 1962) none of the offspring showed cleft palates although the offspring did show a protruding tongue, a short upper jaw and high-domed head. Walker (1971) also reported that prednisolone was not effective in inducing cleft palate in rat embryos although an increased incidence of arched palate was found in the embryos. Dostal and Jelink (1971) proposed that prednisolone was ineffective in inducing a cleft palate in rats because of a placental barrier effect. When prednisolone was injected directly into the amniotic fluid, 44% of the rat embryos showed a partial or complete cleft palate. Blackburn, Kaplin and McKay (1965) reported that 5 mg of prednisolone administered to pregnant rats on a daily basis, resulted in an increased number of resorption sites and dead fetuses. The mean placenta weights of the treated animals were significantly less than the control animals and severe morphological changes occurred in the placentas of the treated rats. The morphological changes that occurred "undoubtedly alter placental function severely and are reflected by a significant increase in intrauterine fetal death" (Blackburn et al., p. 246).

Prednisolone has also been shown to have negative effects on the reproductive capacities of humans. Warrel and Taylor (1968) reported that in the cases of 30 women receiving 2.5 to 30.0 mg of prednisolone daily during their pregnancies, there were eight resulting still births and nine other fetuses which were at "risk during pregnancy or parturition" (p. 117). The cause of risk to

the fetuses appeared to be failure of placental function. Warrel and Taylor (1968) suggested that similar findings in rats (Blackburn et al., 1965) support their hypothesis of failure of placental function. Mancini, Lovieri, Muller, Andrada, and Saraceni (1966) reported that prednisolone had negative effects upon spermatogenesis in men. After treating four normal males with 30 mg of prednisolone for 30 consecutive days, tissue biopsies of the testes showed an arrest of spermatogenesis predominantly at the spermatid stage. The biopsies also showed a sloughing of the germinal cells into the lumens of the seminiferous tubules. No equivalent effects were found in the males given 10 mg of prednisolone daily for the same 30 day period.

The present three experiments are concerned with the effectiveness of prednisolone, at various dosages and sacrifice periods, to induce histopathological changes in the testes and/or epididymides of male CF-1 mice. Histopathological changes in the testes and epididymides of prednisolone treated mice will provide information about the toxicity of the drug as well as a possible teratogenic potential in the spermatazoa of the treated animals.

Experiment 226-5H: Streptomycin Sulfate

In 1944, Schaatz, Bugie and Waksman discussed a new antibiotic by the name of streptomycin. Streptomycin proved to be effective against Mycobacterium tuberculosis, however vestibular disturbances and hearing loss was noted in some of the human patients who were administered the antibiotic. These same negative effects were also

found in some laboratory animals that were administered streptomycin. Molitor, Graessle, Kuna, Mushett, and Silber (1946) reported on the ability of streptomycin to induce vestibular disturbance and/or hearing loss in different species of laboratory animals. Streptomycin was administered to mice, rats, guinea pigs, monkeys, and dogs at various dosages. Only the dogs demonstrated vestibular dysfunction and auditory problems. Hawkins (1947) administered large doses of streptomycin sulfate or streptomycin hydrochloride to 12 rabbits and 2 cats. Both of the cats and six of the rabbits showed a loss of postrotatory nystagmus as well as disturbances of posture and gait. The cats also showed a severe loss of hearing. Stevenson, Alvord and Correll (1947) reported that post mortem examination of three dogs that had received large doses of streptomycin, revealed lesions in the ventral cochlear nuclei of the eighth cranial nerve which would account for hearing loss induced by the antibiotic. Igarashi (1973) administered 50 mg of streptomycin sulfate, daily, to squirrel monkeys until the monkeys demonstrated a vestibular dysfunction as determined by body equilibrium tests. The monkeys were sacrificed and examined for pathological changes six months after the onset of vestibular dysfunction. Post mortem surgery revealed that the streptomycin sulfate had caused injury to type I hair cells of the ampullar cristae of the vestibular apparatus as well as causing injury to the hair cells of the spiral organ in the cochlea. Stevenson et al. (1947) reported that post mortem

examination of human patients who had become partially or totally deaf while receiving large doses of streptomycin for therapy, revealed lesions in the ventral cochlear nuclei of the eighth cranial nerve in each of the five cases. The authors concluded that these pathological changes could contribute to the deafness caused by the streptomycin. Fowler and Seligman (1947) reported on 81 patients who had received streptomycin therapy. Of the 81 patients examined after the streptomycin therapy, 53 patients had audiograms which showed a high tone loss. Three of the 81 patients demonstrated a vestibular disturbance during or shortly after the streptomycin therapy. Northington (1950) reported on 56 patients who experienced vestibular disturbances as a result of streptomycin therapy.

Woltz and Wiley (1945) reported that streptomycin, administered to women near the term of their pregnancies, would pass through the placenta and could be found in the amniotic fluid and in the umbilical cord blood 19 minutes after the mothers had received the intravenous injection of the streptomycin. Due to the effects of streptomycin in causing hearing loss and vestibular disturbances in adult patients and also to the fact that streptomycin was shown to cross the placenta from the mother to the fetus, it was feared that streptomycin administered to pregnant women for tuberculosis could result in vestibular or hearing damage to the infant. Watson and Stow (1948) reported finding no hearing or vestibular problems in the infants born to two mothers who had received 2 grams of streptomycin per day during their

second trimester of pregnancy. The two pregnant women had remained on the streptomycin therapy for 90 and 95 days respectively. Robinson and Combdon (1964) reviewed a number of reports that children may have suffered a hearing loss as a result of the mother being put on streptomycin therapy during pregnancy. The authors believed that sufficient evidence existed to indicate that hearing loss in some children was due to fetal contact with streptomycin that had crossed the placenta from the mother's circulation. However, it was evident from the reports that not all fetuses exposed to streptomycin were born with a hearing loss nor did they develop a hearing loss later. Conway and Birt (1965) reported on 17 children, aged 6-13 years, whose mothers were put on streptomycin during pregnancy for treatment of tuberculosis. Eight of the 17 children were found to have minor abnormalities of the eighth cranial nerve. Of the eight affected children, caloric tests were abnormal in six children and audiograms were abnormal in four, although complete deafness was not found in any of the eight children in the range of the speech frequencies.

Savkovic, Pecevski and Djelineo (1975) treated male mice with streptomycin and then mated the treated males with untreated females. Twelve weeks after birth, the offspring from the matings were examined cytologically for chromosome aberrations. The authors concluded that there "was no mutagenic effect in the animals treated with streptomycin" (p. 206).

The present experiment is concerned with the effectiveness of streptomycin sulfate, at various dosages, to induce histopathological changes in the testes and/or epididymides of male CF-1 mice. The results of this experiment will provide further information about the toxicity and teratogenic capabilities of the antibiotic.

Experiment 226-5L, 226-5K: Mitomycin-C

Mitomycin-C is an antibiotic that has been found to be an effective anti-tumor agent in the treatment of certain cancers. Mitomycin-C inhibits DNA synthesis by producing cross links between the complementary strands of the DNA double helix. Apparently the mitomycin-C cross links prevent separation of the complementary strands during mitosis and cell death results. It is this cross linking property and its cell lethal effect that makes mitomycin-C an effective agent against rapidly dividing tumors (Vig, 1977).

Mitomycin-C was reported to inhibit mitosis and cause breaks and exchanges in the chromosomes of cultured human leukocytes (Cohen & Shaw, 1964; Nowell, 1964). Mitomycin-C has also been shown to induce chromatid exchange in the spermatogonia of mice (Adler, 1973) and chromatid breaks and fragments in the primary spermatocytes of mice. Chromosome aberrations have been induced in monkeys as a result of a single injection of 1 mg/kg dose of mitomycin-C (Ficsor, Malling, Michelmann, Feldman, & Fry, 1979). Allen and Latt (1976) reported that a single 5.0 mg/kg dose of mitomycin-C increased the frequency of sister chromatid exchanges in mouse spermatogonia to

four times that of the controls. Perry and Evans (1975) reported that mitomycin-C induced the frequency of sister chromatid exchanges in cultured Chinese hamster fibroblasts. Seino et al (1978) reported that mitomycin-C gave a positive mutation test using Salmonella typhimurium. Hitotsumachi, & Kikuchi (1976) reported the induction of dominant lethals in mice by mating mitomycin-C treated male mice, seven weeks after treatment, with virgin female mice. Ehling (1970) reported mitomycin-C induced dominant lethal mutations in early spermatids and spermatocytes of mice. Wyrobek and Bruce (1975) reported the induction of abnormal sperm morphology in mice treated with mitomycin-C and Ficsor and Ginsberg (1979) reported that mitomycin-C caused reduced motility of the spermatazoa from the treated animals.

Kratochvilova (1973) reported that male mice injected with a 3.5 mg/kg dose of mitomycin-C had decreased fertilizing ability from day 21 to day 42 post injection, followed by 9 days of complete sterility before the fertilizing ability of the treated mice started to increase. Leonard and Gilliavod treated male mice with a single injection of 5 mg/kg of mitomycin-C noted a significant decrease in testes weight in the animals sacrificed at 50 days post injection. Histological examination of the testes of the treated mice showed a correlation between loss of germ cells and decreased testes weights. Leonard and Gilliavod (1973) postulated that the mitomycin-C killed most of the spermatogonial cells in the treated mouse testes and that spermatazoa, spermatids, and spermatocytes "disappear

progressively from the testes because of the failure of replacement by the spermatogonia" (p.223).

The present experiments is concerned with the potential of mitomycin-C at selected doses and post injection intervals, to induce histopathological changes in the testes and epididymides of treated CF-1 mice. The histopathological results will provide further information about the toxicity and teratogenic potential of mitomycin-C.

Experiment 226-8A: Adriamycin

Adriamycin is an aminoglycosidic antibiotic isolated from Streptomyces peucetius var. cesius and chemically classified as an anthracycline. Adriamycin has been shown to be an effective agent in cancer chemotherapy against several different types of solid tumors (VanDyk, VanDermerwe, Falkson, & Falkson, 1976). Apparently the adriamycin molecules exert their anti-tumor effects by intercalating between two complementary strands of deoxyribonucleic acid (DNA) molecules. The intercalation of the adriamycin molecule between the DNA strands causes distortion and uncoiling of the double helix DNA molecule resulting in the inhibition of nucleic acid synthesis by interfering with the template DNA function (Vig, 1977).

In an animal study, Schwartz and Grindey (1973) reported that adriamycin increased the average survival times of DBA/2 mice that had been inoculated with murine leukemia (p. 288). Barranco and Novak (1974) reported that adriamycin was effective in increasing the

frequency of cell death of cultered Chinese hamster ovary cells that were in a plateau phase period. Barranco and Novak (1974) postulated that since plateau cells in a tumor are generally not killed by anticancer drugs, these cells are probably responsible for regrowth of the tumor, and therefore, their experimental data suggested that adriamycin "may be an effective agent for use on slowly growing solid tumors which contain large fractions of non-dividing cells" (p. 1616). Marks and Venditti (1976) reported that the effectiveness of adriamycin in inhibiting the progress of L1210 lymphoid leukemia in DBA/2 mice was significantly increased when adriamycin was injected into the mice in combination with autoclaved herring sperm DNA.

It is now recognized that many antitumor agents while effectively causing remission of tumors, are themselves carcinogenic causing malignant tummors in laboratory animals (Weisburger, Griswold, Prejean, Casey, Wood, & Weisburger, 1975). Marquardt, Philips and Sternberg (1976) reported that adriamycin was effective in inducing mammary tumors in female rats as the result of a single 5 mg/kg dose of the drug. Marquardt et al. (1976) also reported that adriamycin was able to cause malignant transformation of cultured mouse fibroblasts as well as inducing genetic mutations in Chinese hamster cells. Price, Suk and Skeen (1975) reported that adriamycin had transforming potential in cultured rat embryo cells.

There are numerous scientific articles available that attest

to the genotoxic effects of adriamycin on the chromosomes of treated cells (Vig. 1977). Hsu, Pathak, and Kusyk (1975) reported finding chromatid and chromosome lesions in cultured mouse cell lines after introducing adriamycin into the medium. Perry and Evans (1975) reported finding an increased frequency of chromosome and chromatid aberrations and sister chromatid exchanges (SCE) in cultured Chinese hamster ovary cells after the cells were exposed to adriamycin. Nevstad (1958) reported finding an increased frequency of SCE in cultered human lymphocytes as well as increased chromosomal aberrations as a result of introducing adriamycin into the culture medium. The mutagenic assays using Salmonella typhimurium (Seino, Nagao, Yahagi, Hoski, Kawachi, & Sugimura, 1978).

Lu and Meistrich (1979) reported that a single injection of adriamycin was effective in killing the stem and differentiated spermatogonia in mice that were administered the drug. Au and Hsu (1980) reported that a single injection of adriamycin at doses of 3 or 12 mg/kg caused cell death of the spermatagonial cells in mouse testes and eventual sterility of the seminiferous tubules. Histological examination of the testes of the mice treated with the 12 mg/kg dose, showed sterility of the seminiferous tubules even as late as 120 days post injection. However, the testes of the mice treated with 3 mg/kg of adriamycin, showed a slow recovery of the germinal epithelium between 30 and 70 days after the single injection of the drug. The slow recovery of the germinal epithelium indicates that although a large number of spermatogonial

cells were killed by the 3 mg/kg dose of adriamycin, enough survived to repopulate the seminiferous tubules with germ cell types. Testicular degeneration was also reported in dogs and rhesus monkeys after treatment with adriamycin (Gralla, Fleischman, Luthra, & Stadnicki, 1979).

Adriamycin was reported to be teratogenic in rats but not in rabbits (Thompson, Molello, Strebing, & Dyke, 1978). Necropsy of pregnant rats that had been treated with adriamycin early in their pregnancy, revealed fetuses with esophageal and intestinal atresea, various cardiac anomalies, tracheo-esophageal fistula and hyproplasic of the urinary bladder.

The histopathological results of the present experiment will provide further information about the toxicity and teratogenic capabilities of the antibiotic.

MATERIALS AND METHODS

The animals used in the present project were CF-1 male mice supplied by the Upjohn Company. All of the animals were housed in plastic cages with a commercial bedding of wood shavings. Water and Purina Mouse Chow were supplied ad libitum to all animals. The room in which the animals were housed was kept at a thermostatically controlled temperature of seventy-two degrees Fahrenheit and light periodicity was automated at twelve hours of light followed by twelve hours of darkness.

Animal Dosing and Sacrifice

In the various phases of the present project, the drugs were administered to the animals on an acute or sub-acute dosing schedule. The animals on the sub-acute schedule were administered a single injection of a drug, once per day, for five consecutive days. The animals on the acute schedule were administered a single injection of a drug, one time. Concurrent with the dosing schedules of the treated animals, the control animals were injected with vehicle only.

In reference to the drugs prednisolone, cyproterone acetate, and streptomycin sulfate, this investigator was personally responsible for selecting the drugs, selecting a suitable injection vehicle, weighing out the drugs, mixing the drugs in the vehicle, dosing the animals by subcutaneous injections, taking care of the animals, weighing and recording animal body weights, sacri-

ficing the animals and surgically removing the testes and epididymides. For the drugs hydroxyurea, mitomycin-C, adriamycin and polybrominated biphenyl (PBB), other project personnel were responsible for all of the aforementioned responsibilities. This investigator was also responsible for histologically processing all of the testes and epididymides in this project and assessing any drug related testicular and/or epididymidal histopathology.

In the four experiments using prednisolone or streptomycin sulfate, the animals that were randomly selected for the experiments ranged in age from nine to twelve weeks and had body weights ranging from 35 to 50 grams. An effort was made to use animals as close to the same birthdate as possible. The various dosages used, for the drugs prednisolone and streptomycin sulfate, were calculated so that 0.1 milliliter of the drug-vehicle mixture was sufficient for 50 grams of animal body weight. Prior to each injection, the animals were individually weighed on a triple beam balance and their body weights were rounded off to the nearest five grams. By using a one milliliter tuburculin syringe with ten, one-hundreth milliliter graduations between the one-tenth milliliter graduations, it was a simple task to correlate animal body weight with the correct volume of drug-vehicle mixture. For example, a 50 gram mouse received 0.10 milliliter drug-vehicle mixture; a 45 gram mouse received 0.09 milliliter drug-vehicle mixture; a 40 gram mouse received 0.08 milliliter of drug-vehicle mixture; and a 35 gram

mouse received 0.07 milliliter of drug-vehicle mixture.

Animals were sacrificed by cervical dislocation without anesthetics at a predetermined date which varied with the particular experiment from three to seventy days after the last injection.

Experiment 226-5b: polybrominated biphenyl (PBB). The animals were divided into four groups of nine animals per group with one group serving as control. Each of the three treated groups received either 25, 75, or 150 mg of PBB per kg of animal body weight (mg/kg), per dosing. The treated animals were administered a 0.3 milliliter mixture of the determined dosage of PBB in corn oil. The mixture was injected down the animal's esophagus using a syringe with a small diameter plastic tube over the tip as the gavage. All animals received one oral injection per day for five consecutive days with the control animals receiving 0.3 milliliter oral injections of corn oil. Three animals from each of the four groups were sacrificed at intervals of 7, 28, and 70 days following the fifth oral dosing.

Experiment 226-5c: hydroxyurea. The animals were divided into five groups of four animals per group with one group serving as control. Each of the four treated groups received either 125, 250, 500, or 1,000 mg/kg of hydroxyurea per dosing. The treated animals were injected intraperitoneally with a 0.5 milliliter mixture of the prescribed dosage of hydroxyurea in physiologic saline solution whereas the control animals received 0.5 milliliter intraperitioneal injections of the physiologic saline solution. All animals received

one injection per day for five consecutive days and were sacrificed 35 days following the fifth injection.

Experiment 226-5d: thalidomide. The animals were divided into four groups of four animals per group with one group serving as control. Each of the three treated groups received either 100, 200, or 400 mg/kg of thalidomide per dosing. The treated animals were injected intraperitoneally with a 0.5 milliliter mixture of the prescribed dosage of thalidomide in dimethyl-sulfoxide (DMSO) whereas the control animals received 0.5 milliliter intraperitoneal injections of DMSO only. All animals received one injection per day for five consecutive days and were sacrificed 35 days following the fifth injection.

Experiment 226-5e: prednisolone. The animals were divided into three groups of three animals per group and a fourth group of four animals. Each group of three animals received either 15, 30, or 150 mg/kg of prednisolone per dosing. The one group of four animals served as control. The treated animals were injected subcutaneously with a 0.1 milliliter mixture of the prescribed dosage of prednisolone in sesame oil whereas the control animals received 0.1 milliliter subcutaneous injections of sesame oil. The subcutaneous injections were alternated between the right and left flanks of the animals on the successive days of injections. All animals received one injection per day for five consecutive days and were sacrificed thirteen days following the fifth injection.

Experiment 226-5f: prednisolone. The animals were divided into two groups of five animals per group and two groups of six animals per group. The two groups with five animals per group received either 5 or 50 mg/kg of prednisolone per dosing. One of the two groups with six animals per group received 100 mg/kg of prednisolone per dosing and the other six animal group served as control. The treated animals were injected subcutaneously with a 0.1 milliliter mixture of the prescribed dosage of prednisolone in sesame oil whereas the control animals received 0.1 milliliter subcutaneous injections of sesame oil. The subcutaneous injections were alternated between the right and left flanks of the animals on the successive days of injections. All animals received one injection per day for five consecutive days and were sacrificed thirty days following the fifth injection.

Experiment 226-5g: prednisolone. The animals receiving prednisolone were divided into four groups of four animals per group with one group serving as control. Each of the three treated groups received either 5, 50, or 100 mg/kg of prednisolone. The treated animals were injected subcutaneously with either a 0.1 milliliter mixture of the prescribed dosage of prednisolone in sesame oil whereas the control animals received 0.1 milliliter subcutaneous injections of sesame oil. The subcutaneous injections were alternated between the right and left flanks of the animals on the successive days of injections. All animals received one

injection per day for five consecutive days and were sacrificed three days following the fifth injection.

Experiment 226-5h: streptomycin sulfate. The animals were divided into four groups of four animals per group with one group serving as control. The three treated groups received either 10, 50, or 200 mg/kg of streptomycin sulfate per dosing. The treated animals were injected subcutaneously with a 0.1 milliliter mixture of the prescribed dosage of streptomycin sulfate in distilled water whereas the control animals received 0.1 milliliter injections of distilled water. The subcutaneous injections were alternated between the right and left flanks of the animals on the successive days of injections. All animals received one injection per day for five consecutive days and were sacrificed thirty days following the fifth injection.

Experiment 226-5k: mitomycin-C and hydroxyurea. The animals were divided into three groups of sixteen animals per group with one group serving as control. Each of the two treated groups received either a single 0.5 milliliter intraperitoneal injection of 5 mg/kg of mitomycin-C in physiologic saline solution or a single 0.5 milliliter intraperitoneal injection of 2,000 mg/kg of hydroxyurea in physiologic saline solution. The group of control animals received a single 0.5 milliliter intraperitoneal injection of physiologic saline solution. Four animals from each of the groups were sacrificed at intervals of three, ten, seventeen, and thirty-five days

following the single injection.

Experiment 226-51: mitomycin-C. The animals were divided into four groups of five animals per group and a control group of ten animals. Each of the treated groups received either 0.5, 1.0, 1.5, or 2.0 mg/kg of mitomycin-C per dosing. The treated animals were injected intraperitoneally with a 0.5 milliliter mixture of the prescribed dosage of mitomycin-C in physiologic saline solution whereas the control animals received 0.5 milliliter intraperitoneal injections of physiologic saline solution. All animals received one injection per day for five consecutive days and were sacrificed thirty-five days following the fifth injection.

Experiment 226-8a: adriamycin. The animals were divided into a control group of three animals and a treated group of four animals which received 6.25 mg/kg of adriamycin. The treated animals received a single 0.5 milliliter intraperitoneal injection of 6.25 mg/kg adriamycin in physiologic saline solution whereas the control animals received a single 0.5 milliliter intraperitoneal injection of physiologic saline solution. All animals were sacrificed fifty-three days following the single injection.

Histological Processing

At sacrifice, the testes and epididymides were surgically removed from each animal in the project. The testes were weighed and then placed in Helly's fixative (see Appendix A for complete derivation) along with the caput portions of the epididymides.

A pilot study performed by this investigator prior to the present project, established that an incision through the tunica albuginea of the testis prior to fixation, caused severe disruption of the relative organization of interstitial tissue and seminiferous tubules. In an effort to prevent the introduction of histologic artifact to the internal morphology of the testis and thereby assure a more accurate assessment of any drug induced histopathology to the testis, the testes were placed in the fixative with the tunica albuginea (testicular capsule) intact except where the capsule was incised at the testis-epididymis (caput) junction. The movement of the Helly's fixative through the capsule and through the testis-caput incision was rapid enough to assure a thorough fixation of the mouse testis without apparent autolytic artifact.

The testes and epididymides remained in Helly's fixative for a period of 40 to 48 hours and were then transferred to 10% formalin-saline to prevent excessive hardening. Depending on the experiment being run, the tissues remained in 10% formalin-saline for 7 to 28 days and were then rinsed in running water for at least nine hours before being placed on an automatic tissue processing machine (auto-technicon: model-2A). While on the autotechnicon, the tissues were transferred through an ascending ethanol dehydration series (70% ethanol to 80% ethanol to 95% ethanol to 100% ethanol), followed by clearing with xylene and finally infiltrated with paraffin (Tissue Prep, M.P. 56.5°C). Both the testes and epididymides from the same

animal were then embedded in paraffin within the same tissue mold (Tissue Tek). The tissue blocks were then placed in a -15°C freezer compartment for storage until they could be sectioned.

All tissue blocks were sectioned at six micrometers (microns) using an American Optical rotary microtome (model No. 820) and an American Optical 180 millimeter double plano microtome knife. Six microns was determined as the most suitable thickness for sectioning since some of the tissues fixed in the above manner would fractionate when sectioned at five microns. The tissue sections were floated on a 50°C gelatinized water bath and then mounted on 25 millimeter X 75 millimeter No. 1 thickness glass slides using Mayer's egg albumen as the mounting adhesive. The slides were then placed on their edge in a 60°C oven for at least twelve hours or until most of the paraffin was removed from the slides.

Six slides, with three to four tissue sections per slide, were prepared for each animal in the project with the two best slides being selected for staining. One of the two slides was passed through a hematoxylin (Harris) and eosin-B staining series (see Appendix B for complete derivation) while the other slide was passed through a modified trichrome (Masson's) staining series (see Appendix C for complete derivation). The slides were coverslipped with 22 millimeter X 40 millimeter No. 1 thickness cover glasses. A xylene soluble synthetic resin mounting medium (Permount) made by Fisher Scientific Company, was used as an adhesive between the slides and the coverslips.

Histological Evaluation

It was the responsibility of this investigator to examine the histology of the testes and epididymides of each project animal and to determine if any histopathology was appearing in the tissue cross-sections. The criteria used to evaluate the epididymides were based on three histological phenomena observed in the epididymides of untreated CF-1 mice. These phenomena were, (a) the extensive volume of sperm present in the ductule lumen of the distal caput and proximal corpus epididymis, (b) the appearance of a small number of sloughed germ cells in the ductule lumen of the epididymis, and (c) the morphology of the ductule epithelium in the epididymis. The criteria used to evaluate the testes of project animals were based on two histological phenomena observed in the testes of untreated CF-1 mice. The two phenomena were, (a) the absence of sterile seminiferous tubules, and (b) the morphology of the seminiferous epithelium. The following is a more complete explanation of the above criteria that were used to evaluate the testes and epididymides of project animals for the presence of histopathology.

Volume of sperm in the ductule lumen of the epididymis. In the epididymides of the untreated mice, it was estimated by this investigator that fifty to ninty percent of the cross-sectional areas of the distal caput ductule lumen and proximal corpus lumen were occluded by a vast number of spermatazoa (Figure 1). Cross-sections of the proximal caputs revealed that the lumens of the ductus epididymidis and efferent

ductules were void of spermatazoa. The failure of spermatazoa to appear in the cross-sections of the ductus epididymidis and efferent ductules may be due to the same reason that spermatazoa do not often appear in cross-sections of the tubuli recti and rete testes, namely that in the live animal, the passage of spermatazoa through tubuli recti and rete testis is very rapid and as a consequence, difficult to observe in cross-section. Cross-sections of epididymides from project mice that did not display an extensive volume of spermatazoa in the lumens of the distal caput and proximal corpus ductule, were scored as representative of a histopathological condition.

Appearance of germinal cells in the epididymis. It is apparently normal for a small number of spermatids of primary spermatocytes to be lost from the seminiferous epithelium of the testes, and subsequently to appear in the ductule lumen of the epididymides. The examination of a number of epididymides from untreated CF-1 mice revealed a small number of spermatids intermixed with the spermatazoa in the ductule lumens of the distal caputs and proximal corpus epididymides. In addition to the spermatids a very small number of primary spermatocytes were also observed in the ductule lumens, however the primary spermatocytes appeared even less frequently in the cross-sections of the epididymides than did the spermatids. Cross-sections of epididymides from project mice that displayed large numbers of spermatids and/or large numbers of other types of pre-spermatazoa germ cells in the ductule lumens, were scored as

representative of a histopathological condition.

Morphology of the ductule epithelium. The ductule epithelium of the epididymis is composed of a pseudostratified columnar epithelium. The nuclei of the epithelial cells are predominantly located near the basement membrane of the epithelium and tufts of stereocilia extend into the ductule lumen from the adluminal surfaces of the cells. The height of ductule epithelium varies from one region of the epididymis to the adjacent regions. In the present study, three adjacent anatomical regions of the epididymides were examined. In the proximal caput, the ductule epithelium is composed primarily of tall columnar cells. In the distal caput, the ductule epithelium is composed primarily of columnar cells which are shorter than the epithelial cells of the proximal caput regions. The ductule epithelium of the proximal corpus region is composed of columnar cells even shorter than the epithelial cells of the distal caput region. Project animals that displayed ductule epithelium that was morphologically different from the ductule epithelium found in untreated CF-1 mice, were scored as representative of a histopathological condition.

Abscence of sterile seminiferous tubules in the testes. Microscopic examination of the testes from untreated CF-1 mice did not display sterile seminiferous tubules in the testicular cross-sections. Sterile seminiferous tubules were defined as tubules which were void of all germinal cell types found in the untreated

animals (i.e. spermatogonia, spermatocytes, spermatids, spermatazoa). Although lacking the germinal cells, the Sertoli cells were still visible in these sterile seminiferous tubules. In the untreated testes, it appeared that small groups of sterile tubules were present in the region where the testis joined the caput epididymis, but these groups of tubules were in fact cross-sections of straight tubules. The reason for the sterile appearance of the straight tubules is that in the transition zone where the seminiferous tubule merges with the straight tubule, the seminiferous epithelium disappears and only Sertoli cells remain giving the same appearance as sterile tubules. Project animal testes that displayed sterile seminiferous tubules in cross-sections, were scored as representative of a histopathological condition.

Morphology of the seminiferous epithelium. The testes of the untreated mice displayed seminiferous epithelium continuous from the basement membrane to the lumen of the tubule (Figure 2). No matter which cell association appeared in the seminiferous tubules of the untreated testes, the seminiferous epithelium did not appear vacuolated or fragmented. Fragmented seminiferous epithelium is characterized by the appearance of pre-spermatazoa germ cell types in the lumen of the tubule and discontinuous seminiferous epithelium due to the premature release of spermatids, spermatocytes and spermatagonia. Project animals that displayed vacuolated or fragmenting seminiferous epithelium were scored as representative of a histopathological condition.

Figure 1. Cross-section of the distal caput epididymis of untreated CF-1 mouse. Note the extensive volume of spermatozoa in the ductule lumens. X 200.

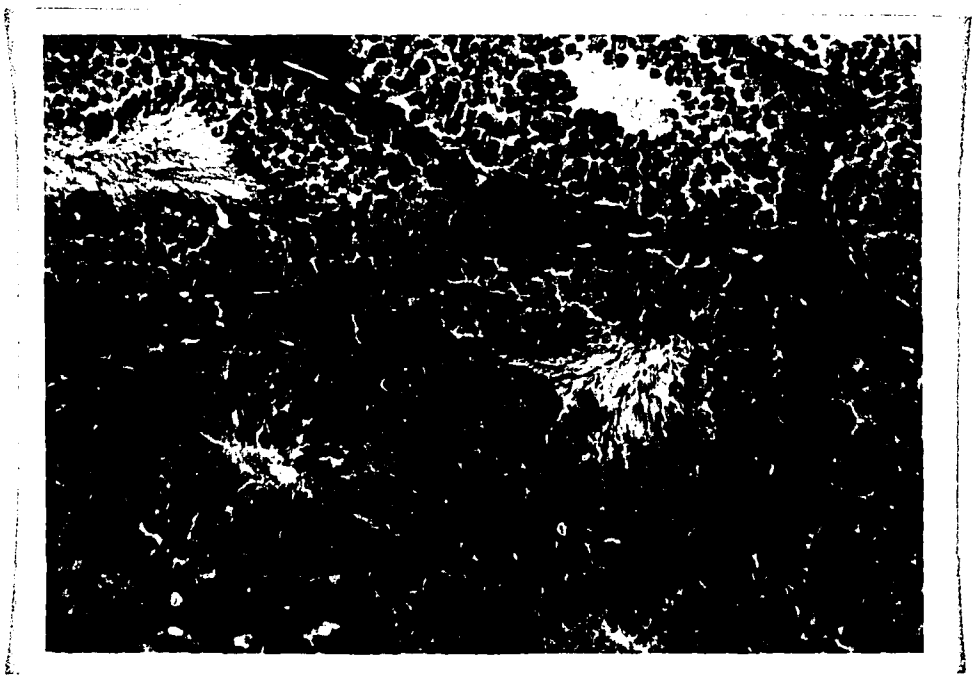

Figure 2. Cross-section of the testis of an untreated CF-1 mouse. Note the continuity of the seminiferous epithelium in the seminiferous tubules. X 200.

Statistical Analysis

Statistical analysis was performed on the testes weights of the treated and control animals in each experiment, by using a one-tailed analysis of variance. Differences between the mean testes weights of the control and treated mice were reported at a significance level of $P<0.05$.

RESULTS

Experiment 226-5B: Polybrominated Biphenyl (PBB)

Using the criteria selected by this investigator, there was no histopathology observed in the epididymides or testes of mice treated with PBB at any of the selected doses and post injection times (Table 1).

Experiment 226-5C: Hydroxyurea

In hydroxyurea treated animals there was histopathology in some of the epididymides and testes (Table 2). The extent of the histopathology was positively correlated with the total dose of the drug administered to the animals. At a total dose of 625 mg/kg there was no histopathology observed in either the epididymides or testes of any animals but a total dose of 1250 mg/kg caused histopathology in both the epididymides and the testes in two of the three treated animals. The histopathology consisted of a reduced volume of spermatozoa, an increased number of germ cells in the ductule lumen of the epididymides, and seminiferous tubules with fragmented germinal epithelium.

At a total dose of 2500 mg/kg, histopathology was found in the epididymides and testes of all three treated mice. Each animal had decreased volumes of spermatozoa and increased numbers of germ cells in the ductule lumens of the epididymides. As many as 25 germ cells (principally spermatids) were observed in a single cross-section of the ductule lumen. In the testes of the three

Table 1

Experiment Number 226-5B: Polybrominated Biphenyl (PBB)

Number of animals processed	Dose (mg/kg) 1 inj/day for 5 days	Interval from last injection to sacrifice (days)	Number of animals displaying positive histopathology					Mean testes weight (mg)
			Epididymidis			Seminiferous Tubules		
			Reduced volume of sperm in ductule lumen	Increased number of germ cells in ductule lumen	Atrophy of ductule epithelium	Presence of sterile tubules in testes x-sections	Fragmenting germinal epithelium	
3	0	7	0	0	0	0	0	227
3	25	7	0	0	0	0	0	195
3	75	7	0	0	0	0	0	230
3	150	7	0	0	0	0	0	227
3[a]	0	28	0	0	0	0	0	201
3[a]	25	28	0	0	0	0	0	239
3[b]	75	28	0	0	0	0	0	240
3	150	28	0	0	0	0	0	280
3[a]	0	70	0	0	0	0	0	245
3	25	70	0	0	0	0	0	274
3	75	70	0	0	0	0	0	274
3	150	70	0	0	0	0	0	249

[a]One animal died before processing.

[b]Two animals died before processing.

Table 2

Experiment Number 226-5C: Hydroxyurea

Number of animals processed	Dose (mg/kg) 1 inj/day for 5 days	Interval from last injection to sacrifice (days)	Number of animals displaying positive histopathology					Mean testes weight (mg)
			Epididymidis			Seminiferous Tubules		
			Reduced volume of sperm in ductule lumen	Increased number of germ cells in ductule lumen	Atrophy of ductule epithelium	Presence of sterile tubules in testes x-sections	Fragmenting germinal epithelium	
4[a]	0	35	0	0	0	0	0	279
4	125	35	0	0	0	0	0	220
4[a]	250	35	2	2	0	0	2	246
4[a]	500	35	3	3	0	2	3	163[c]
4[b]	1000	35	3	3	0	3	3	154[c]

[a]One animal died before processing.

[b]It is suspected that one of the four animals was not given the prescribed dosage of the drug.

[c]$P \leq 0.025$, significantly different from controls.

treated animals the germinal epithelium was fragmented in several of the seminiferous tubules and sterile seminiferous tubules were observed in two of the three treated animals. All of the sterile tubules were void of any germ cell types although Sertoli cells were still visible.

Three of the four animals that received a total dose of 5,000 mg/kg of hydroxyurea showed extensive histopathology in the cross-sections of their epididymides and testes. These three animals showed a very small volume of spermatazoa as well as increased numbers of germ cells in the ductule lumen of their epididymides (Figure 1). These same three mice showed a vast number of sterile and fragmented seminiferous tubules in their testes (Figure 2). However, one of the four animals originally selected to receive this dose, did not show histopathology in either its testes or epididymides.

Experiment 226-5D: Thalidomide

Using the criteria selected by this investigator, there was no histopathology observed in the epididymides or testes of the mice that received total doses of 500 or 2,000 mg/kg of thalidomide (Table 3). One of the three mice that received a total dose of 1,000 mg/kg did, however, show a noticeable loss of spermatazoa as well as an increase of germ cells present in the ductule lumens of the epididymides. In the testes of this animal, there were a few seminiferous tubules that showed fragmented epithelium.

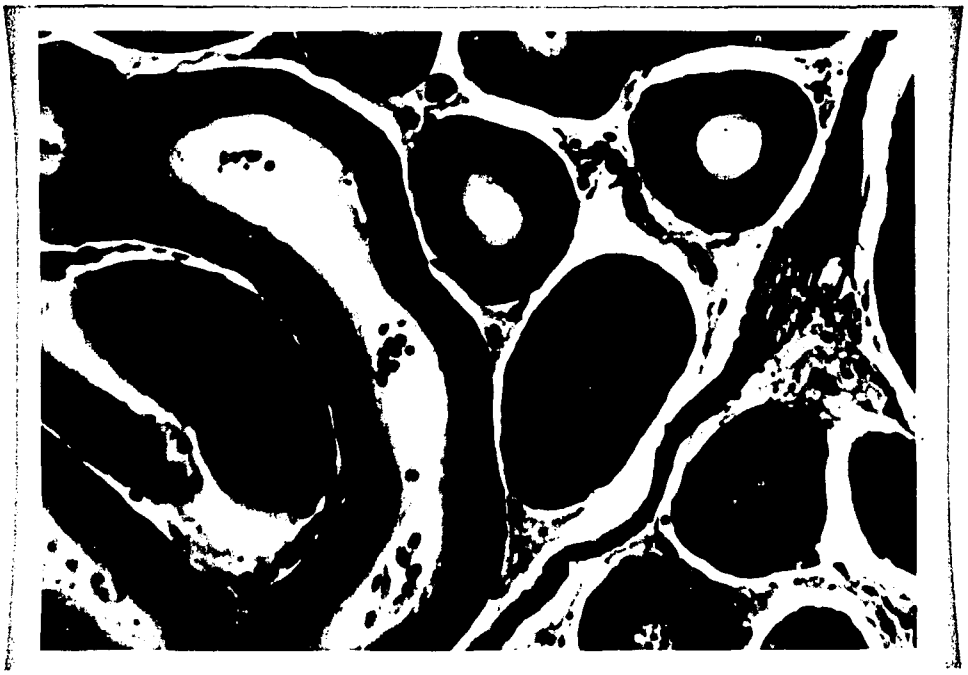

Figure 1. Cross-section of the distal caput epididymis of a CF-1 mouse that received a total dose of 5000 mg/kg of hydroxyurea. Note the lack of spermatozoa and the presence of germ cells in the lumen of the ductule.

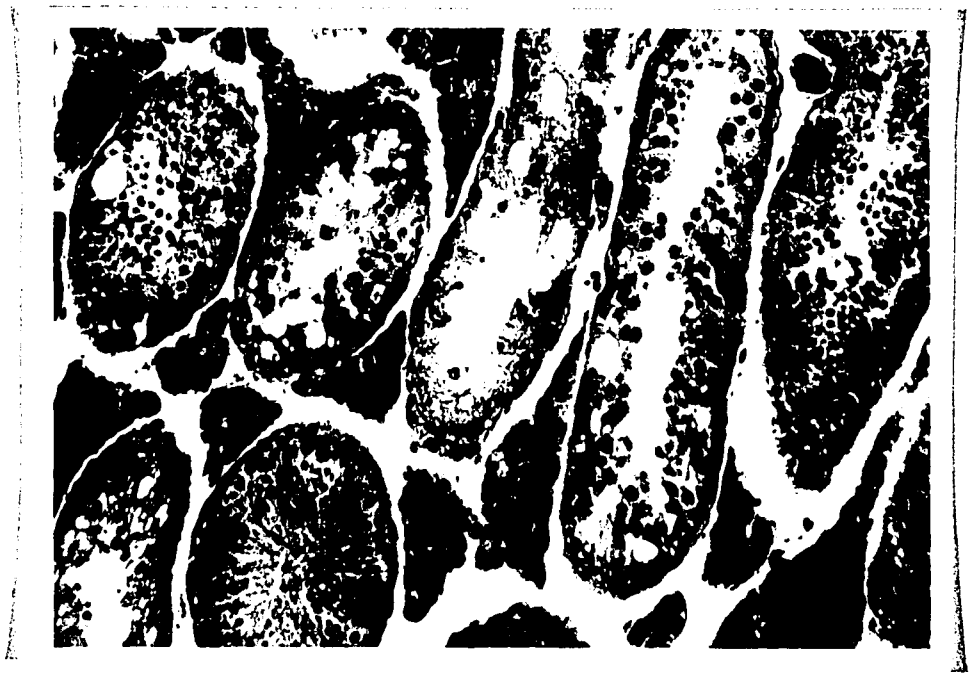

Figure 2. Cross-section of the testis of a CF-1 mouse that received a total dose of 5000 mg/kg of hydroxyurea. Note the fragmenting germinal epithelium in the seminiferous tubules. The upper center tubule has almost become a sterile tubule in which all of the germ cell types have been lost.

Table 3

Experiment Number 226-5D: Thalidomide

Number of animals processed	Dose (mg/kg) 1 inj/day for 5 days	Interval from last injection to sacrifice (days)	Number of animals displaying positive histopathology					Mean testes weight (mg)
			Epididymidis			Seminiferous Tubules		
			Reduced volume of sperm in ductule lumen	Increased number of germ cells in ductule lumen	Atrophy of ductule epithelium	Presence of sterile tubules in testes x-sections	Fragmenting germinal epithelium	
4	0	35	0	0	0	0	0	266
4[a]	100	35	0	0	0	0	0	343
4[a]	200	35	1	1	0	1	1	314
4[b]	400	35	0	0	0	0	0	312

[a]One animal died before processing.

[b]Two animals died before processing.

Experiment 226-5E: Prednisolone

Using the criteria selected by this investigator, there was no histopathology observed in the epididymides or testes of mice that received total doses of 150 or 750 mg/kg of prednisolone and were sacrificed 13 days following the last injection (Table 4). One of the two mice that received a total dose of 75 mg/kg and also sacrificed by the same schedule did, however, show a noticeable loss of spermatazoa as well as an increase of germ cells present in the ductule lumens of the epididymides. In the testes of this animal, there were a few sterile seminiferous tubules and also a large number of seminiferous tubules that showed fragmented and vacuolated germinal epithelium.

Experiment 226-5F: Prednisolone

Using the criteria selected by this investigator, there was no histopathology observed in the epididymides or testes of mice treated with total doses of 75, 150, or 750 mg/kg of prednisolone and sacrificed 30 days following the last injection (Table 5).

Experiment 226-5G: Prednisolone

Using the criteria selected by this investigator there was no histopathology observed in the epididymides or testes of mice that received a total dose of either 25 or 500 mg/kg of prednisolone (Table 6). One of the four mice that received a total dose of 250 mg/kg of prednisolone did show a severely decreased volume of spermatazoa, a large number of germ cells in the ductule lumens

Table 4

Experiment Number 226-5E: Prednisolone

Number of animals processed	Dose (mg/kg) 1 inj/day for 5 days	Interval from last injection to sacrifice (days)	Number of animals displaying positive histopathology					Mean testes weight (mg)
			Epididymidis			Seminiferous Tubules		
			Reduced volume of sperm in ductule lumen	Increased number of germ cells in ductule lumen	Atrophy of ductule epithelium	Presence of sterile tubules in testes x-sections	Fragmenting germinal epithelium	
4	0	13	0	0	0	0	0	b
3[a]	15	13	1	1	0	1	1	b
3	30	13	0	0	0	0	0	b
3	150	13	0	0	0	0	0	b

[a]One animal died before processing.

[b]No testes weights recorded for this experiment.

Table 5

Experiment Number 226-5F: Prednisolone

Number of animals processed	Dose (mg/kg) 1 inj/day for 5 days	Interval from last injection to sacrifice (days)	Number of animals displaying positive histopathology					Mean testes weight (mg)
			Epididymidis			Seminiferous Tubules		
			Reduced volume of sperm in ductule lumen	Increased number of germ cells in ductule lumen	Atrophy of ductule epithelium	Presence of sterile tubules in testes x-sections	Fragmenting germinal epithelium	
6	0	30	0	0	0	0	0	251
5	5	30	0	0	0	0	0	261
5	50	30	0	0	0	0	0	275[b]
6[a]	100	30	0	0	0	0	0	288[b]

[a]One animal died before processing.

[b]$P < .05$, significantly different from controls.

Table 6

Experiment Number 226-5G: Prednisolone

Number of animals processed	Dose (mg/kg) 1 inj/day for 5 days	Interval from last injection to sacrifice (days)	Number of animals displaying positive histopathology					Mean testes weight (mg)
			Epididymidis			Seminiferous Tubules		
			Reduced volume of sperm in ductule lumen	Increased number of germ cells in ductule lumen	Atrophy of ductule epithelium	Presence of sterile tubules in testes x-sections	Fragmenting germinal epithelium	
4	0	3	0	0	0	0	0	241
4	5	3	0	0	0	0	0	244
4	50	3	1	1	0	1	2	281
4	100	3	0	0	0	0	0	285

of the epididymides and several sterile and fragmented seminiferous tubules (Figure 3 & 4). One of the other three animals that received 250 mg/kg total dose of prednisolone, also had fragmented germinal epithelium in the seminiferous tubules but no additional histopathology was found in either the testes or epididymides. All animals in experiment 226-5g were sacrificed three days after the last injection.

Experiment 226-5H: Streptomycin Sulfate

Using the criteria selected by this investigator, there was no histopathology observed in the epididymides or testes of mice treated with streptomycin sulfate at any of the selected doses with a post final injection sacrifice time of 30 days (Table 7).

Experiment 226-5K: Mitomycin-C and Hydroxyurea

Using the criteria selected by this investigator, there was no histopathology observed in the epididymides or testes of mice treated with a single 2,000 mg/kg dose of hydroxyurea and sacrificed at post injection intervals of 3, 10, 17, and 35 days (Table 8). Also there was no histopathology observed in the epididymides or testes of mice treated with a single five mg/kg dose of mitomycin-C and sacrificed 3 and 10 days post injection. However, at 17 days after the injection of a single five mg/kg dose of mitomycin-C, three of the four animals injected, showed a few sterile seminiferous tubules dispersed in the testes and a few seminiferous tubules with fragmented germinal epithelium. At 35 days after a

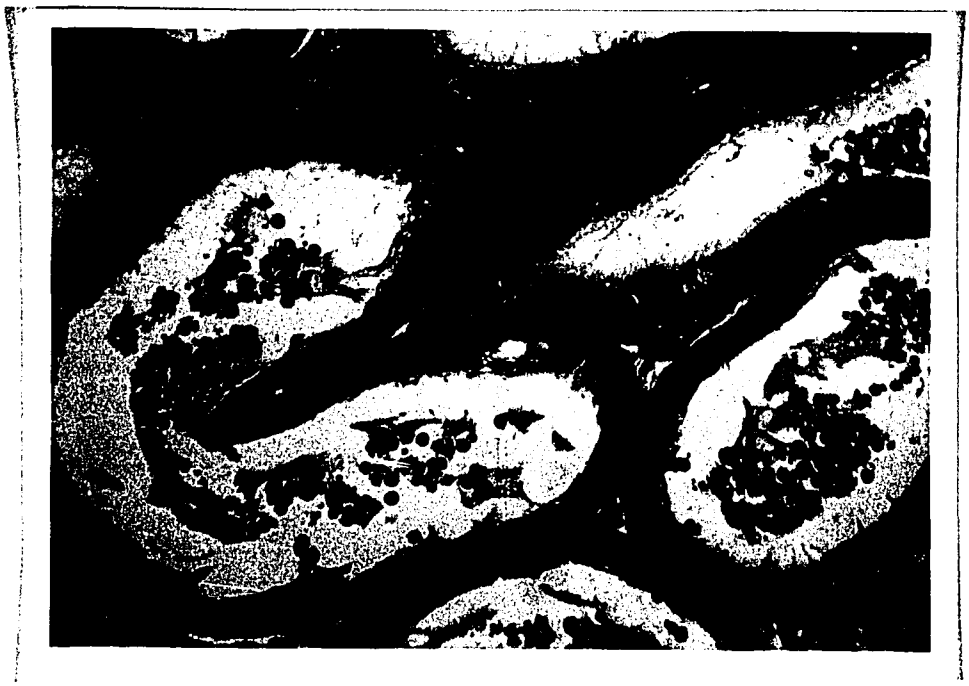

Figure 3. Cross-section of the distal caput epididymis of a CF-1 mouse that received a total dose of 250 mg/kg of prednisolone. Note the lack of spermatozoa and the presence of a vast number of germ cells lost from the germinal epithelium.

Figure 4. Cross-section of the testis of a CF-1 mouse that received a total dose of 250 mg/kg of prednisolone. Note the fragmented germinal epithelium due to loss of germ cells. The germ cells entering the lumens will now move into the ductule of the epididymis.

Table 7

Experiment Number 226-5H: Streptomycin Sulfate

Number of animals processed	Dose (mg/kg) 1 inj/day for 5 days	Interval from last injection to sacrifice (days)	Number of animals displaying positive histopathology					Mean testes weight (mg)
			Epididymidis			Seminiferous Tubules		
			Reduced volume of sperm in ductule lumen	Increased number of germ cells in ductule lumen	Atrophy of ductule epithelium	Presence of sterile tubules in testes x-sections	Fragmenting germinal epithelium	
4	0	30	0	0	0	0	0	255
4	10	30	0	0	0	0	0	273
4	50	30	0	0	0	0	0	277
4	200	30	0	0	0	0	0	296

Table 8

Experiment Number 226-5K: Mitomycin-C and Hydroxyurea

Drug & number of animals processed	Dose (mg/kg) 1 injection	Interval from last injection to sacrifice (days)	Number of animals displaying positive histopathology					Mean testes weight (mg)
			Epididymidis			Seminiferous Tubules		
			Reduced volume of sperm in ductule lumen	Increased number of germ cells in ductule lumen	Atrophy of ductule epithelium	Presence of sterile tubules in testes x-sections	Fragmenting germinal epithelium	
Mitomycin-C								
4	0	3	0	0	0	0	0	218
4	5	3	0	0	0	0	0	192
4	0	10	0	0	0	0	0	226
4	5	10	0	0	0	0	0	182[a]
4	0	17	0	0	0	0	0	228
4	5	17	0	0	0	3	3	163[a]
4	0	35	0	0	0	0	0	267
4	5	35	3	3	0	3	3	185[a]
Hydroxyurea								
4	0	3	0	0	0	0	0	218
4	2000	3	0	0	0	0	0	237
4	0	10	0	0	0	0	0	226
4	2000	10	0	0	0	0	0	221
4	0	17	0	0	0	0	0	228
4	2000	17	0	0	0	0	0	186
4	0	35	0	0	0	0	0	267
4	2000	35	0	0	0	0	0	229

[a] $p < .05$, significantly different from controls.

single five mg/kg injection of mitomycin-C, three of the four animals injected showed a decreased volume of sperm and increased numbers of germ cells in the ductule lumen of the epididymides. These same three 35 days post injection animals also showed a few dispersed sterile seminiferous tubules and several seminiferous tubules which had fragmented germinal epithelium due to the loss of germ cells.

Experiment 226-5L: Mitomycin-C

In mitomycin-C treated animals, which received five sequential injections and were sacrificed at 35 days post final injection, showed histopathology in the epididymides and testes at all of the selected doses. The extent of the histopathology observed, was positively correlated with the total dose of the drug administered to the animals (Table 9). At a total dose of 2.5 mg/kg the histopathology was observed primarily in the epididymides. Four of the five mice that received a total dose of 2.5 mg/kg showed an increased number of germ cells in the ductule lumens of the epididymides but only one of these five mice showed a decreased volume of spermatazoa in the ductule lumens of the epididymides. Only one of the five mice given a total dose of 2.5 mg/kg showed any histopathology of the testes and this was in the form of a few seminiferous tubules with fragmented germinal epithelium.

At a total dose of five mg/kg all five of the treated mice showed a decreased volume of spermatazoa and a large number of germ cells in the ductule lumens of their epididymides. It was also

Table 9

Experiment Number 226-5L: Mitomycin-C

Number of animals processed	Dose (mg/kg) 1 inj/day for 5 days	Interval from last injection to sacrifice (days)	Number of animals displaying positive histopathology					Mean testes weight (mg)
			Epididymis			Seminiferous Tubules		
			Reduced volume of sperm in ductule lumen	Increased number of germ cells in ductule lumen	Atrophy of ductule epithelium	Presence of sterile tubules in testes x-sections	Fragmenting germinal epithelium	
5	0	35	0	0	0	0	0	255
5	0.5	35	1	4	0	0	1	240
5	1	35	5	5	0	5	5	198[a]
5	1.5	35	5	5	5	5	5	124[a]
5	2	35	5	5	5	5	5	118[a]

[a]$P<0.05$, significant.

observed in the testes of these same five mice, that several seminiferous tubules were sterile as well as several seminiferous tubules with fragmented germinal epithelium.

A total doses of 7.5 mg/kg and 10 mg/kg of mitomycin-C all of the treated mice showed an almost complete absence of spermatozoa as well as a vast number of germ cells in the ductule lumens of their epididymides (Figure 5). At 7.5 mg/kg and 10 mg/kg total doses of mitomycin-C, histopathology in the ductule epithelium of the epididymis was observed. The simple columnar epithelium of the ductule was atrophic in many regions of the epididymis, a condition which was not seen in any other animals in the entire project. In the testes cross-sections prepared from the mice treated with total doses of 7.5 mg/kg or 10 mg/kg of mitomycin-C, between 40 and 90 percent of the seminiferous tubules were sterile (Figure 6). Most of the remaining seminiferous tubules were nearly sterile or showed severe loss of germinal epithelium, however, there were a few seminiferous tubules that showed very little damage to the germinal epithelium. A greater number of sterile seminiferous tubules were observed in the testes cross-sections of the mice that received a total dose of 10 mg/kg of mitomycin-C than in the testes cross-sections of the mice that received a total dose of 7.5 mg/kg of the drug.

Experiment 226-8A: Adriamycin

In comparison to all of the other mice which were given various

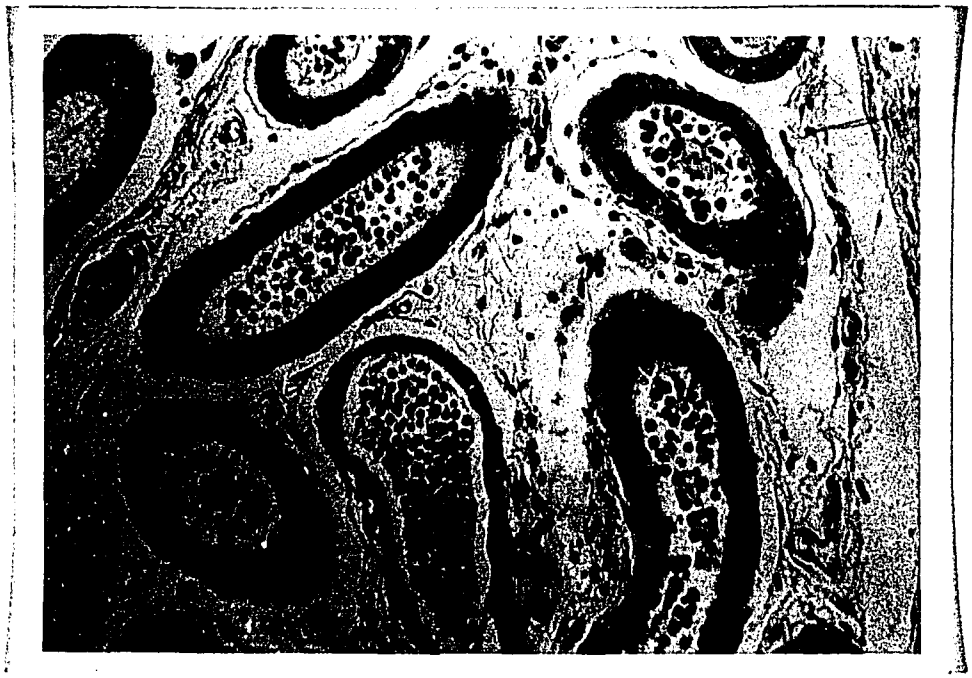

Figure 5. Cross-section of the distal caput epididymis of a CF-1 mouse that received a total dose of 10 mg/kg of mitomycin-C. Note the lack of spermatozoa and the large number of germ cells filling the ductule lumen. Note also the atrophic ductule epithelium in the lower center cross-section of the ductule.

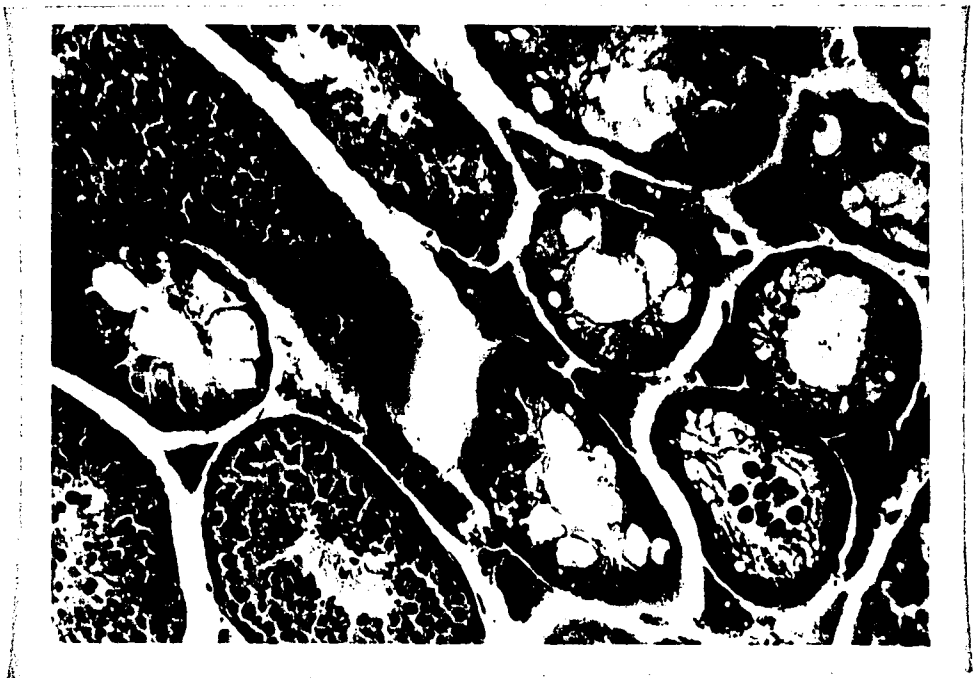

Figure 6. Cross-section of the testis of a CF-1 mouse that received a total dose of 10 mg/kg of mitomycin-C. Note the sterile seminiferous tubules and the seminiferous tubules with fragmenting germinal epithelium.

drugs in this project, the mice treated with adriamycin at a total dose of 6.25 mg/kg, showed the most extensive histopathology in the testes. The seminiferous tubules of the treated animals were devastated with almost every seminiferous tubule being sterile or very near to that point (Table 10). All that could be seen in the sterile seminiferous tubules were the Sertoli cells with their cytoplasmic processes (Figure 8). In only a very few seminiferous tubules could small areas of germinal epithelium be located.

In the epididymides of the treated animals, there was a complete absence of spermatazoa in the ductule lumen and contrary to even the control animals, there were no germ cells found in the ductule lumen (Figure 7). The lack of germ cells in the ductule lumen is understandable since only a few germ cells could be located in the testes of the treated animals. Unlike the mice that were treated with mitomycin-C at 7.5 mg/kg and 10 mg/kg total dose, the adriamycin treated mice showed no degeneration of the ductule epithelium in the epididymides.

Table 10

Experiment Number 226-8A: Adriamycin

Number of animals processed	Dose (mg/kg) 1 inj/day for 5 days	Interval from last injection to sacrifice (days)	Number of animals displaying positive histopathology					Mean testes
			Epididymidis			Seminiferous Tubules		
			Reduced volume of sperm in ductule lumen	Increased number of germ cells in ductule lumen	Atrophy of ductule epithelium	Presence of sterile tubules in testes x-sections	Fragmenting germinal epithelium	
3	0	53	0	0	0	0	0	247
4	6.25	53	4	4	0	4	4	61[a]

[a] $P < .001$, significant.

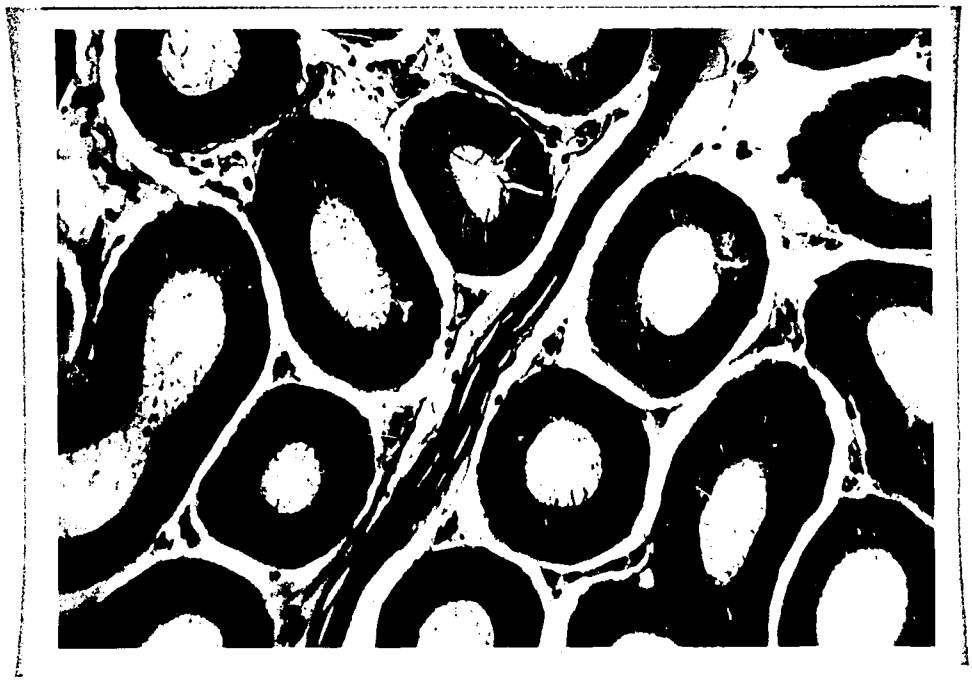

Figure 7. Cross-section of the caput epididymis of a CF-1 mouse that received a total dose of 6.25 mg/kg of adriamycin. Note the complete absence of spermatozoa and germ cells in the ductule lumen. X 200.

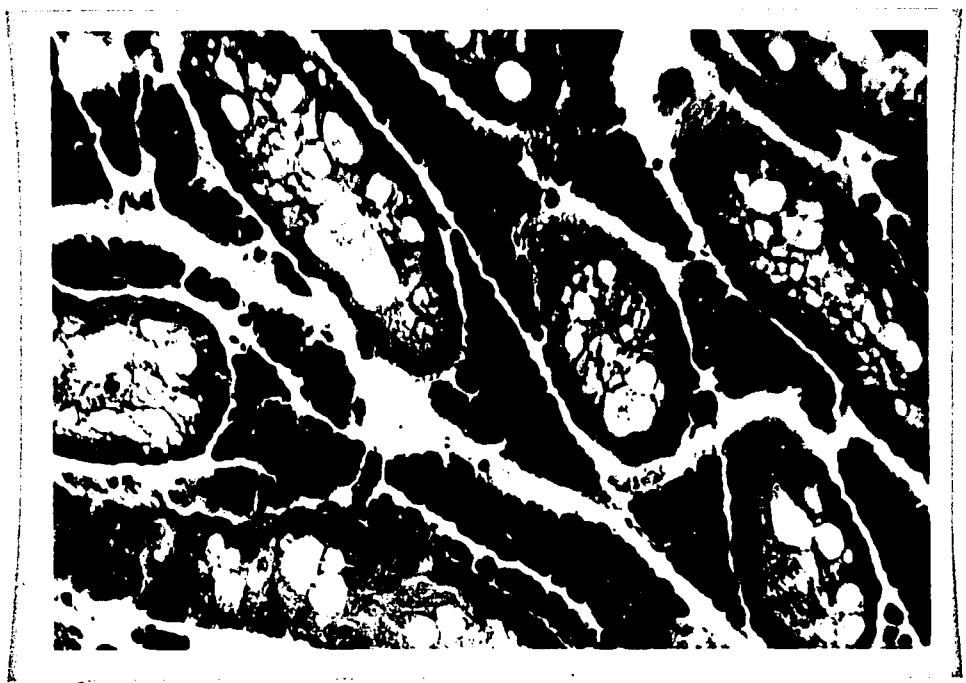

Figure 8. Cross-section of the testis of a CF-1 mouse that received a total dose of 6.25 mg/kg of adriamycin. Note that all of the seminiferous tubules are sterile.

DISCUSSION

Experiment 226-5B: Polybrominated Biphenyl (PBB)

Although single doses of 400 and 800 mg PBB/kg were shown to be embryolethal and teratogenic in pregnant rats (Beaudoin, 1977), the present experiment shows that subacutely administered dosages of PBB as high as 750 mg PBB/kg, were not apparently toxic to the testes and epididymides of CF-1 mice. A possible species specific action of PBB has been suggested (Corbett et al., 1975), and it is possible that even much higher dosages of PBB then those used in the present experiment, would not have resulted in histopathological changes occurring in the testes of the treated CF-1 mice.

Experiment 226-5C, 226-5K: Hydroxyurea

Experiment 226-5k: hydroxyurea. The results of this experiment show that a single injection of hydroxyurea at a dose of 2,000 mg/kg, was not apparently toxic to the germ cells of the testes or epithelium cells of the ductule in the epididymides of CF-1 mice that were examined at intervals of 3, 10, 17, and 35 days post injection.

Experiment 226-5c: hydroxyurea. The results of this experiment show that hydroxyurea, at a total dose of 625 mg/kg, was not apparently toxic to the germ cells of the testes or epithelium cells of the ductule in the epididymides of CF-1 mice that were examined 35 days post final injection. However, at total doses of 1250, 2500, and 5000 mg/kg, dose-dependent histopathology was observed in the testes and epididymides of the treated mice. Speculation by this investigator as to why a single 2,000 mg/kg dose of hydroxyurea (experi-

ment 226-5K) caused no histopathology in the testes or epididymides of treated mice whereas sub-acute dosing of hydroxyurea at a total dose of 1250 mg/kg did, may be related to the fact that hydroxyurea has been shown to be excreted in the urine in large unchanged percentages within a very short period of time after administration of the drug (Timpson, 1975). The sub-scute dosing schedule (experiment 226-5c) may actually have provided a greater exposure of the germ cells to hydroxyurea than in the case of the acute dosing (experiment 226-5k). Another possible explanation is that the sub-acute dosing schedule exposed a greater number of cycling spermatogonia to the hydroxyurea at the damaging S phase of their cell cycles.

Since hydroxyurea blocks synthesis of DNA in mitotically dividing cells, it is likely that the spermatogonial cells are affected by the drug. Since there was no chronological examination of the testes of hydroxyurea treated mice in this experiment, (experiment 226-5c), no evidence is available to suggest that hydroxyurea was toxic to spermatocytes, spermatids or spermatazoa in the treated mice. The fact that several seminiferous tubules in the testes had germinal epithelium remaining, at a post injection interval of 35 days, suggests that many stem spermatogonia were not affected by the hydroxyurea, at the selected doses, and normal germinal epithelium cycles were not interrupted in many of the seminiferous tubules of the treated mice.

Experiment 226-5D: Thalidomide

The results of this experiment show that although thalidomide is an effective teratogen in the females of several species, it is not very effective in inducing histopathological changes in the testes or epididymides of treated male CF-1 mice. No histopathology was observed in the epididymides or testes of the mice that received total dosages of 500 or 2,000 mg/kg of thalidomide. However, one of three mice that received a total dose of 1,000 mg/kg did show a noticeable loss of spermatazoa as well as an increase of germ cells present in the ductule lumens of the epididymides and a few seminiferous tubules with fragmented germinal epithelium in its testes.

Each of the three treated groups of mice in this experiment suffered no significant loss of testes weight, signifying no great loss of germ cells, as a result of the thalidomide treatments.

Experiment 226-5E, 226-5F, 226-5G: Prednisolone

The results show that prednisolone was not effective in inducing histopathological changes in the testes or epididymides of CF-1 mice administered a total dose of either 75, 150 or 750 mg/kg and then sacrificed 30 days after the last injection. There were also no histopathological changes found in the testes or epididymides of the mice that received a total dose of either 150 or 750 mg/kg of prednisolone and sacrificed 13 days after the last injection. Likewise, the mice that received a total dose of either 25 or 500 mg/kg of prednisolone and sacrificed 3 days after the last injection showed no histopathological changes in the testes or epididymides.

Although the three experiments show principally negative

results, there is some evidence, albeit inconsistent, that prednisolone can induce histopathological changes in the testes and epididymides of CF-1 mice. One of the two mice that received a total dose of 75 mg/kg of prednisolone and was sacrificed 13 days after the last injection, did show extensive loss of germinal cells and spermatazoa from the seminiferous tubules and there was an increased number of germinal cells in the ductule lumens of the epididymides. In addition, two of the four mice that received a total dose of 250 mg/kg and were sacrificed three days following the last injection showed fragmented and sterile seminiferous tubules. One of these four mice also showed a large number of germinal cells in the ductule lumens of the epididymides.

Prednisolone was shown to induce histopathology in the testes of men who received 30 mg of prednisolone for 30 consecutive days (Mancini et al., 1966). This calculates to a total dose of prednisolone of 900 mg or 13.2 mg/kg if the men averaged 150 pounds of body weight. In comparison, the mice in the present experiments received total doses of prednisolone as high as 500 or 750 mg/kg and still did not show histopathology in the testes. An obvious explanation for the discrepancy in the results for men and mice is that prednisolone is species specific in its capacity to induce histopathology in the testes. Another variable to be considered is that the positive results for the men may be related to the fact that the men were chronically dosed for 30 days whereas the mice were chronically dosed for only five days.

This investigator can offer no explanation for the fact that

some prednisolone treated mice, at some dosage levels, displayed testicular and/or epididymidal histopathology while other animals in the same sample displayed no evidence of pathology. There is also no clear explanation why the testes and epididymides of mice receiving the highest doses of prednisolone were free of pathology.

Experiment 226-5H: Streptomycin Sulfate

The results of this experiment show that streptomycin sulfate, at the dosages selected, was not effective in inducing histopathological changes in the testes or epididymides of the treated male mice. The results of this experiment are in agreement with the results of Molitor et al., (1946) who reported finding no pathological changes in mice that were treated with streptomycin.

The treated male mice in this experiment, suffered no significant loss of testes weight as a result of the streptomycin treatments. Such a weight loss in the testes would be indicative of a germ cell loss from the germinal epithelium and would have been detected in the histological cross sections of the testes and epididymides of the treated mice (See Results p. 49).

Experiment 226-5L, 226-5K: Mitomycin-C

Experiment 226-5L: Mitomycin-C. The mice were treated for five consecutive days with a single injection of mitomycin-C at doses of either 0.5, 1.0, 1.5, or 2.0 mg/kg per injection and then sacrificed 35 days post final injection. In this case, histological examination of the testes at 35 days post final injection revealed

the germ cell proliferation of the spermatogonial cells treated with mitomycin-C 35-40 days prior. The amount of histopathology observed in the testes and epididymides of the treated mice increased with the total dose administered to the animals. The mean testes weights of the treated groups decreased in a dose dependent fashion signifying greater losses of germ cells the greater the total dose of the drug administered. The positive histopathology found in the testes of the treated animals means that mitomycin-C has killed many spermatogonial cells, however, since all of the seminiferous tubules are not sterile, some spermatogonia have survived and are starting to repopulate the seminiferous tubules. These histopathological results of the testes, are in agreement with the results of Leonard and Gilliavod (1973).

It is significant that mitomycin-C of all the drugs used in this project, was the only drug to induce atrophy of the ductule epithelium in the epididymides of the treated mice. This investigator can offer no explanation as to how mitomycin-C exerted a toxic effect on the ductule epithelium of the epididymis.

Experiment 226-5k: mitomycin-C. In this experiment, the male mice were administered a single 5 mg/kg injection of mitomycin-C and then sacrificed at intervals of 3, 10, 17, and 35 days post injection. No histopathology was observed at 3 day post injection, however, positive histopathology was found in the testes at 17 and 35 days post injection. There is also a significant loss of mean testes weight in the treated group as compared to the mean testes

weight of the control group. The fact that there was no significant drop in testes weight at 3 days post final injection, suggests that the lethal effects of the drug on the spermatogonia have not yet resulted in significant depletion of the germinal epithelium of the testes in this treated group of mice.

Only at 35 days post injection, is histopathology observed in the epididymides suggesting that the full impact of germ cell loss from the seminiferous tubules is now being observed as reduced volumes of spermatazoa and increased numbers of sloughed germ cells find their way into the ductule lumen of the epididymids.

The results of this experiment is in agreement with the results of Leonard and Gilliavod (1973).

Experiment 226-8A: Adriamycin

The results of this experiment show that a single injection of adriamycin, at a dose of 6.25 mg/kg induced almost complete sterility in the testes of the treated mice. Histological examination of the testes and epididymides of the treated mice, 53 days post injection, showed that almost every seminiferous tubule in the testes was either sterile or very near to that point and that no spermatazoa or germ cells could be found in the ductule lumen of the epididymids. Since small areas of germinal epithelium could be located in a few of the seminiferous tubules of the treated mice, we cannot make the all encompassing statement that a single 6.25 mg/kg dose of adriamycin killed all stem spermatogonia in the testes. If this statement were true than we would have

found no germinal epithelium in any of the seminiferous tubules at 53 days post injection time. The observation of a small amount of germinal epithelium in the seminiferous tubules at 53 days post injection time reveals that a very small number of stem spermatogonia survived the adriamycin treatment and are slowly repopulating the seminiferous tubules with germ cell types. The testicular results of this experiment support the results reported by Au and Hsu (1980).

Unlike certain mitomycin-C treated mice, there was no observed degeneration of the ductule epithelium in the epididymides of adriamycin treated mice. These results do not preclude the possibility that the ductule epithelium of the adriamycin treated mice has regenerated itself since necropsy of these mice was performed 53 days post injection of adriamycin and the aforementioned mitomycin-C treated animals were sacrificed at 35 days post final injection.

CONCLUSIONS AND RECOMMENDATIONS

To those persons who may wish to pursue an extension of the present project or to those persons who may wish to use parts of the methods presented in this paper, I direct the following recommendations. Helly's fixative was selected as the primary fixative for the tissues used in this project because Helly's fixative provides excellent cytoplasmic and nuclear detail as well as enhancing the results of trichrome stains, one of which was used in the present project. If this investigator were to repeat this project, the testes and epididymides would not be allowed to remain in Helly's fixative for a period of 40 to 48 hours, as was done. Unnecessary hardening of the testes and epididymides resulted from leaving the tissues in the fixative for this extended period of time. Instead, 10% formalin-saline would be used as the primary fixative since the tissues could be left in this fixative for months without undue hardening. The tissues could then be transferred to Helly's fixative for a six hour period prior to histological processing. The benefits from following such a fixing procedure would be better and easier sectioning of the tissues with less fragmentation, without sacrificing any of the trichrome staining qualities.

It is also a recommendation that the sectioned tissues not be allowed to float out in a 50°C water bath as was done in this project. A lower temperature of 44°-46° would be preferable when

paraffin with a 56° melting point is used for infiltrating the tissues. Because of the hardness found in several of the tissues after being left in Helly's for 40 hours, curling and fragmentation of the tissues occurred upon sectioning. This investigator found that a water bath set at 50°C removed the curling in the tissue sections better than a lower temperature setting, however, close monitoring of the tissue sections while in the water bath is necessary since undue spreading of the tissues will result.

Although the method used for dosing the mice in the prednisolone and streptomycin sulfate experiments was fast and simple, some arguments can be made about the accuracy of the method. The total experimental error entailed in the dosing ranged from 15.3% to 22.0% depending on the weight of the animals used. Since the animals body weights were rounded to the nearest 5.0 grams, there is a possibility of including a rounding error of 2.5 grams for each weighing. An experimental error of 5.3% is introduced when a 47.5 gram mouse is rounded up to 50.0 grams (i.e. 2.5 grams rounding error/47.5 grams x 100 = 5.3%), and an experimental error of 7.7% is introduced when a 32.5 gram mouse is rounded up to 35.0 grams (i.e. 2.5 grams rounding error/32.5 grams x 100 = 7.7%). In addition to the weighing error, many of the one milliliter plastic syringes used for administering prednisolone and streptomycin sulfate had imprecise alignments of the rubber syringe plungers with the graduations on the syringe barrel. Because of this imprecise alignment as well as the difficulty in differentiating

between the 0.01 milliliter graduations during the injections, this investigator believes that a 0.01 milliliter error was incurred during the dosing of many of the animals. The imprecise plunger alignment would introduce an additional experimental error of as much as 10% for a 50 gram mouse (i.e. 0.01 milliliter graduation error/ 0.10 milliliter of drug-vehicle injection x 100 = 10%), and as much as 14.3% for a 35 gram mouse (i.e. 0.01 milliliter graduation error/0.07 milliliter of drug-vehicle injection x 100 = 14.3%). The total experimental error incurred with the dosing of prednisolone and streptomycin sulfate could therefore have ranged from a low of 15.3% (5.3% + 10%) for a mouse whose weight was rounded from 47.5 grams to 50.0 grams, to a high of 22.0% (7.7% + 14.3%) for a mouse whose weight was rounded from 32.5 grams to 35.0 grams. This investigator believes that the experimental error incurred by rounding the weights of the animals is acceptable but the experimental error incurred by using plastic syringes with imprecise plunger alignments is not acceptable. An effort should be made to make sure the plunger alignment is precise in the plastic syringes prior to injections or elect to use glass syringes with a more precise plunger alignment.

In summary of the results obtained in this project, the drugs adriamycin, hydroxyurea, and mitomycin-C caused extensive histopathology in the testes and epididymides of treated mice.

The drugs, PBB, prednisolone, streptomycin sulfate and thalidomide caused no or minimal histopathology in the testes and epididymides of treated mice. This investigator believes it significant that the three drugs that gave positive histopathological results are drugs capable of mutagenic changes to chromosomes, whereas the four drugs causing no or minimal histopathology to the testes and epididymides are drugs not renowned for their mutagenic potentials. However, this is not to suggest that only drugs with mutagenic potential can harm the germ cells in the mammalian testes as evidenced by the effect of PBB on bulls (Jackson & Halbert, 1974) and the effects of the antispermatogenic agent idenopyridine (Matter, Jaeger, Suter, Tsuchimoto & Deyssenroth, 1979).

Finally, this investigator believes that a better understanding of the effect that the drugs, adriamycin, hydroxyurea, and mitomycin-C have on the germ cells in the mammalian testes, could be obtained by an ultrastructural examination of the gap-junction that exists between the Sertoli cells and the spermatogonia and primary spermatocytes (McGinley, Posalaky, Porvaznik, & Russel, 1979). The gap-junction contains intercommunicating pores between the Sertoli cells and the germ cells and may be the method by which some nutrients and cell macromolecules are communicated to the spermatogonia and primary spermatocytes from the Sertoli cells. It may also be the method by which some drugs gain entry to spermatocytes in the adluminal compartment of the testes. The changes that occur

in the gap-junction, as spermatogonia and spermatocytes are affected by chemical teratogens, mutagens and other antispermatogenic drugs, could provide clues to effects of the drugs on the germ cells.

APPENDIX - A

Fixative solutions

Helly's Fixative

Stock solution:
- Potassium dichromate - - - - 25 grams
- Mercuric chloride - - - - - 50 grams
- Sodium sulfate - - - - - - 10 grams
- Distilled water - - - 1000 milliliters
- Dissolve all the salts together with gentle heat.

For use:
- stock solution 100 milliliters
- formalin 5 milliliters

10% Formalin-saline

- Formalin - - - - - - - - 100 milliliters
- Sodium chloride - - - - - - - - 9 grams
- Distilled water - - - - 900 milliliters

APPENDIX - B

Harris Hematoxylin and Eosin-B Staining Series

1)	Xylene	5 Minutes
2)	Xylene	2 Minutes
3)	100% ethanol	2 Minutes
4)	95% ethanol	2 Minutes
5)	70% ethanol	2 Minutes
6)	Distilled water	1 Minute
7)	Lugol's iodine solution	10 Minutes
8)	Running water	3 Minutes
9)	Sodium thiosulfate solution	3 Minutes
10)	Running water	5 Minutes
11)	Distilled water	1 Minute
12)	Harris hematoxylin	5 Minutes
13)	Running water	1 Minute
14)	70% ethanol	2 Minutes
15)	1% HCL in 70% ethanol	8 dips, 5 seconds each
16)	1% NH_4OH in 70% ethanol	3 Minutes
17)	Running water	3 Minutes
18)	80% ethanol	2 Minutes
19)	Eosin-B	2 Minutes
20)	80% ethanol	3 dips
21)	80% ethanol	3 dips
22)	95% ethanol	3 dips
23)	95% ethanol	3 dips
24)	100% ethanol	2 Minutes
25)	Xylene	5 Minutes
26)	Xylene	5 Minutes
27)	Coverslip with permount	

Solutions

1) Lugol's Iodine:
   - 400 milliliters distilled water } mix first
   - 8 grams potassium iodide } mix first
   - 4 grams iodine crystals } add to above using heat

2) Sodium thiosulfate solution:
   - 400 milliliters distilled water
   - 20 grams sodium thiosulfate

APPENDIX - C

Lillie's Modification of Mallory's Modification of Masson's Trichrome Staining Series

1)	Xylene	5 Minutes
2)	Xylene	2 Minutes
3)	100% ethanol	2 Minutes
4)	95% ethanol	2 Minutes
5)	70% ethanol	2 Minutes
6)	Distilled water	1 Minute
7)	Lugol's iodine solution	10 Minutes
8)	Running water	3 Minutes
9)	Sodium thiosulfate solution	3 Minutes
10)	Running water	5 Minutes
11)	70% ethanol	2 Minutes
12)	95% ethanol	2 Minutes
13)	Picric acid	5 Minutes
14)	Running water	7 Minutes
15)	Distilled water	1 Minute
16)	Weigert's iron hematoxylin	3 Minutes
17)	Running water	5 Minutes
18)	Biebrich scarlet	1 Minute
19)	Running water	5 Minutes
20)	Equal parts of 5% phosphotungstic acid and 5% phosphomolybdic acid. Agitate slides continuously.	1 Minute
21)	Fast green	2 Minutes
22)	1% acetic acid	1 Minute
23)	95% ethanol	1 dip
24)	100% ethanol	1 dip
25)	100% ethanol	1 Minute
26)	Xylene	5 Minutes
27)	Xylene	5 Minutes
28)	Coverslip with permount	

Solutions

1) 6% picric acid in 95% ethanol
2) 1% biebrich scarlet in 1% acetic acid
3) 5% phosphotungstic acid (aqueous)
4) 5% phophomolybdic acid (aqueous)
5) 2.5% fast green (FCF) in 2.4% acetic acid
6) 1% aqueous acetic acid solution
7) Lugol's iodine solution (see Appendix B)
8) Sodium thiosulfate solution (see Appendix B)

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