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Title INFLUENCE OF CERTAIN CHEMICALS ON THE SENSITIVITY OF RAT EMBRYOS TO X-IRRADIATION

Abstract approved

The effect of 2-aminoethyl-2-thiopseudourea· Br· HBr (AET), 2-mercaptoethylamine· HCl (MEA) and trisodium calcium chelate of diethylenetriaminepentaacetic acid ($CaNa_3DTPA$) on the radiosensitivity of rat fetus was investigated. A total of 1256 fetuses were critically examined.

Exposure of rats to 200 r whole-body X-irradiation at 9.5 days of gestation resulted in a high incidence of uterine resorption (50%) and eye defects in fetuses (90%) when examined at 19th day of gestation, and only about 27% of the fetuses survived to term.

However, when AET (50 mg per rat) or MEA (25 mg per rat) was given to rats through I. P. injection before X-irradiation, the incidence of uterine resorption and fetal abnormalities was significantly reduced. The irradiated pregnant rats receiving AET or MEA prior to irradiation were able to give birth to young of normal litter size and birth weight. These offspring, though some still carried

eye defects, survived beyond puberty and showed apparent normal growth and reproduction. Greater protection to fetuses against X-irradiation was obtained when AET and MEA were given simultaneously to pregnant rats shortly before irradiation than when either of the two chemicals was administered separately.

The study also revealed that the chelating agent $CaNa_3DTPA$, which is now increasingly used in plant and animal nutrition and in removing toxic elements from the human body, had a detrimental effect on fetal development. It induced uterine resorption and eye defects in fetuses when administered to rats at 9.5 days of gestation. At a low dose level (62.5 mg per rat) it protected the fetus slightly against the irradiation effect. Unfortunately, a synergistic action in damaging of the rat fetuses was observed when large doses of $CaNa_3DTPA$ were administered to pregnant rats prior to 200 r whole-body X-irradiation. The findings should warrant a reappraisal of the use of DTPA in animal nutrition and human therapy.

INFLUENCE OF CERTAIN CHEMICALS ON THE SENSITIVITY OF RAT EMBRYOS TO X-IRRADIATION

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INFLUENCE OF CERTAIN CHEMICALS ON THE SENSITIVITY OF RAT EMBRYOS TO X-IRRADIATION

INTRODUCTION

Radiation has long been known to harm embryos which are more sensitive to radiation than is any subsequent stage in the ontogeny of the organism. The cells are most radiosensitive during the stages of differentiation although the range of susceptibility to acute damage among cells during these early stages is not as great as it is in adults (52; 102, p. 36; 106).

With the increasing use of radiation in both diagnosis and therapy and with the application of atomic energy to agriculture, industry and research, one may predict that more and more embryos and fetuses of various species will be exposed to different types of radiation. This has stimulated the interest of many investigators to study the possibility of using chemicals to protect the embryos or fetuses against radiation injuries.

During recent decades some sulfhydryl compounds were found to be very effective in protecting animals against radiation damages. However, the effectiveness of these compounds as radioprotectants may vary, not only between closely related species such as rats and mice, but also in animals from different strains of the same species. Some compounds gave excellent protection only if a minute amount was used (1) while others, when used in high dosages, increased

radiation sensitivity or even gave a synergistic effect with radiation (7).

It has been reported that the thio compounds, aminoethylisothiourea (AET) and mercaptoethylamine (MEA) were effective in reducing the lethal effect of radiation and protecting fetal mice and rats irradiated in utero. The chelating agent ethylenediamine tetraacetic acid (EDTA), which has been used in plant and animal nutrition and in hastening the excretion of radioelements, was also found to protect mice against irradiation. Recently diethylene triamine pentaacetic acid (DTPA) was proved to be more effective than EDTA in combining with metal agents.

This study was, therefore, designed to investigate the effect of AET, MEA and trisodium calcium chelate of diethylene triamine pentaacetic acid ($CaNa_3DTPA$) on the radiosensitivity of fetuses from a strain of rats exposed to whole body X-irradiation. The information so obtained may lead to a better understanding of the radiation effect and to the developing of techniques for radiation protection.

REVIEW OF LITERATURE

Effects of Ionizing Radiation on the Embryo and Fetus

The embryo and fetus are considered the most radiosensitive stages in the entire life history of any living organism. However, the embryo or fetus have certain powers which are not found at other periods in their life history, that is the powers of repair, regeneration, or reconstitution. During the early developing stage, they possess phagocytes which are quite active and ready to absorb and remove cellular detritus, and necrotic cells which have been injured by ionizing radiation. With these out of the way, the remaining unharmed and undifferentiated primordial cells will be called upon by the organism to fill in the deficiencies as best they can. As a result the embryo will be formed topographically normal but reduced through cellular deletion. This will lead to stunting, microphthalmia, microcephalia, and other evidences of loss of formative materials. Therefore the fetus or newborn may be normal, even though miniature or reduced (102, p. 37).

Job et al. (59) studied the effect of X-irradiation to determine the critical periods in the development of rat embryo and fetus. They described that with the proper doses of X-irradiation on the ninth day post-conception, a hydrocephalic condition can be induced in the

young. The defects of eyes and jaw can be produced if X-rays are given on the tenth and the eleventh days of gestation. Resorption of dead embryonic or fetal material occurred rapidly in the rat. No abortion occurs even if the dosage of X-rays sufficient to kill all fetuses is given as late as the eighteenth day of gestation. They also stated that at certain doses of X-irradiation male individuals seemed to be more susceptible to X-rays than the females.

Wilson and his associates have reported a series of their studies dealing with the effect of X-irradiation on the rat fetuses (130; 131; 132; 133; 134; 135; 136; 137; 138). Exposure to 50 r X-irradiation on the tenth day of gestation had no effect on embryonic development. A dosage of 100 r increased slightly the incidence of intrauterine death, and caused retarded or anomalous development of eyes in 60 percent of the embryos. With 200 r treatment results were similar but with more intense effects: higher death rate, slower growth rate, and more severe developmental defects. Exposure to 200 r on the ninth day usually caused death of all embryos, whereas on the tenth day a dosage of 400 r was required to kill all fetuses within 24 hours (130; 131; 132; 137). In another study Wilson and Karr (134) irradiated the fetuses with doses ranging from 50 to 400 r. They found a localized retardation of growth with respect to the eyes, brain, aortic arches, lung, liver, and urinary organs. On the eighth day of gestation the effect of X-irradiation on rat embryos

was more limited than was a similar treatment on the ninth or tenth days (136; 138). The dosages of 100 r given on the eighth day only caused retardation of growth. They suggested that this difference in reactivity seemed to be dependent upon the change in the susceptibility of the cells to the various biological effects of irradiation, as the embryo passes from the undifferentiated state on the eighth day into the early stages of differentiation which begin on the ninth day.

After exposure to the higher doses of X-rays (100 r and 200 r) of the nine-day fetus Wilson and his associates (132; 133; 135) found abnormal growth of compact masses of nerve-like cells in mesenchyme surrounding the brain. They described these as neoplastic growths. The small cluster consisting of a few cells seen on the second day showed varied capacities of growth as the embryo grew older. Some grew and receded, others remained compact and static or grew rapidly and killed the embryo. The lack of a blood supply appeared to be a factor in limiting growth.

From intensive histological studies, Hicks (46; 47; 48; 49; 50; 51) reported that 200 to 600 r whole-body irradiation given to pregnant rats the second or third week of gestation caused acute necrosis of the rapidly growing parts of the brain, spinal cord, and retinas of their fetuses. This could be selectively and extensively destroyed by irradiation with virtually no destructive effect on any other organ systems. He believes that the neuroblast is as sensitive as is the

hematopoietic elements in the embryo or fetus, due to the fact that it is an actively differentiating cell. The neuroblast, is actively involved in the production of sulfhydryl enzymes, nucleic acids, and proteins necessary in differentiating growth which transforms it into an immature neuron.

In studying the rat and mouse embryos Hicks (48; 49) found that no deformities occurred following irradiation in the first eight days of gestation. Even when the exposure was as high as 400 r some of the litter still survived. There is no organ primordia during this period. Anencephaly and multiple head defects occurred on the ninth day. Irradiation of the fetus on the tenth day resulted in somewhat better development of the brain and the face, but the eye was still malformed. In many cases the nervous part of the eye was absent and only an abortive lens and lid were formed. Exposure of X-irradiation on the eleventh day showed still more normal development of the brain except for a hydrocephalus and a narrow aqueduct. The retina was malformed, but the optic nerves appeared to be quite normal. The deformities produced by irradiation on the twelfth day were in the brain, skull, and skeleton. The brain stem was reduced in volume, the dorsal cervical cord showed some jumbling of neurons. The skeleton was small and the toes incompletely formed. The eyes were very small, and the retina was almost completely wiped out. Following irradiation on the thirteenth through sixteenth days showed severe cerebral

and striatal malformations. Little or no corpus callosum developed following irradiation on any day from the ninth to nineteenth days. Striatal, hippocampal and cortical defects were decreasingly severe as injury was induced on successive days in the latter third of gestation. The cerebellum began to show increasing damage as radiation was given on those days approaching term and in the postnatal period. Skeletal and eye deformities occurred especially in the ninth to fourteenth day period.

Recently Brent and McLaughlin (14) and Brent (15) studied the indirect effect of 400 r irradiation on rat embryos. They showed that irradiation of the placenta (with the rest of the body shielded) did not change the fetal growth or fetal mortality. If only the mother was irradiated it resulted in resorption and fetal death. It suggested that fetal mortality was increased by maternal irradiation and not affected by the irradiation of the placenta.

The psychological effects of irradiation on the rat fetuses as indicated by postnatal behavior have been studied (102, p. 62; 110; 111; 112). Rat fetuses given 300 to 600 r on the eleventh and nineteenth days of gestation showed more emotion and nervousness in the maze situation than did the controls. During the last days in the maze, there was teeth-chattering, persistent scratching, face washing, defecation, and urination. This was not shown in the controls. The rats irradiated in utero were very difficult to tame. Their learning ability was

decreased in proportion to the amount of radiation given (102, p. 62). Sikov et al. (110, 111, 112) X-irradiated pregnant rats with 20 to 100 r after ten days of gestation and with 50 or 185 r after 15 days. The greatest incidence of severe neurological deficit was produced by treatment with 185 r after 15 days of gestation. These neurological deficits were often detectable at birth as a diminution of grasp and balancing reflexes. In many cases, the neurological deficit progressed so that adults displayed a syndrome of hind limb ataxia, loss of hopping and placing reaction, and blindness or decreased response to visual stimuli. Some animals displayed nystagmus, backing-up, forced circling and titubations of the head. Many of them displayed myoclonic jerks in response to sensory stimuli. Many males developed a priapism at the time of maturity and persisted until death.

Quite recently Meier and Foshee (76) studied the indirect effect of X-irradiation (200 r to 400 r) at mid gestation of fetuses on their postnatal behavior. They found the maximal effects in the young born to mothers which had received total body irradiation. It had slightly less effect in the fetus-shielded young, and markedly less in the fetus-exposed young.

Timetable of Radiation Malformations in Rat[1]

Time of Radiation	Brain Defects	Other Defects
First eight days of gestation	None	None
Ninth day	Anencephaly	Skeleton, eye
Tenth day	Anencephaly, encephalocele, severe head deformities	Skeleton, eye, viscera
Eleventh day	Narrow aqueduct, hydrocephalus, encephalocele	Skeleton, retina
Twelfth to fourteenth day	Porencephaly, severe forebrain defects, cord anomalies	Skeleton, retina
Fifteenth to eighteenth day	Decreasing degree of microcephaly. Malformations of striatum, hippocampus, cerebral, cortex and callosum	Decreasing skeletal and eye defect
Sixteenth day to new born	Increasing cerebellar defects	Stunted growth

[1]Adapted from Hicks (49, p. 290).

Chemical Protection Against Ionizing-Radiation

The General Concept of Chemical Protection

The terms protection and protective agent were introduced by Dale (26) and first applied by Patt et al. (82). They found that animals could be partially protected against the lethal effects of ionizing radiation by pre-administration of cysteine and glutathione. Since then the concept of chemical protection has been concerned mainly with the complex biochemical system of living organisms.

The ideal protective substances should have the following pharmacological characteristics (119, p. 17). The chemical should be active when taken orally; it must be quickly absorbed from the intestinal tract and distributed to the tissue; it should not produce undesirable side effects or show cumulative effects on repeated administration, and finally the margin of safety should be reasonably great.

Unfortunately, the chemical protectants so far known do not satisfy all of these requirements. Many compounds are known to meet some of the above requirements, but most of them are effective only when doses given are close to producing serious toxic effects in experimental animals (119, p. 17).

The term "chemical protection" should be used only when the

chemical is present in the system before or at the time of irradiation, and in some manner is capable of reducing or preventing radiation lesions (66). Maisin and Doherty (72) proposed that chemicals should be considered protective only when it decreases the 30-day mortality at an LD_{100} radiation dose by at least 50 percent or increases the $LD_{50/30}$ dose by at least 30 percent of its value in a given species. Doherty (30, p. 49) evaluated the effectiveness of a chemical as poor protection with 40 percent or less survival, as fair with 40 to 85 percent survival, and as good with 85 to 100 percent survival 30 days based on $LD_{100/30}$ of unprotected animals.

Route of Administration

Intraperitoneal injection is the most common and easiest technique used for most compounds. The next easiest ways of administration are probably subcutaneous and intramuscular injection. Intravenous and oral administration require more time and experience. The chemical is much more rapidly distributed among the tissues of the body by intravenous injection than those given by other routes and animals are able to detoxify or excrete the agent more rapidly via the kidneys. In contrast, in other routes of administration, the absorption of the chemical into the blood stream is required. The absorption is usually rapid by way of intraperitoneal injection, because of the large surface area within the peritoneal cavity. It is slower after

subcutaneous injection and slowest after oral administration. The toxicity of the compound also varies with the route of administration. It is most toxic in intravenous injection, followed by intraperitoneal, intramuscular, subcutaneous, and oral administrations (119, p. 29).

Protective Agents

As mentioned earlier sulfhydryl containing compounds, such as 2-amino-ethyl-isothiourea (AET) and 2-mercapto-ethylamine (MEA) are the most radioprotective agents so far known. This review is confined only to the protective effect of these two chemicals against radiation. The possibility of protection against radiation damage by chemicals and other means has been reviewed thoroughly by Bacq and Alexander (6, p. 457-479), Bond and Cronkite (13, p. 299-328), Doherty (30, p. 45-86), Maisin and Doherty (72), Ord and Stocken (81, p. 356-386), Patt (83, p. 35-76; 84, p. 51-80), and Pihl and Eldjarn (86, p. 437-474).

Mercaptoethylamine. In 1951, Bacq et al. (3) first reported the radioprotective effectiveness of cysteamine, the decarboxylation product of cysteine. Cysteamine is also known as β-mercaptoethylamine, 2-aminoethanethiol, 2-aminoethylmercaptan, cysteinamine and mercamine. A permanent survival of 97 percent was observed in mice if a dose of three mg of cysteamine was applied intraperitoneally shortly before $LD_{100/15}$ X-irradiation (4; 5; 95). Cysteamine was

about five times as efficient as cysteine on a molar basis. At the dosage of 150 mg/kg cysteamine afforded an $LD_{50/30}$ in the range of 1000 r (118). Cysteamine was also considerably more toxic than cysteine, so that the irradiation therapeutic indices would be about the same. Cysteamine was more effective than cysteine when administered intraperitoneally rather than intravenously. The former also possessed some effectiveness when given orally (119, p. 60).

Rugh and Clungston (96) found that, under conditions of continuous exposure to lethal radiation at either 365 or 1500 r/min, three mg of cysteamine per mouse given via I. P. injection prior to irradiation caused the animals to die more quickly than did the controls. However, at the higher dose rate, if the pre-treated animals were exposed to 66, 000 r, they tended to live more than twice as long as the controls. Cysteamine given after the administration of 66, 000 r at 1500 r/min tended to add also a mild toxicity in the mice. Mewissen (77) found that $LD_{50/30}$ of cysteamine treated mice was increased from 416 r to 700 r, provided the radiation exposure was given in two fractions, at a five day interval.

Straube and Patt (118) showed that liver shielding combined with cysteamine gave about 60 percent of rat survival at 1000 r, a dose that was lethal to over 95 percent of unshielded animals within 30 days. The combination of cysteamine pretreatment and hypoxia also was found to increase the 30 day survival over chemical treatment alone

(27). The studies of Lothe and Devik (68) showed that cysteamine seemed to give some additional protection when administered to the ears of a rabbit irradiated in a complete anoxic state, which indicated that cysteamine and anoxia to some degree acted along different pathways. Baldini and Ferri (8) found that the combination of pantothenic acid and cysteamine strengthened the protective action of the latter against the effect of radiation, and also showed that cysteamine administered intravenously gave good protection to guinea pigs.

The biological effects in irradiated animals protected with cysteamine have been extensively studied by Bacq et al. (4; 5) and by Peterson and DuBois (85) as well as many others. Mandl (74) showed that pre-treatment with 30 mg of cysteamine partially protected the reduction in the number of spermatogonia and resting pre-spermatocytes following X-irradiation of the scrotum of adult rats to 230 to 460 r. More recently Luning et al. (69) reported that cysteamine might give protection against genetic damages by decreasing post-implantation deaths and improving the rate of implantations. The rate of mutation decreases to 75 percent in males receiving cysteamine as compared with those receiving physiological saline only.

Rugh and Wolff (100) found that cysteamine decreased the sterilizing effect of X-irradiation in female mice. Wang et al (125) concluded that injection of cysteamine prior to X-irradiation was very effective in preventing transient sterility in male mice during the first

two or three months following X-irradiation given to the whole body or to the testes alone. On the other hand Kaplan and Lyon (60) found no effect of cysteamine to protect the male germ cell of mice against radiation death.

Chutny et al. (21) compared the protective capacity of a series of other cysteamine salts, in addition to the cysteamine HCl, which has been used in most experiments. At a dosage of 900 r the rats receiving cysteamine HCl prior to X-irradiation gave the highest percent of survival (80 percent), followed by those receiving cysteamine salicylate, cysteamine succinate, N-acetylcysteamine, cysteamine base, cysteamine HCl (base) and cysteamine tartrate which gave the lowest survival percentage (23 percent).

Bacq et al. (4) reported the clinical use of cysteamine HCl and cysteamine salicylate after irradiation in an attempt to eliminate radiation sickness. They claimed that a single intravenous injection of 200 mg of cysteamine given to patients was enough to alleviate the symptoms of radiation sickness (nausea, vomiting, diarrhea, general weakness, etc.) in 24 hours after irradiation. Similar results were obtained from oral administration (300 mg three times a day) of cysteamine salicylate in gelatin capsules. Brown (19) and Healy (45), however, found no value of cysteamine and cystamine in the prevention of radiation sickness in human patients. Pretreatment with cysteamine and cystamine has been reported to reduce anorexia, nausea

and malaise in human patients who received radiation therapy (9).

Aminoethylisothiourea. S, 2-aminoethylisothiourea dihydrobromide (AET) is one of the most effective chemicals in radiation protection (119, p. 65). AET in aqueous solution at neutral pH (or when injected in animals) will be changed to mercaptoethylguanidine (MEG), by a process of intratransguanylation (6, p. 461). Doherty and Burnett (29) first showed that AET protected mice against X-irradiation. Greater chemical stability, more effective tissue distribution, less toxic and probably greater reactivity with free radicals, made AET superior to both MEA and cysteine (29; 53; 119, p. 66). Preston et al. (88) found 80 percent survival in rats receiving AET via intraperitoneal injection shortly before exposure to 900 r at a dose of 300 mg/kg. A level of 400 mg/kg not only failed to increase but also decreased survival as compared to that of untreated controls. Oral administration of AET at levels of 400 or 800 mg/kg of body weight shortly prior to X-irradiation had no effect on survival. AET given orally to mice showed a significant protective effect at two, three and five hours after ingestion, but not at 16 and 24 hours, to 770 r of X-irradiation (25). AET has been shown by Urso et al. (121) to reduce the effect of 900 r on the bone marrow, peripheral blood leukocytes, spleen, thymus, body weight, hematocrit and histology of the hematopoietic organs in mice. Chemical protection by AET of the gastrointestinal tract of mice

against radiation doses of 900 r, 1500 r and 2000 r also has been reported (73). Upton et al. (120) reported that AET treatment shortly before exposure to 150 or 300 r inhibited the induction of granulocytic leukemia and thymic lymphoma in mice. The drug treated animals also exhibited less shortening of the life span by radiation than did untreated controls.

The modification of radiation injury by AET has been reported widely in other animal species. In the monkey, Macaca mulatta, Crouch and Overman (24) reported that pretreatment of AET at doses of 200 to 250 mg/kg of body weight was capable of protecting the animals from X-irradiation death at 650 r. A single dose of AET above 250 mg/kg, is lethal in the monkey. Benson et al. (12) claimed that AET given in a single intravenous injection at doses from 50 to 450 mg/kg was lethal to mice, rats, rabbits, guinea pigs and cats. When given by intraperitoneal injection the lethal range was 350 to 650 mg/kg. The lethal range was 250 to 1000 mg/kg when given orally as a single dose. AET is highly toxic to dogs so the use of this chemical for radiation protection is of little value in this species (12).

A study of the effect of AET in man as reported by Condit et al. (22) indicated that man is very sensitive to the compound. Nausea and vomiting were commonly observed after either oral or intravenous doses of 10 to 20 mg per kilogram of body weight. A slight

depression in blood pressure occurred in most patients. It might be possible that tolerance could be brought about in man by repeated administration, since monkeys can be adapted to AET by daily administration of progressively increasing doses (24).

In recent studies Ehling and Doherty (34) found that AET is capable of protecting the reproductive capacity of female mice against 50 r whole body X-irradiation. The length of the reproductive period, the number of litters per female and the number of offspring per female were also increased when compared to irradiated controls.

Protection of Embryos Against Radiation

Information on the possible protection of the mammalian fetus against the damaging effect of ionizing radiations is very limited. Russell et al. (105) first found that hypoxia gave some protective action in mice fetuses against X-irradiation. Rugh and Clungston (98) used cysteamine 3 mg/mouse before 300 r X-irradiation to the 14.5 day mouse fetuses and before 700 r to the 17.5 day fetuses. They found an increase of survival by 79 percent in the drug-treated groups, while all fetuses irradiated without cysteamine died within the first ten days after delivery. Later Rugh (99) found that optimum survival of controls exposed at 15.5 and 17.5 days postconception to 700 r X-irradiation was 19 percent at 30 days, while those given cysteamine prior to irradiation showed 50 percent survival. This has

been confirmed by Woolam and Millen (140), and similar results also have been reported in rats (61).

Quite recently Ershoff et al. (37) administered radioprotective agents AET, cysteamine and MEG to rats prior to 150 r X-irradiation on the fourteenth day of gestation. At doses of 100 mg/kg these agents largely prevent the occurrence of foot deformities and a gait defect in young rats. Starkie (116) demonstrated that pretreatment of the pregnant rats with cysteamine (30 mg/rat) shortly before 100 to 150 r X-irradiation on the seventeenth and twentyfirst day of gestation partially inhibits the deleterious effect of irradiation on the testes of the offspring.

Rugh and Grupp (103) used AET and 14 other agents to study their protective effect of embryo against X-irradiation. They found no protective value from that agent to protect the 8.5 day mouse embryo against X-irradiation, and AET alone was found even to be harmful to the unirradiated embryos.

Mechanisms of Chemical Protection

There are three major theories of the possible protective mechanisms of cysteamine and AET against radiation injury (6, p. 465-477).

Anoxia theory. Anoxia has been reported as giving protection against radiation in rats and mice (31; 89), guinea pigs, rabbits (62)

and chicks (117). Bacq (6, p. 471) reported that a large dose of cysteamine given intravenously or intraperitoneally decreased the oxygen tension in the venous blood. But he concluded that anoxia induced by a sulfhydryl compound was not clarified by experimental evidence and should be considered only as a contributing factor of radioprotection.

Free radical theory. Since the cell is 75 percent water, it is apparent on the basis of quantity alone that more energy is absorbed by the water molecule than by any other molecule in the cell. The peroxide and free radicals that result from the ionization of water are among the most important toxic agents involved in the ultimate inactivation. These agents may act directly on the target molecule or indirectly via the reactive products of their action on other molecules by diffusing through the water of the cell (54).

When in the presence of protective agents free radicals can be trapped or react with the agent before they are able to react with the target molecule. β-mercaptoethylamine has been found to be an excellent competitor to react with the free radicals. The protection may not occur by competition but by repairing of the initial chemical lesion before a biochemically critical molecule can be altered (6, p. 111, 475).

The mixed disulphide hypothesis. This hypothesis of protection applies only to the sulfhydryl-containing compounds. These

substances are believed to react with -SH or -S-S groups of proteins to form mixed disulphides, which may shield the protein of the blood and tissues from the attack of free radicals in the water (6, p. 473).

These three major theories just described are the possible mechanisms known at the present time. Even though there is strong argument against them, it is hoped that a clarification of the mechanism of protection against radiation of sulfhydryl compounds will be found in the near future.

Chelation

The term chelation is derived from the Greek "chele" meaning claw, and is descriptive of the claw-like hold of a group of organic compounds on various metallic ions (32, p. 3). When a metallic ion combines with an electron donor, the resulting substance is termed a complex. If the substance combining the metal contains two or more donor groups so that one or more ring compounds are formed, the resulting structure is called a chelate and the donor a chelating agent (75, p. 1).

There are a number of known chelating agents, but the one most currently used in biology and medicine is ethylenediamine tetra-acetic acid (EDTA) and more recently diethylenetriaminepenta-acetic acid (DTPA). Although most of the metallic elements are capable of forming chelates, they vary considerably in the ease with

which they combine with the chelating agent, as indicated by the stability constant (K) of the resultant metal chelates (33, p. 8). The constant (K) indicates the ratio of a metallic chelate in equilibrium with a free-metallic ion. The higher the log K value means the greater the tendency to chelate formation. Likewise, the greater the log K value, the more stable the chelate.

The uses of chelating agents are many and varied, and cover a wide range of application. They have been employed in analytical chemistry as titrating agents; in agriculture to improve soils; in industry as water softeners; scale removers; food preservers and many others; and in medicine to detoxify heavy metal, remove traces of radioactive elements and to preserve blood (32, p. 12-15; 33, p. 18-26).

Chelating agent as radioprotector. Alexander et al. (1) reported that diethyldithiocarbamate was as good a protector in mice as was cysteamine. A dose of 4×10^{-5} mole not only gave 100 percent protection of mice against 700 r but gave 40 percent protection against 900 r. Ethylenediaminetetraacetic acid (EDTA) was found to give some protection. Quite recently Rixon and Whitfield (91) found that injection of Na_2-EDTA (300 mg/kg) shortly before or after irradiation (740 r) significantly increased the 30 day survival. The injection of Ca-EDTA immediately before irradiation did not, however, give any significant protection.

Radiation sensitizers. Bridges and Horne (17) described a sensitizing agent as "a substance which enhances the effects of radiation without itself being toxic; or if it is toxic, the resulting effect should be more than the sum of the radiation and the agents effect separately." Any good protective compound at high doses might act as a sensitizer. In many cases there was an additive toxicity of radiation and the drugs (119, p. 26). Eldjarn and Pihl (36, p. 238) suggested that the phenomena of protection and sensitization are closely linked aspects of radiosensitivity.

Rixon and Whitfield (91) reported the synergistic action between the chelating agent and radiation in rats when large amounts of Na_2-EDTA (350 to 450 mg/kg) were administered. The synergistic effect of EDTA and radiation also has been observed in Viciafaba, Trandescantia, yeast and Habrobracon oöcytes.

Toxicity. The DTPA toxicity is actually of the same order as EDTA. Intraperitoneal administration of $CaNa_3DTPA$ in mice gave an LD_{50} of about 2.8 gm/kg (41). CaDTPA in single intraperitoneal doses as high as 1.8 gm/kg caused no death or paralyses in rats. Intravenous administration of CaDTPA in doses of 250 mg/kg in rats also produced no toxic effects (40).

The new chelating agent DTPA has proved to be superior to EDTA in its capacity to increase the urinary excretion of many heavy metals (38; 39; 40; 43).

Such chelating agents have been used very extensively in removing stable or radioactive toxic elements from the human body (18), and also have been found to amplify the sensitivity of organisms to ionizing radiation (91). The study of the effect of this agent at sublethal doses to pregnant rats is of considerable interest.

MATERIAL AND METHODS

Experimental Animals

Sprague-Dawley rats obtained from Northwest Rodent Co., Pullman, Washington strain, each weighing 250 to 300 grams were used in this investigation. They were housed in the Small Animal Laboratory of Oregon State University under uniform environmental conditions.

The pregnancies of rats were obtained by placing a male with five females in the same cage at 5 p. m. and leaving them overnight. At 9 a. m. of the next morning females were examined by vaginal smears for the presence of spermatozoa. Those with sperm present were isolated and regarded as one day of gestation 24 hours after sperm were found. In most rats copulation occurs between 10 p. m. and midnight (104) and fertilization is complete approximately 13 hours after the beginning of heat. Nine a. m. of the following morning should represent a fair approximation of the mean time of fertilization in rats (134). Since in this study the exposure of pregnant rats to X-irradiation was carried out at approximately 8 p. m. on the tenth day postcoitus, the estimate of this time as 9. 5 days of gestation should be reasonably accurate.

Chemicals and Preparation

The chemical compounds were obtained from commercial sources. The protective thiol compounds, 2-aminoethyl-2-thio-pseudourea dyhydrobromide (AET) from Matheson Coleman and Bell, 2-mercaptoethylamine (MEA) from Nutritional Biochemicals Corporation, and a chelating agent trisodium calcium chelate of diethylene triamine pentaacetic acid ($CaNa_3DTPA$) (calcium Chel 330 Geigy, Geigy Pharmaceuticals) were used in these studies.

Solutions of AET and MEA were prepared by dissolving these compounds in physiological saline solution. The solutions were immediately adjusted to a pH of 6 to 7 by adding 1.0 N NaOH. The chelating agent $CaNa_3DTPA$ was available in ampules containing four ml of 25 percent of the trisodium salt of the calcium chelate adjusted to pH 7.5 with HCl. Isotonic sodium chloride solution was used to dilute the $CaNa_3DTPA$ concentration.

For protection studies one ml of freshly prepared solution of AET, MEA or $CaNa_3DTPA$ was injected intraperitoneally 15 to 20 minutes before irradiation. The injection doses and variety of treatments are listed in Tables 1A, 1B, 2A and 2B. Control animals were injected with one ml of normal saline. The fetuses were examined either at 19 days of gestation or at term.

Table 1. Studies on the effect of AET and MEA on radiosensitivity of fetuses from rats exposed to 200 r whole body X-irradiation at 9.5 days of gestation.

A. Studies at 19-days of Gestation

Treatment	Dose Administered (mg/rat) AET	MEA	No. of Pregnancies	No. of Embryos
1. Saline control	-	-	4	52
2. Starvation*	-	-	4	51
3. X-rays (200 r)	-	-	7	85
4. AET	50	-	3	36
5. MEA	-	25.0	2	27
6. AET + MEA	50	25.0	2	24
7. X-rays + AET	25	-	3	41
8. X-rays + AET	50	-	4	45
9. X-rays + MEA	-	12.5	3	36
10. X-rays + MEA	-	25.0	4	47
11. X-rays + AET + MEA	25	12.5	4	51
12. X-rays + AET + MEA	50	25.0	4	52

*Because a decrease in food consumption has been observed in irradiated rats, food restriction controls (giving them none on the first, 1/4 on the second, 1/2 on the third and the whole of their normal requirement on the fourth days) were included for comparison.

B. Studies at Term

Treatment	Dose Administered (mg/rat) AET	MEA	No. of Pregnancies	No. of Young
1. Saline control	-	-	5	49
2. AET + MEA	50	25	3	39
3. X-rays (200 r)	-	-	5	12
4. X-rays + AET	50	-	4	41
5. X-rays + MEA	-	25	4	46
6. X-rays + AET + MEA	50	25	2	24

Table 2. Studies on the effect $CaNa_3DTPA$ on radiosensitivity of fetuses from rats exposed to 200 r whole body X-irradiation at 9.5 days of gestation.

A. Studies at 19 Days of Gestation.

	Treatment	Dose Administered mg/rat	No. of Pregnancies	No. of Embryos
1.	Saline control	-	4	52
2.	X-rays (200 r)	-	7	85
3.	$CaNa_3DTPA$	62.5	2	17
4.	X-rays + $CaNa_3DTPA$	62.5	4	49
5.	$CaNa_3DTPA$	125.0	7	76
6.	X-rays + $CaNa_3DTPA$	125.0	7	87
7.	$CaNa_3DTPA$	187.5	4	51
8.	X-rays + $CaNa_3DTPA$	187.5	4	48
9.	$CaNa_3DTPA$	250.0	4	49
10.	X-rays + $CaNa_3DTPA$	250.0	2	25

B. Studies at Term

	Treatment	Dose Administered mg/rat	No. of Pregnancies	No. of Young
1.	Saline control	-	5	49
2.	X-rays (200 r)	-	5	12
3.	$CaNa_3DTPA$	62.5	5	61
4.	X-rays + $CaNa_3DTPA$	62.5	3	28
5.	$CaNa_3DTPA$	125.0	5	58
6.	X-rays + $CaNa_3DTPA$	125.0	3	0
7.	$CaNa_3DTPA$	187.5	4	36
8.	$CaNa_3DTPA$	250.0	3	13

Irradiation

X-irradiation was carried out with a General Electric Maximar Unit operating at 250 kvp, 15 ma, delivering an air exposure of 14.3 r/min. The filtration employed was three mm Al inherent filtration, and Al one mm, and Cu one-half mm added filtration. This resulted in a first HVL of 1.25 mm Cu and a second HVL of 4.25 mm Cu. The TSD was 115 cm. Pregnant rats were irradiated in individual plastic boxes placed on a rotating turntable below the X-ray tube. In every irradiation the dose was monitored by a Victoreen Condenser R-meter (25 r range) with its thimble chamber placed on the beam axis on the center of the rotation turntable about the height of the plastic box above the turntable. The total X-rays exposure given to each rat was 200 r in a single exposure delivered at 9.5 days gestation.

Observations at 19 Days of Gestation

At 19 days postconception the females were sacrificed by ether vapor and the gravid uterus was removed. The number of implantations, resorption, and dead and live fetuses were recorded. The young were weighed and examined for the presence of deformities.

The major deformities encountered included, microphthalmia,

and anophthalmia. Other defects also noted, were central nervous system anomalies and deformed tails and toes.

Preparation of Specimen

For histological studies the young were fixed in Bouin's fluid, embedded in paraffin and stained with hematoxylin and eosin. The serial frontal sections of the brain were examined histologically. Some of the 19-day fetuses from each litter of the $CaNa_3DTPA$ treated group were prepared for a cleared skeletal specimen study by clearing in alkali and glycerin. The fetal skeleton was stained with alizarin, according to the method described by Weesner (127, p. 100-101).

Observations at Term

Some experimental rats were allowed to go to term. The number of young, both alive and still born, were counted, weighed and examined for various defects. At weaning, the offspring from these treatments (except $CaNa_3DTPA$ treated groups) were weighed and the males were separated from the females to prevent mating. At eight weeks of age half of the offspring were sacrificed. Body weight and weights of testes, ovaries, adrenals, pituitary and brain were recorded. These organs were fixed in Bouin's fluid for histological studies. The remaining half of the offspring was reared for

the fertility test.

Fertility Test

After reaching puberty the offspring from rats which received chemical protectants prior to X-irradiation were allowed to mate for a period of five consecutive days. Each morning the females were checked for pregnancy by examining the vaginal smears for the presence of spermatozoa. Those which became pregnant were kept in individual cages. Their young were counted, weighed and examined for any possible deformity. The mothers were sacrificed and the number of corpora lutea and implantation sites were examined.

Blood Analysis

The blood samples were obtained by heart punctures from four pregnant control rats and four pregnant $CaNa_3DTPA$ (250 mg/rat) treated rats, 24 hours after they received $CaNa_3DTPA$ at 9.5 days of pregnancy. A blood sample of about four ml was collected from each rat into an oxalated tube to prevent coagulation (126). The calcium and iron contents of the blood from the control and $CaNa_3DTPA$ treated rats were compared. The calcium of whole blood was determined by flame spectrophotometer as described by

Chapman and Pratt (20). The iron content of the blood was calculated on the basis of the hemoglobin level given by direct reading in a Spencer Haemoglobinometer as described in detail by Oldfield (80, p. 68).

RESULTS

The Effect of AET (Studies at 19 Days of Gestation)

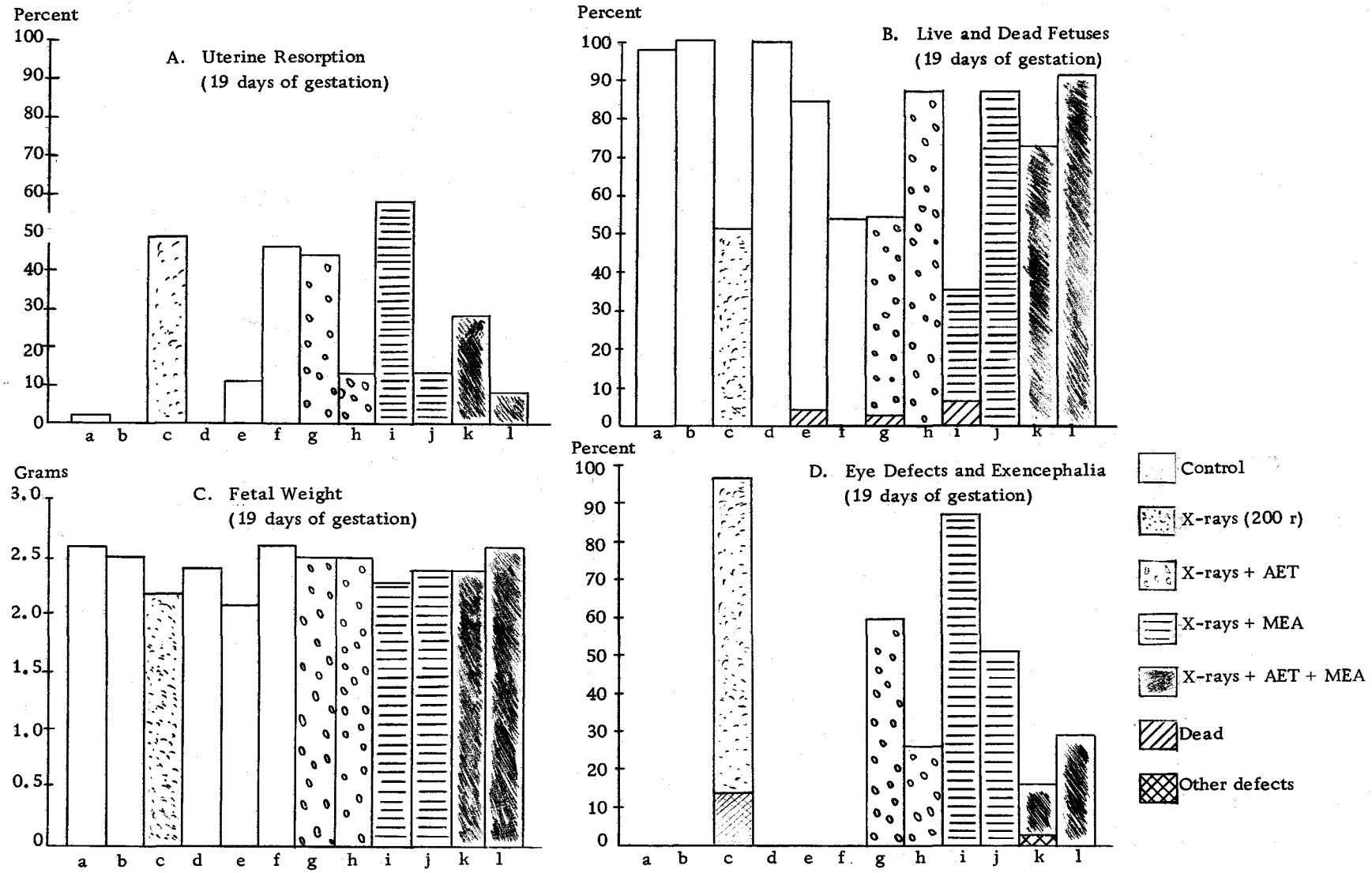
The experimental data from AET treatments are presented in Appendix Table 4. It is clear that the exposure of rats to 200 r whole-body X-irradiation at 9.5 days of gestation did not affect implantation. This was due to the fact that in the rat implantation usually occurs about 4.5 days after fertilization, and since in this study the animals were irradiated on 9.5 days postconception, the rate of implantation was not affected.

In this report both the resorption and the dead embryos were tabulated and graphed separately (Appendix Table 4, Figures 1A and 1B). This made it possible to distinguish between those that survived to a late fetal life before dying and those which died at an earlier stage. Resorption of the early dead embryos were determined from the mark of the implantation site by a residual resorption mass of clotted blood. The dead fetuses were identifiable as formed embryos but were dead on examination of the gravid uterus.

It appears (Appendix Table 4) then that the administration of AET to rats prior to irradiation protects against the lethal effects of 200 r of X-irradiation on the nine and one-half days old fetus. At dosages of 25 mg or 50 mg per rat AET was able to increase the survival of

Figure 1. Effect of X-irradiation on fetuses of rats receiving AET prior to 200 r whole body irradiation at 9.5 days of gestation.

a. Control
b. Starvation
c. X-rays
d. AET 50 mg
e. MEA 25 mg
f. $AET_{50mg} + MEA_{25mg}$
g. X-rays + AET_{25mg}
h. X-rays + AET_{50mg}
i. X-rays + $MEA_{12.5mg}$
j. X-rays + MEA_{25mg}
k. X-rays + $AET_{25mg} + MEA_{12.5mg}$
l. X-rays + $AET_{50mg} + MEA_{25mg}$


Percent
A. Uterine Resorption
(19 days of gestation)
100
90
80
70
60
50
40
30
20
10
0
a b c d e f g h i j k l
Percent
B. Live and Dead Fetuses
(19 days of gestation)
100
90
80
70
60
50
40
30
20
10
0
a b c d e f g h i j k l
Grams
C. Fetal Weight
(19 days of gestation)
3.0
2.5
2.0
1.5
1.0
0.5
0
a b c d e f g h i j k l
Percent
D. Eye Defects and Exencephalia
(19 days of gestation)
100
90
80
70
60
50
40
30
20
10
0
a b c d e f g h i j k l
Control
X-rays (200 r)
X-rays + AET
X-rays + MEA
X-rays + AET + MEA
Dead
Other defects

fetuses as examined at the ninteenth day of gestation by 3. 0 percent and 36 percent respectively over those receiving X-irradiation but without AET protection (see Figure 1B and Appendix Table 4). Starvation or AET treatment alone did not induce fetal mortality or resorption.

The fetuses from rats which received AET or AET with 200 r X-irradiation were significantly larger ($P < 0.05$) than those from rats receiving X-irradiation but without the administration of AET (see Figure 1C and Appendix Table 4).

As it can be seen (Figure 1D and Appendix Table 4) fetuses from rats exposed to whole body X-irradiation showed a high incidence of eye defects which included anophthalmia and microphthalmia of one or both eyes, or a combination of the two eye anomalies. Other defects including edema, missing toes and short tail or tailless were also observed. Eye defects however were the major fetal deformity found in this study.

In the X-irradiated group 95 percent of the fetuses showed eye defects. When AET was administered to pregnant rats shortly before irradiation the eye anomalies were reduced to 59 percent (25 mg AET per rat) or 25 percent (50 mg AET per rat) depending upon the dose of AET administered. Exencephalia was found in fetuses from X-irradiated controls but was not seen in AET treated groups.

The Effects of MEA (Studies at 19 Days of Gestation)

The data obtained for the effect of MEA against the lethal effects of 200 r X-irradiation on nine and one-half day old rat fetuses are shown in Appendix Table 5. MEA seemed to have a mild toxic effect on rat fetuses. A dose of 12.5 mg per rat prior to X-irradiation not only failed to increase but actually decreased the fetal survival by 14 percent below that of the X-irradiated controls. However, when MEA was given at a level of 25 mg per rat, prior to irradiation fetal survival increased by 37 percent over those exposed to X-irradiation but without MEA protection (Figure 1B).

The fetal weight seemed also to be affected by 25 mg per rat of MEA. When the rats received 200 r of X-irradiation, MEA at either 12.5 mg or 25 mg per rat, the fetal weight was significantly greater ($P < 0.05$) than those of the irradiated controls (Figure 1C).

The eye defects of the fetuses were reduced by ten percent in those rats receiving 12.5 mg per rat of MEA prior to X-irradiation and by 44 percent in the 25 mg per rat group. No other defects were observed in the offspring of these drug treated animals (Figure 1D).

The Effects of AET and MEA Given Simultaneously

It was shown in Appendix Table 5 that MEA was slightly toxic

to the 9.5 days old rat fetuses. The purpose of using the simultaneous administration of the AET and MEA was to study whether AET would reduce the adverse effect of MEA on fetuses and whether a combination of the two agents could give a greater protection to rat fetuses against X-irradiation. The experimental findings are presented in Appendix Table 6.

The simultaneous administration of these two drugs (AET_{50mg} + $MEA_{25mg/rat}$) to rats at 9.5 days of gestation showed no greater toxic effect on the fetuses than that seen in the irradiated rats. No exencephalies appeared in fetuses from rats treated by the drugs. When the agents were given simultaneously to pregnant rats before X-irradiation there was definite evidence of protection in terms of fetal survival, reduction in fetal anomalies and resorption (Figures 1A, 1B, 1C and 1D). Fetuses from irradiated rats without chemical protection exhibited 95.5 percent eye defects compared with 16.2 percent to 29.2 percent of these defects found in fetuses from rats which received a combination of AET and MEA prior to X-irradiation (Figure 1D). The fetal resorptions were reduced from 49.4 percent as seen in the irradiated controls to 27.5 percent and 7.7 percent in rats receiving low and high levels of these drugs respectively (Figure 1A). The results clearly indicate that the combined use of these two drugs gave a greater protection to fetuses against X-irradiation than when either of the

two drugs was used separately.

As indicated in Figures 1A, 1B, 1C and 1D and Appendix Tables 4, 5 and 6, AET, MEA and the combination of these two at high levels on the ninteenth day of gestation proved most effective in regard to the number of survival and resorption of fetuses. Only these three treatments were studied at term (Figures 2A, 2B and 2C and Appendix Table 7A).

The mean number of offspring per female was raised from 2.4 in the X-irradiated group to 10.3, 11.5 and 12.0 in the AET, MEA and AET + MEA pretreated groups respectively (Figure 2A). There were 9.0 percent stillborn in the X-irradiated groups, but none occurred in those groups treated with the drugs (Appendix Table 7A). The pretreatments with drugs were able to maintain the birth weight of fetuses at normal average size (over 6.0 gm) as compared with only 5.5 gm in irradiated controls (Figure 2B). Eye defects were reduced from 100 percent in the X-irradiated group to 48.8, 28.3 and 20.8 percent in AET, MEA and AET + MEA treated groups respectively. One tailless animal was observed in the AET treated group. There was no apparent incidence of either brain or other defects (Figure 2C). The offspring which suffered with eye abnormalities grew and developed normally. All offspring survived to weaning and 95 percent lived beyond eight weeks of age.

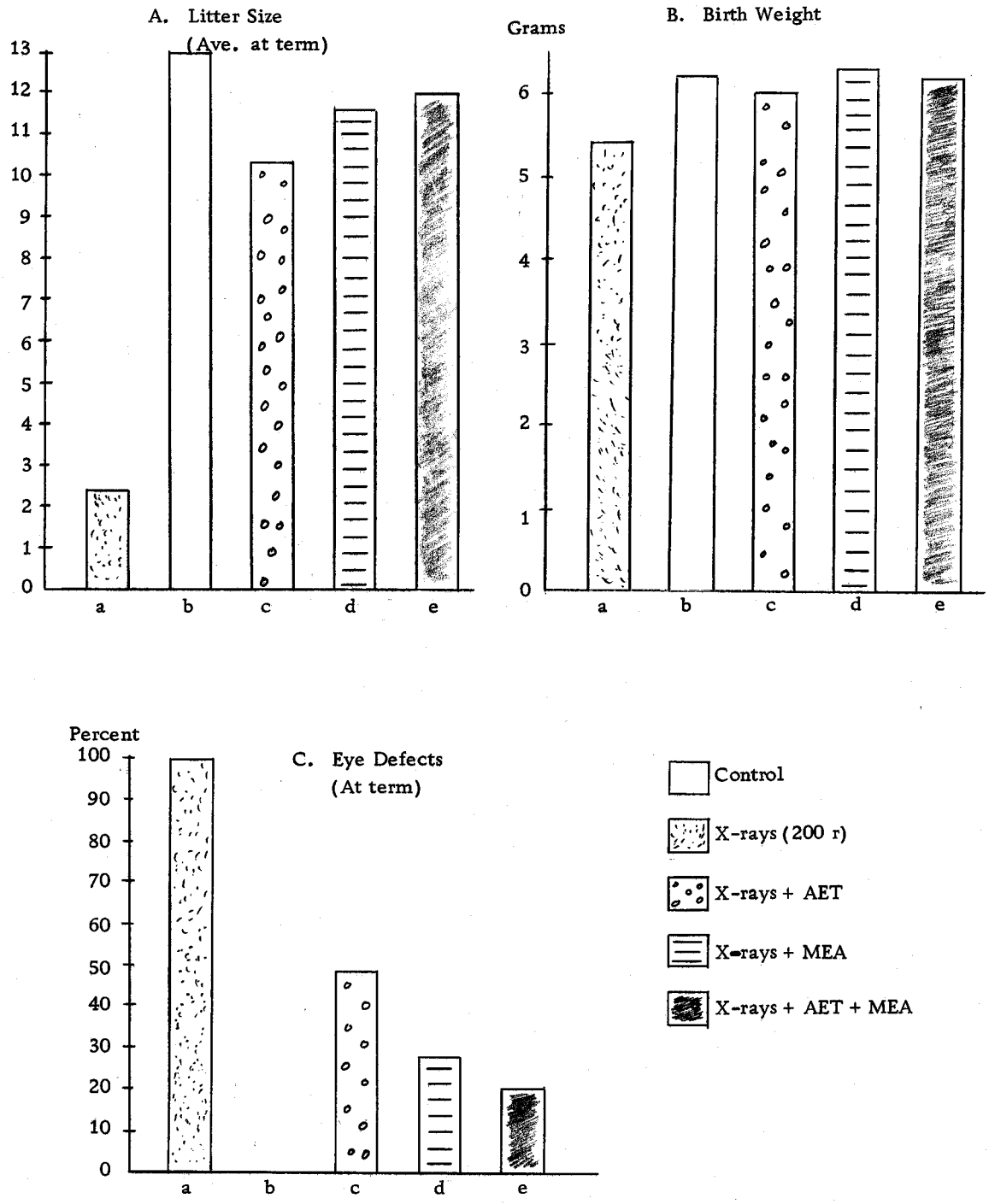


Figure 2. Effect of X-irradiation on fetuses of rats receiving AET prior to 200 r whole body irradiation at 9.5 days of gestation.

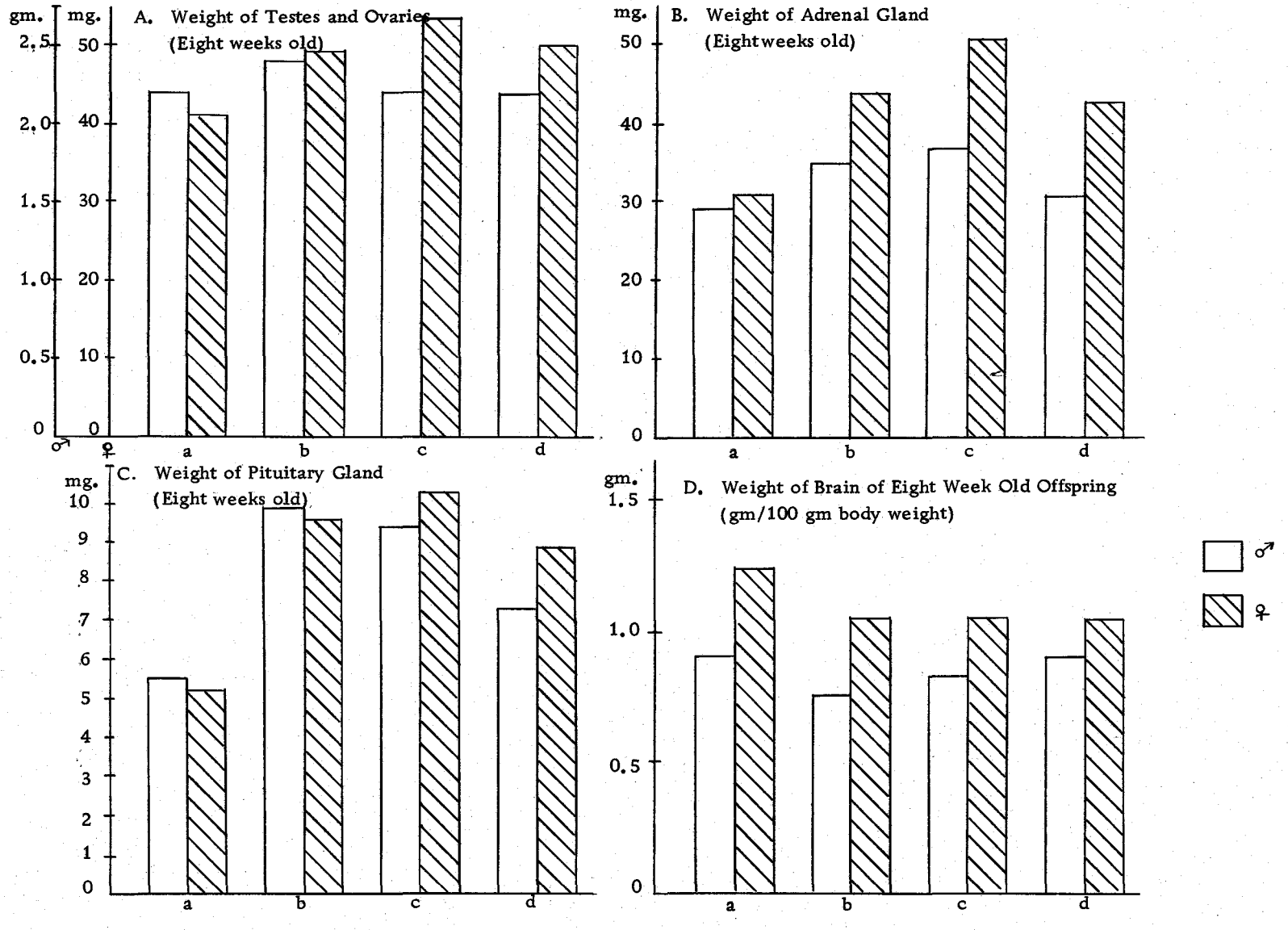
a. X-rays
b. AET_{50mg} + MEA_{25mg}
c. X-rays + AET_{50mg}
d. X-rays + MEA_{25mg}
e. X-rays + AET_{50mg}

Studies of Organ Weight in Eight Week Old Rats

On the average the male in the drug pretreated groups was heavier than in the controls (Appendix Table 7B). As shown in Figure 3A and Appendix Table 7B there was no difference in testicular weight in the fetuses from all groups. The female offspring from the rats receiving both X-irradiation and chemical protectants were slightly smaller than those from the non-irradiated controls. It is interesting to note that their ovaries were significantly larger ($P > 0.05$) than those from the controls (Figure 3A). Most of the female offspring from the irradiated rats receiving chemical protection seemed to be in estrus by the time of examination at eight weeks of age as indicated by the size of the ovaries and the appearance of follicles and corpora lutea. The adrenals and pituitary glands (Figures 3B and 3C) of the young of both sexes from the drug pretreated groups were significantly larger ($P < 0.05$) than those from the young of the controls. The brain weights (Figure 3D and Appendix Table 7D) of rats expressed as gram per 100 grams of body weight were 0.91 for male offspring from the controls and 0.75, 0.82 and 0.91 for the male offspring in AET, MEA and AET + MEA treated groups respectively. The brain weights of the female rats expressed as grams per 100 grams body weight were 1.24 for the offspring from the controls and 1.04 for the offspring of AET, MEA

Figure 3. Effect of X-irradiation on gonadal, adrenal, pituitary and brain weights of young from rats receiving AET and MEA prior to 200 r whole body irradiation at 9.5 days of gestation.

a. Control
b. X-rays + AET_{50mg}
c. X-rays + MEA_{25mg}
d. X-rays + AET_{50mg} + MEA_{25mg}


A. Weight of Testes and Ovaries
(Eight weeks old)
gm.
mg.
2.5
2.0
1.5
1.0
0.5
0
50
40
30
20
10
0
♂
♀
a
b
c
d
B. Weight of Adrenal Gland
(Eightweeks old)
mg.
50
40
30
20
10
0
a
b
c
d
C. Weight of Pituitary Gland
(Eight weeks old)
mg.
10
9
8
7
6
5
4
3
2
1
0
a
b
c
d
D. Weight of Brain of Eight Week Old Offspring
(gm/100 gm body weight)
gm.
1.5
1.0
0.5
0
a
b
c
d
♂
♀

or AET + MEA treated groups. It is interesting to note that there were more female than male offspring in all drugs pretreated groups (54 percent in AET, 56 percent in MEA and 61 percent in AET + MEA).

Fertility Studies

In order to determine the effectiveness of AET and MEA in protecting rat fetuses against X-irradiation, the fertility was tested of offspring from the irradiated rats receiving the chemicals prior to irradiation. The results from this study are shown in Figures 4A, 4B, and 4C and Appendix Table 7C. At the end of a five-day mating period 50 percent of the females in each treatment were found to be pregnant. Since the mating period was limited to five consecutive days and the number of females tested was small (eight to ten) it was rather difficult to predict their life time reproductive performance. The test, however, indicated that the young from irradiated rats which received chemical protection may reproduce normally. The histological examination of ovaries and testes of the offspring from irradiated rats receiving chemical protection and those from non-irradiated controls revealed no differences. Neither was there any significant differences in the number of implants, litter size and birth weight of the young in the chemical protected and the non-irradiated controls (Figures 4A-4C).

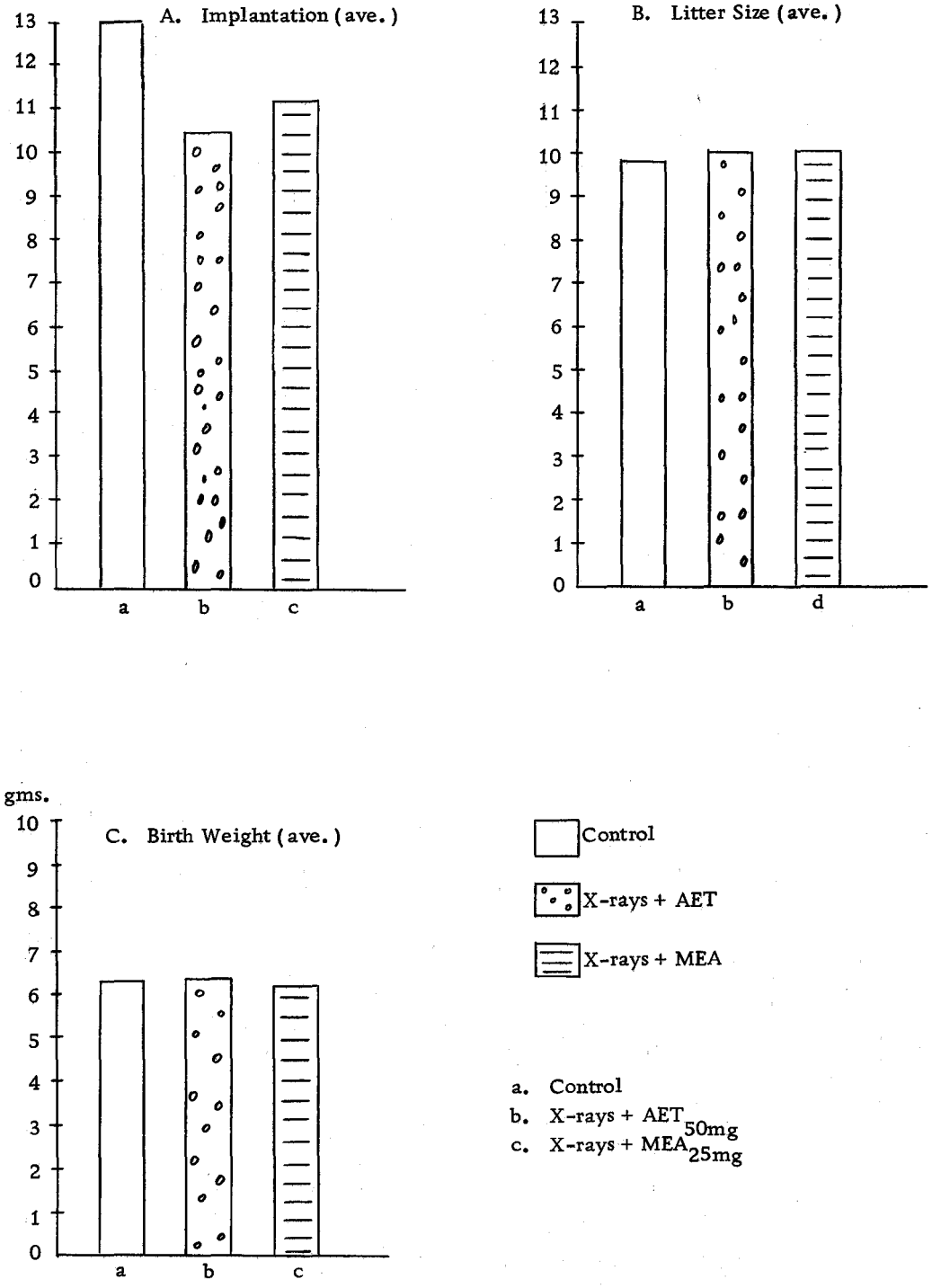


Figure 4. Effect of X-irradiation on fertility of young from rats receiving AET and MEA prior to 200 r whole body irradiation at 9.5 days of gestation.

The Effects of $CaNa_3DTPA$ Without X-rays

At all dose levels used in this study $CaNa_3DTPA$ seemed to cause pain to the rats as indicated by the stretching of their body and limbs during the time of intraperitoneal injection of the agent especially at high doses. This painful expression of the animal lasted for about two minutes after injection. There was a significant increase ($P < 0.05$) in the fetal resorption rate in all $CaNa_3DTPA$ treated groups (Figure 5A and Appendix Table 8). The resorption rate seemed to increase as the dose went up. However, the fetal weights from those receiving $CaNa_3DTPA$ were not significantly different from those of the controls.

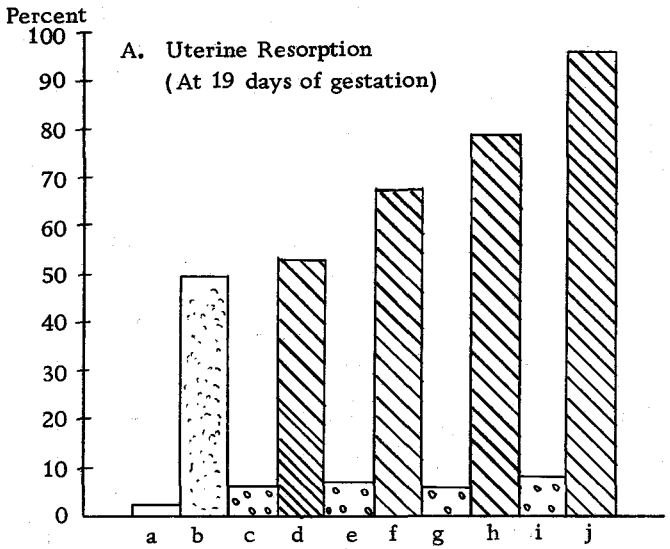
The number of eye defects tended to go up as the doses of $CaNa_3DTPA$ increased, but it leveled off at a dosage of 250 mg per rat (Figure 5C and Appendix Table 8). Exencephalia (2.2 percent) was observed in fetuses from rats receiving $CaNa_3DTPA$ (250 mg per rat). This deformity was not observed in the other groups.

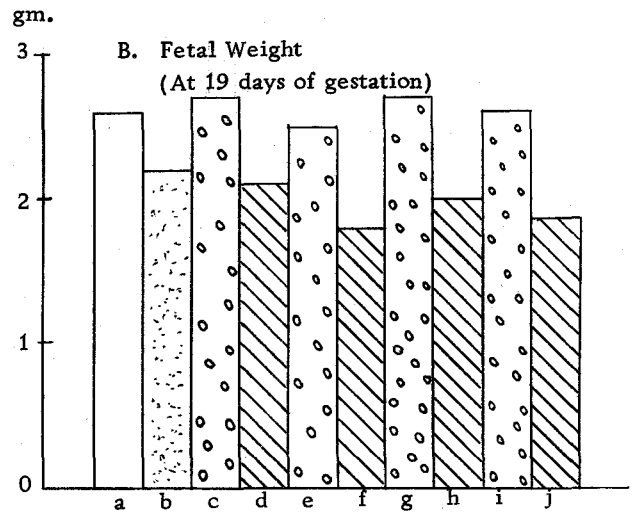
The Effects of $CaNa_3DTPA$ With X-rays

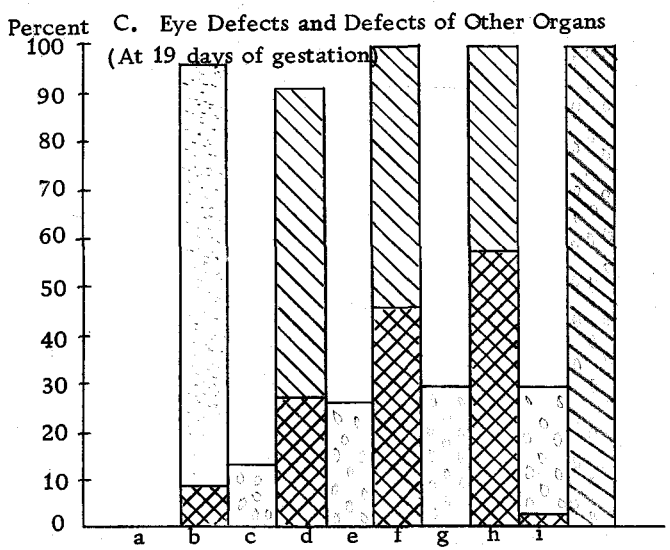
The administration of $CaNa_3DTPA$ to rats exposed to whole body irradiation at 9.5 days of gestation seemed to enhance the deleterious effect of radiation on rat fetuses (Figures 5A, 5B, 5C and Appendix Table 9A). The synergistic action occurred when large

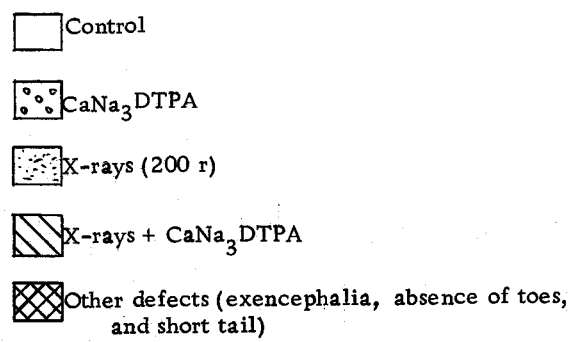
Figure 5. The effect of X-irradiation on fetuses of rats receiving $CaNa_3DTPA$ prior to 200 r whole body irradiation at 9.5 days of gestation.

a. Control
b. X-rays
c. $CaNa_3DTPA_{62.5mg}$
d. X-rays + $CaNa_3DTPA_{62.5mg}$
e. $CaNa_3DTPA_{125mg}$
f. X-rays + $CaNa_3DTPA_{125mg}$
g. $CaNa_3DTPA_{187.5mg}$
h. X-rays + $CaNa_3DTPA_{187.6}$
i. $CaNa_3DTPA_{250mg}$
j. X-rays + $CaNa_3DTPA_{250mg}$


Percent
A. Uterine Resorption
(At 19 days of gestation)
100
90
80
70
60
50
40
30
20
10
0
a b c d e f g h i j


gm.
B. Fetal Weight
(At 19 days of gestation)
3
2
1
0
a b c d e f g h i j


Percent
C. Eye Defects and Defects of Other Organs
(At 19 days of gestation)
100
90
80
70
60
50
40
30
20
10
0
a b c d e f g h i


Control
CaNa3DTPA
X-rays (200 r)
X-rays + CaNa3DTPA
Other defects (exencephalia, absence of toes, and short tail)

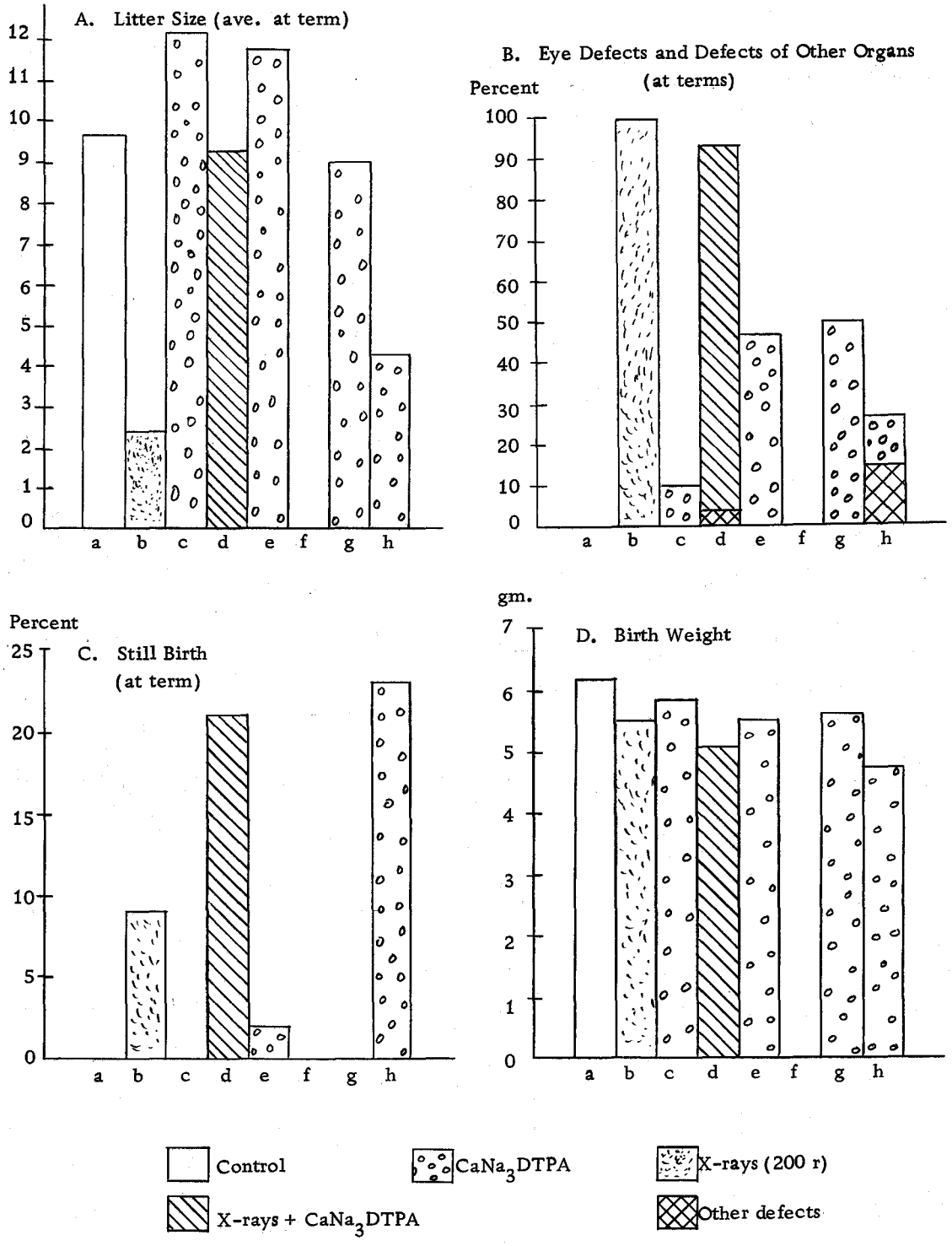
amounts of $CaNa_3DTPA$ (125. 0 to 250 mg per rat) were employed together with X-irradiation. The fetal resorption (Figure 5A and Appendix Table 9A) was 49. 4 percent in the X-irradiated groups and 8. 2 percent in rats receiving 250 mg of $CaNa_3DTPA$. Fetal resorption was 96. 0 percent in rats receiving 250 mg of the chelating agent prior to X-irradiation.

The synergistic action of $CaNa_3DTPA$ on X-rays or vise versa was also evident when one examined the eye defects of the fetuses as well as other defects of a developmental nature. The incidence of the eye defects (Figure 5C and Appendix Table 9A) was increased from 95. 5 percent in fetuses from irradiated rats to 100. 0 percent in the $CaNa_3DTPA$ pretreated group. Other defects (exencephalia, edema, missing toe and short-tail and tailless) were also increased from 8. 7 percent in those receiving X-irradiation alone to 57. 1 percent in fetuses from rats subjected to both treatments. It was not surprising that there was no incidence of other defects found in the fetuses from X-irradiated rats receiving 250 mg of $CaNa_3DTPA$ prior to irradiation, because the damage was so severe (as shown in percent resorption) that the injured fetuses were dead or resorbed before the nineteenth day of gestation.

$CaNa_3DTPA$ alone undoubtedly reduced the litter size in rats. The litter size was significantly reduced ($P < 0.05$) at a dose of 250 mg per rat (Figure 6A and Appendix Table 9B). The number of

Figure 6. The effect of X-irradiation on fetuses of rats receiving $CaNa_3DTPA$ prior to 200 r whole body irradiation at 9.5 days of gestation.

a. Control
b. X-rays
c. $CaNa_3DTPA_{62.5mg}$
d. X-rays + $CaNa_3DTPA_{62.5mg}$
e. $CaNa_3DTPA_{125mg}$
f. X-rays + $CaNa_3DTPA_{125mg}$
g. $CaNa_3DTPA_{187.5mg}$
h. $CaNa_3DTPA_{250mg}$


A. Litter Size (ave. at term)
12
11
10
9
8
7
6
5
4
3
2
1
0
a b c d e f g h
B. Eye Defects and Defects of Other Organs
(at terms)
Percent
100
90
80
70
60
50
40
30
20
10
0
a b c d e f g h
Percent
C. Still Birth
(at term)
25
20
15
10
5
0
a b c d e f g h
gm.
D. Birth Weight
7
6
5
4
3
2
1
0
a b c d e f g h
Control
CaNa3DTPA
X-rays (200 r)
X-rays + CaNa3DTPA
Other defects

stillbirths was also increased from none at low level to 23. 1 percent at high levels of the agents (Figure 6C and Appendix Table 9B). The compound slightly reduced the weight of the young at birth (Figure 6D and Appendix Table 9B). The incidence of eye defects (Figure 6B and Appendix Table 9B) in fetuses was increased from 9. 8 percent in rats receiving $CaNa_3DTPA$ at a level of 62. 5 mg per rat to 50. 0 percent in 187. 5 mg per rat. The eye defects then leveled off to 27. 3 percent in the 250 mg per rat treated group in which 15. 4 percent of the fetuses had other defects. The offspring in all treatments survived beyond weaning, except in the 250 mg per rat groups whose offspring died within one week after birth.

At the lowest dosage (62. 5 mg per rat) $CaNa_3DTPA$ seemed to give some protection to the fetuses against X-irradiation as far as the litter size and eye defects were concerned (Figures 6A and 6B and Appendix Tables 9A and 9B). The litter size in irradiated rats receiving 62. 5 mg per rat of $CaNa_3DTPA$ was 9. 3 as compared with 2. 4 in the irradiated group and 9. 8 in the saline controls. The pretreatment with $CaNa_3DTPA$ before irradiation reduced eye defects by 8. 0 percent from that of the irradiated controls. Some 23 percent of the offspring managed to live through weaning. Unfortunately $CaNa_3DTPA$ administered at a level of 125 mg per rat before irradiation resulted in high resorption rate of the fetuses (Appendix Tables 8A and 8B). Since this dosage of $CaNa_3DTPA$ administered to rats

prior to X-irradiation caused high uterine resorption in rats, the same results should be expected when higher doses of the agent are used. The results suggested that $CaNa_3DTPA$ at high dose levels may increase the radiation effect on rat fetuses.

DISCUSSION

Although the mechanism of radiation action was not the main objective of this study, the biochemical events occurring in rats which received the chemical agents prior to irradiation is of interest. There are two possible mechanisms of the effect of radiation on living organisms (119, p. 5). One of these is the direct inactivation of some essential component of the cells. The second is the indirect action in which the molecule does not absorb the energy but receives this by transfer from another molecule.

It has been estimated that within 10^{-12} seconds after the passage of a particle or photon-induced electrons through an aqueous medium, the free radicals H^{o} and OH^{o} are formed. In less than a microsecond, these radicals have either (a) recombined to form water, (b) combined with an identical radical to yield molecular hydrogen and hydrogen peroxide or (c) reacted with solutes present in the system (6; 119; 129). If, however, these radicals are caused to recombine or to interact with competing agents instead of with critical biological materials, the sequence of events would be interrupted. Andrews and Sneider (2) called this phenomenon an "antienergistic effect" or in other words a protective effect. The interruption by a protective compound is accomplished by decreasing or abolishing the effect of radiation on another solute. Since finding that AET and MEA

were the most active thiols in providing radiation protection to mice, rat and other species, it raised the question of what mechanisms are responsible for the added protection.

Bacq and Alexander (6, p. 475) found that MEA was an excellent competitor. AET has also been reported to have more active antienergistic effects than MEA and reduced toxicity for the recipient at the same time (2).

Oxygen has been recognized to increase the radiosensitivity of organisms (129, p. 133-140). Reduction of oxygen tension has been reported to give protective action against X-irradiation (31; 117). DiStefano et al. (28) found that the blood pressure and respiratory activity were changed in dogs, rabbit and cat after given low doses of AET. In the rabbit five mg/kg of AET produced hypotension, whereas in the dog hypotension was followed by hypertension from a dose of only 2.5 mg/kg. The fall in blood pressure was found also in cats following a dose of 2.5 mg/kg of AET. The effect of AET was by no means identical with that of MEA. They concluded that hypertension following these drugs resulted from a stimulation of sympathetic receptors.

Mundy and Heiffer (78) studied the pharmacology of MEA in dogs. They suggested that MEA caused an increase of sympathetic nervous system activity followed by an increase in catechol amine and blood sugar levels, and the severe hypotension causing the

production of relative anoxia in the body system might contribute to radioprotection.

Vittorio et al. (122; 123) studied the effect of MEA and AET on thyroid activity in non-irradiated and X-irradiated rats. They found that AET or MEA decreased I^{131} uptake by the thyroid and the serum protein-bound iodine. In an X-irradiated sample AET appeared to lower I^{131} uptake slightly more than MEA. The combination of AET and MEA appeared to have a synergistic effect in lowering the serum protein-bound I^{131}. They suggested that AET and MEA might slow down the movement of I^{131} into the thyroid, since the decreased uptake of I^{131} by the thyroid was followed by a decreased protein-bound I^{131} value. If a lowered protein-bound I^{131} value is indicative of a lowered basal metabolic rate, the decreased metabolic rate could play a role in protection against X-irradiation.

From studies of the effect of the sulfhydryl groups on irradiated enzymes and DNA, Hutchinson (56) suggested sulfhydryl groups (SH) might react with enzyme or DNA molecules and alter their response to radical attack. Pihl and Eldjarn (86, p. 463) called this reaction the "mixed disulfide mechanism." They reported (35) that cysteamine (MEA) was found to be bound to intra- and extracellular proteins and to other blood constituents 30 minutes after interperitoneal administration, the period in which the compound offered optimum radiation protection.

Recently Shapiro et al. (109) found that protein bound S^{AET} was the major chemical form in all tissues 20 minutes after AET-S^{35} was injected peritoneally in mice. They concluded that the protective form of AET in the animal was either GED (disulfide of AET) itself or protein-bound S^{AET} or both. Protein-bound S^{AET} might protect by shielding the protein sulfhydryl groups of the tissue as described by Pihl and Eldjarn (86, p. 463).

Another possibility of protection by MEA was described by Baldini and Ferri (8). They stated that MEA might be considered as a component of pantotheine, a basic constituent of co-enzyme A. Furthermore the fact that co-enzyme A seemed to interfere with the oxidation of the two carbon chain during glucose metabolism, might represent a factor in radio-resistance.

Radiation Protection in Fetuses

There are few studies concerning to what extent the harmful effects of radiation on fetuses can be prevented and what mechanism is involved by administration of radioprotective agents. Rugh and Clungston (98) suggested that since related sulfhydryl compounds have been demonstrated to give high protective value in adults the same conclusion should be made if they were applied to the fetus.

Brent et al. (16) managed to protect against the lethal and growth-retarding effects of 200 r X-irradiation to rat fetuses on the

eighth and ninth days of gestation by uterine vascular clamping of the pregnant rat uterus for 45 minutes. This operation no doubt cut down the blood supply to the fetuses and therefore reduced the levels of oxygen tension. Hypoxia has also been shown by Russell et al. (105) and by Rugh and Grupp (103) to protect the growth-retardation, malformation, and lethal effects of irradiation of mouse embryos. Their results suggest that rat fetuses may be protected from X-irradiation by hypotension induced by AET or MEA. In spite of the conclusion that hypoxia resulting from AET and MEA pretreatment may play an essential role in protecting the irradiated fetus, the other hypotheses which has been accepted by many investigators cannot be excluded. AET and MEA may act as free radical interceptors and protein-binding agents and then alter the response of the embryos to irradiation.

Protective Effect of AET

AET has been reported to provide significant protection against lethal doses of X-irradiation in adult mice (29), rat (88), monkey (24), rabbit, guinea pigs and cats (12). Few attempts have been made to study its effect in protecting fetuses. Ershoff et al. (37) found that AET at a level of 100 mg/kg administered before 150 r X-irradiation at 14 days of gestation in rats prevented the occurrence of foot deformities and a gait defect. The same dose was without

effect in reducing the high incidence of anopthalmia and microphthalmia observed in the young of rats when exposed to a single dose of 150 r X-irradiation on the tenth day of pregnancy. AET was active however in preventing testicular degeneration and failure of spermatogenesis in the young of rats exposed to the same dose of X-irradiation on the eighteenth day of gestation. On the other hand Rugh and Grupp (103) found no protective effect of AET to the 8.5 day mouse fetuses against X-irradiation. At the dose of nine mg per mouse (or equivalent to 450 mg/kg) of the agent alone without X-irradiation resulted in 24.5 percent resorption and ten percent exencephaly, but in combination with irradiation no counteracting effect on X-ray damage was observed.

The results from the present investigation showed that AET at a dosage of 50 mg per rat (equivalent to 200 mg/kg) when given before 200 r X-irradiation on 9.5 days of gestation significantly increased ($P < 0.05$) increased the percentage survival and fetus weight and reduced the incidence of anopthalmia and microphthalmia. Brain defects in fetuses were not observed in pregnant rats receiving AET prior to irradiation. AET treatment significantly increased the litter size and birth weight over that of the irradiated controls. Eye defects were significantly reduced by AET pretreatment. The offspring of the irradiated rats receiving AET were apparently fertile. At a lower dose level (25 mg per rat), however, AET was not

effective in increasing the percentage of survival of rat fetuses.

Rugh and Grupp (103) did not find any protective value of AET in mice. The dose of AET used by these investigators might have been too high. On the other hand the failure of AET to reduce the incidence of eye defects in rat by Ershoff et al. (37) might have been because the dose used was too low.

The toxic effect of AET seemed to be varied in different species. The larger size animals tended to have lesser tolerance to AET. Benson et al. (12) reported that the LD_{100} of AET for rats, mice, guinea pigs, rabbits, cats and dogs was 550, 580, 400, 350, 600 and 25 mg/kg respectively when given by intraperitoneal injection. Man was even more sensitive to AET and could tolerate a maximum dose of only about 10 to 15 mg/kg (22; 23). In the present investigation AET at the level of 50 mg per rat showed no apparent toxic effect on pregnant rats.

Protective Effect of MEA

The results from the present study showed that MEA at a dosage of 12.5 mg per rat (about 50 mg/kg) did not protect 9.5 days rat fetuses against X-irradiation damage. However, the present study clearly indicated that a dose of 25 mg per rat (about 100 mg/kg) MEA was as effective as AET in increasing the percentage survival and fetal weight. AET was superior to MEA for the reduction of eye

defects. Both AET and MEA prevented sterility in the offspring of X-irradiated rats.

As suggested by Rugh (94) and Starkie (116), if 40 percent or more of the tubules in the testicle contained spermatogonia and primary spermatocytes as well as spermatids the testes were considered to be normal. The present findings showed that more than 50 percent of normal tubules were found in testes of the offspring from the pretreated groups which clearly indicated that they probably were all fertile (Figure 13 in Appendix).

It is of interest to compare the present findings with those reported by other investigators. Maisin et al. (70) found that MEA gave some protection to the fetuses when given intraperitoneally at a level of ten mg per rat on 15 and 18 days of pregnancy before 300 r X-irradiation. Recently Ershoff et al. (37) found that MEA at a level of 100 mg/kg was able to prevent the incidence of foot deformities and a defect in the gait in rat fetuses when given before 150 r total body X-irradiation on the fourteenth day of pregnancy. But it failed to give any protection against the high incidence of eye defects when the same dose of MEA was administered on the tenth day of pregnancy.

Protection of MEA to pregnant mice was also reported. Rugh and Clunston (98; 99) found that MEA given before X-irradiation at 14.5 to 17.5 days of gestation in mice increased the percentage

survival and weight increment of the young during the first month after birth. A reduction in abnormalities (micromelia, anophthalmia, hydrocephalus and cleft palate) in 12 day old mouse fetuses treated with MEA at a level of four mg per mouse before 300 r X-irradiation also has been reported by Woolam and Millen (140). Rugh and Grupp (103) found that MEA (three mg mouse or about 150 mg/kg) reduced uterine resorption and the incidence of all the adverse effects and increased the number of normal fetuses when given intraperitoneally 30 minutes before 200 r X-irradiation on 8.5 days of gestation.

Rugh and Wolff (100) reported that administration of three mg per mouse of MEA delayed the onset of sterility in female mice given 50 r of X-irradiation. Ehling and Doherty (34) demonstrated that AET (seven mg per mouse) before 50 r X-irradiation gave some protection to reproductive capacity of female mice when compared to irradiated controls. Wang et al. (125) found that MEA (three mg per mouse) was a very effective compound in protecting the presperma-togenic elements from X-ray damage. Luning et al. (69) showed that MEA was also effective in protecting mice against radiation induced genetic damage. They found that pretreatment of male mice with MEA (four mg per mouse) reduced the postimplantation death of embryos sired shortly after irradiation to about 75 percent of that for males receiving no MEA pretreatment.

Some points from this investigation however, did not fully

agree with the previous findings by others. This difference may arise from factors such as species differences and differences in stages of development of embryos at the time of treatment.

Protective Effect of AET and MEA Simultaneously

Jacobus (58) suggested that the combination of drugs of dissimilar toxicity was the principle way to make possible the protection of large animals. He was successful in protecting against a lethal dose of radiation in dogs by combining treatment of MEA (100 mg/kg) and cysteine (300 mg/kg). AET and MEA have been shown to give synergistic effect (123). Wang et al. (124) reported that serotonin combined with either AET or MEA offered 100 percent protection against 800 r in mice, but the combinations containing AET and MEA failed to demonstrate appreciable protection in this species.

In this study a simultaneous administration of AET and MEA to pregnant rats seemed to give synergistic effect in radioprotection. At low doses the combined use of AET (25 mg per rat) and MEA (12. 5 mg per rat) was able to increase the percentage of fetal survival from 50. 6 percent in irradiated controls to 72. 6 percent in the AET and MEA pretreated group. At these doses AET or MEA alone offered only slight protection (Appendix Tables 4 and 5). MEA is slightly toxic when given alone at high doses (25 mg per rat), but at this level it offered a highly protective effect when given before

X-irradiation. When AET and MEA combined at high doses the toxic effect is comparable to the effect of 200 r X-irradiation based on the incidence of uterine resorption. But, this toxic effect seemed to disappear after combining with 200 r X-irradiation. The combination of these two drugs gave good protective effect in all aspects. It may be of interest to recall the similar findings observed by others. While studying the way to protect fish from the effect of radiation by cold treatment, Gros et al. (55) found that radiation protected fish against the killing effects of cold. From the above finding Hulse (55) studied the use of X-irradiation to prevent the killing effects of a lethal dose of MEA. He found that 100 rads of X-irradiation significantly reduced the LD_{50} caused by MEA (350 mg/kg), but found no effect after doses above 500 rads. The small doses of radiation seemed to be stimulating and larger doses depressing. However, the mechanism involved in reducing the toxic effect of MEA by radiation is still not clearly understood.

Ershoff et al. (37) reported that the ratio of brain weight to body weight was reduced in both male and female offspring in irradiated rats and that administration of AET, MEA, or MEG showed no significant effect in counteracting the reduction in brain weight. Present findings (Appendix Table 7D) confirmed the report of Ershoff and his associates. The ratio of brain weight to body weight of both male and female offspring in drug treated groups was smaller than

that of the controls. AET pretreatment was less effective than that of MEA in maintaining brain weight in male offspring. The combination of AET and MEA pretreatment was the most effective in maintaining the same brain weight as that of the control (0.91 $\pm$ 0.03). In female offspring AET and MEA given singly or in combination gave similar effects in maintaining brain weight of the fetuses.

The Effect of Chelating Agent

Detrimental Effect of $CaNa_3DTPA$ on Rat Fetuses

The findings from the present investigation indicated that doses of $CaNa_3DTPA$, which showed no apparent toxic effect in adults, may induce eye (Figure 15 in Appendix) and brain defects in fetuses from pregnant rats receiving this agent. Uterine resorption also was increased, even when a dose as low as 62.5 mg per rat was used. The results from this finding indicated the need for further investigation on the effect of chelating agents on embryos or fetuses in order to avoid such incidence possibly occurred in species other than rat.

Ever since the introduction of the new chelating agents EDTA and DTPA, it has stimulated the interest for effective use of these compounds for plants and animals including man. The use of chelates as vehicles carrying needed metals to desirable sites has been investigated along several lines. Jacobson (57) reported that iron

deficiencies in tomato and other plants grown in nutrient solution could be overcome by a single addition of an Fe-EDTA chelate for an entire experiment.

The addition of disodium ethylenediaminetetraacetic acid (Na_2EDTA) to a diet containing isolated soybean protein has been shown to increase the availability of zinc for turkey poults (63; 64). Zinc in sesame seed meal also was more available to chicks by the addition of EDTA to the diet (67). The similar treatment should apply to other metal ion deficient animals.

In man the uses of EDTA and DTPA for the removing of radioactive isotopes and poisoning metal ions from the body have been reported (11; 18; 42; 44; 90; 93; 115). Chelating agent DTPA has been proved to be more effective in combining metal ion than EDTA (38; 39; 40; 43; 115).

In spite of their toxicity (40; 41; 108) and practical limitations the use of DTPA and EDTA for medical purposes is still an useful tool. Studies reported by others have been concerned chiefly with the effect of chelating agents on adult organisms. The present findings of the teratogenic effect of $CaNa_3DTPA$ on rat fetuses will lead to a more cautious use of the chelating agents and to a more extensive study of their effect on embryonic development.

Radioprotective Effect of $CaNa_3DTPA$

The present result showed that the administration of $CaNa_3$ DTPA at a low dose (62.5 mg per rat or about 250 mg/kg) before 200 r X-irradiation at 9.5 days of pregnancy only partially protected rat fetuses. The result seemed to be comparable with the finding of Rixon and Whitfield (91) who reported that injection of Ca-EDTA shortly before X-irradiation did not significantly increase survival of rats above that of the controls.

However, EDTA has been reported to protect mice when injected before irradiation (1). Intraperitoneal injection of 300 mg/kg of Na_2-EDTA shortly before 740 r X-irradiation significantly increased the 30 day survival from 33 to 73 percent. Administration immediately after irradiation also increased survival according to Rixon and Whitfield (91). They suggested that the protective action of appropriate doses of Na_2-EDTA was related to the chelation of serum calcium. The calcium mobility may be caused by the acting of Na_2-EDTA via parathyroid hormone. Parathyroid extract which has been known as a material to mobilize calcium from bone also has been reported to give a protective effect when 50 and 200 USP were given five minutes before 800 r X-irradiation in rats (92). Support for this concept is provided by the observation that calcium (as calcium gluconate) increases the survival of X-irradiated rat

thymocytes and conidia of Neurospora crasse (79; 128).

It has been found that $CaNa_2$-EDTA caused a drop in blood pressure in hypertensive rats (107). Similar results were reported by Spencer et al. (114) in hypercalcemia patients after either Na or Ca-EDTA were given. This hypotensive effect might be responsible for the irradiation protection, since in the case of calcium chelate the immobility of calcium may not be affected.

Synergistic Action of $CaNa_3$DTPA with X-irradiation

In their studies of radiation effect on yeast Bair and Hungate (7) found that EDTA appeared to augment the action of radiation. They found that the higher the concentration of sodium EDTA, the greater synergistic effects obtained with radiation. The results from the present study showed that the chelating agent $CaNa_3$DTPA increased the radiation effect on rat fetuses. It is not possible at present to specify the mode of action by which $CaNa_3$DTPA amplifies the radiation effect. Bair and Hungate (7) believed that the increase of radiosensitivity of yeast to irradiation in the presence of EDTA might be due to a general change in the electrolyte balance of the cell rather than by a specific reduction of calcium.

In the case of rats the effect of $CaNa_3$DTPA on fetuses was not due to a deficiency of Ca or Fe induced by this agent. Blood analysis (Table 3) showed no difference in blood calcium and iron in

the $CaNa_3DTPA$ treated animals from that of the controls. There are no visible abnormalities of the skeletal system in all the $CaNa_3$ DTPA groups treated alone when examined in cleared skeletal specimens.

Table 3. Blood Ca and Fe 24 hours after intraperitoneal administration of $CaNa_3DTPA$.

Treatment	No. of Animals	Hb gm/100 ml	Fe mg/100 ml	Ca mg/100 ml
Control	4	11.8 ±0.53	39.53±1.78	11.46±0.89
$CaNa_3DTPA$	4	12.13±0.09	40.62±2.95	11.41±0.09

Other mechanisms have been discussed by various authors to interpret the effect and synergistic action of a chelating agent with X-irradiation. It has been suggested (65) that a number of enzymic pathways could be affected owing to the chelating of Fe, Cu, Zn or Mn ions. The presence of a chelating agent might amplify the damage of irradiation through the inhibition of the enzyme catalase and peroxidase. On the other hand Barber (10) reported that EDTA decreased the amount of catalytic iron available for the production of peroxides.

Rixon and Whitfield (92) showed that there was a synergistic action between the chelator and radiation in rats when larger amounts of Na_2EDTA (350 to 450 mg/kg) were given. EDTA in the form of sodium chelate seemed to increase the number of intestinal deaths.

They further suggested that, since the administration of Ca-EDTA did not increase the incidence of early death, it was reasonable to assume that Na_2EDTA has sensitized the intestinal tissue to X-irradiation by the removal of calcium and possibly magnesium.

It has been reported that the intravenous injection of EDTA at a dosage of 100 mg/kg caused a sharp fall in the serum ionic calcium level resulting in hypocalcemia tetany death in rabbit (87). This effect can be prevented by using the disodium salt of the calcium chelate instead of the acid (113). The absence of hypocalcemia in pregnant rats receiving DTPA in this study may be due to the chelating agent employed in this investigation being in the form of its Ca- salt.

Furthermore, from available evidence it appears that the trace metal complexes of a normal Co-enzyme system is of such stability that therapeutic doses of DTPA do not create deficiency states. But if the system is abnormal, metal deposits may be removed. Fahey et al. (38) reported that $CaNa_3DTPA$ given intravenously at doses of 2.5 to 4.0 gms resulted in iron excretion of up to 109 mg per 24 hours in three patients with clinical hemochromatosis. Two controls showed no evidence of increased urinary iron after the doses of a chelating agent were given.

The synergistic action of chelating agents with irradiation may be explained at the subcellular basis. From the hypothesis that

chromosomes are held together by the ionic bonds formed by the divalent cation of calcium and magnesium, the breakage of this bond will cause the aberration of chromosomes. Wolff and Luippold (139) demonstrated that low concentration of EDTA could produce chromosome breakage of Vicia faba. The combination of EDTA and X-irradiation increased the total aberration yield by two fold, which suggests a synergistic effect.

SUMMARY

1. The effect of 2-aminoethyl-2-thiopseudourea dyhydrobromide (AET), 2-mercaptoethylamine HCl (MEA) and trisodium calcium chelate of diethylene triamine pentaacetic acid ($CaNa_3$DTPA) on fetuses was studied in rats with and without exposure to 200 r whole body X-irradiation at 9.5 days of gestation.

2. The administration of AET and MEA either singly or in combination to pregnant rats shortly before irradiation proved to be highly protective against the effect of X-irradiation on their fetuses.

Either AET (50 mg per rat) or MEA (25 mg per rat) given through I.P. injection before X-irradiation allowed 87 percent of the 9.5 day embryos to survive to at least 19 days of gestation while the survival rate of fetuses from irradiated rats which received no chemical protectants was only 51 percent. In the chemically protected groups there was also considerable reduction in eye defects among the fetuses and no anomalies of exencephalia was observed. AET at the level of 25 mg per rat gave only slight protection. MEA at the level of 12.5 mg per rat was slightly toxic to fetuses and gave no apparent protection against X-irradiation effect.

Simultaneous administration of AET (50 mg per rat) and MEA (25 mg per rat) to rats at 9.5 days of gestation resulted in high fetal resorption as examined at 19 days of gestation. This was probably

due to the toxic effect of these drugs to the fetus. When rats were exposed to 200 r whole body X-irradiation at 9.5 days of gestation, there was only about 27 percent of the fetuses survived to term. But the rats which received AET (50 mg per rat) and MEA (25 mg per rat) either singly or in combination via the I.P. injection shortly before X-irradiation gave birth to young with normal litter size and birth weight. The offspring were apparently normal in most respects, except some of them suffered eye defects. Most of them survived beyond puberty and showed no apparent disturbance in reproduction.

3. The chelating agent $CaNa_3DTPA$ alone reduced the number of survivals of rat fetuses when given to pregnant rats at 9.5 days of gestation. The incidence of uterine resorption and the defect of eyes in the fetuses seemed to increase with the increase of the dose of the agent.

At 62.5 mg per rat, $CaNa_3DTPA$ showed only partial protection of the rat fetuses against X-irradiation. Unfortunately, synergistic effect in damaging of the rat fetuses was observed when large doses of $CaNa_3DTPA$ were given to pregnant rats prior to 200 r X-irradiation.

4. With the increasing use of chelating agents and atomic power, the toxicity of $CaNa_3DTPA$ to fetuses and the synergistic action between this chelating agent and X-irradiation revealed important problems which warrant further investigation.

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APPENDIX

Table 4. The effects of X-irradiation on fetus in pregnant rats receiving AET prior to X-irradiation. Studies at 19-days of gestation (1)

	Saline Control	Starvation Control	AET (50 mg)	X-irradiation (200 r)		
				No AET	AET (25 mg)	AET (50 mg)
No. of pregnancies	4	4	3	7	3	4
Ave. no. of implantation	13.0	12.8	12.0	12.1	13.7	11.3
Dead fetuses (%)	0	0	0	0	2.4	0
Resorption (%)	1.9	0	0	49.4	43.9	13.3
Live fetuses (%)	98.1	100.0	100.0	50.6	53.7	86.7
Ave. fetal wt. (gm)	2.6	2.5	2.4	2.2	2.5	2.5
Eye defects (%)	0	0	0	95.5	59.1	25.6
Other defects (%)	0	0	0	8.7(2)	0	0

(1) See Table 1 A.

(2) Exencephalia

Table 5. The effects of X-irradiation on fetuses in pregnant rats receiving MEA prior to irradiation. Studies at 19-days of gestation[1]

	Saline Control	Starvation Control	MEA (25 mg)	X-irradiation (200 r)		
				No MEA	MEA (12.5 mg)	MEA (25 mg)
No. of pregnancies	4	4	2	7	3	4
Ave. no. of implantation	13.0	12.8	13.5	12.1	12.0	11.8
Dead fetuses (%)	0	0	3.7	0	5.6	0
Resorption (%)	1.9	0	11.1	49.4	58.3	12.8
Live fetuses (%)	98.1	100.0	85.2	50.6	36.1	87.2
Ave. fetal wt. (gm)	2.6	2.5	2.1	2.2	2.3	2.4
Eye defects (%)	0	0	0	95.5	86.7	51.2
Other defects (%)	0	0	0	8.7[2]	0	0

(1) See Table 1 A.

(2) Exencephalia

Table 6. The effects of X-irradiation on fetuses in pregnant rats receiving AET and MEA prior to irradation. Studies at 19-days of Gestation[1]

	Saline Control	Starvation Control	$AET_{50\,mg}$ + $MEA_{25\,mg}$	X-irradiation (200 r)		
				No AET or MEA	AET + MEA 25 mg 12.5 mg	AET + MEA 50 mg 25 mg
No. of pregnancies	4	4	2	7	4	4
Ave. no. of implantation	13.0	12.8	12.0	12.1	12.8	13.0
Dead fetuses (%)	0	0	0	0	0	0
Resorption (%)	1.9	0	45.8	49.4	27.5	7.7
Live fetuses (%)	98.1	100.0	54.2	50.6	72.6	92.3
Ave. fetal wt. (gm)	2.6	2.5	2.6	2.2	2.4	2.7
Eye defects (%)	0	0	0	95.5	16.2	29.2
Other defects (%)	0	0	0	8.7[2]	2.7[2]	0

(1) See Table 1 A

(2) Exencephalia

Table 7. The effects of X-irradiation on fetuses in pregnant rats receiving AET and MEA prior to irradiation.

A. Studies after Parturition[1]

	Saline Control	X-rays Control	$AET_{50\ mg}$ and $MEA_{25\ mg}$	X-irradiation (200 r)		
				$AET_{50\ mg}$	$MEA_{25\ mg}$	$AET_{50\ mg}$ and $MEA_{25\ mg}$
No. of pregnancies	5	5	3	4	4	2
Stillborn (%)	0	9.0	0	0	0	0
Ave. litter size	9.8	2.4[2]	13.0	10.3	11.5	12.0
Ave. birth wt. (gm)	6.3	5.5	6.2	6.0	6.3	6.2
Eye defects (%)	0	100.0	0	48.8	28.3	20.8
Other defects (%)	0	0	0	2.4[3]	0	0

(1) See Table 1 B.

(2) All but one died within one week.

(3) Tailless

Table 7. B. Organ Weight of 8 Weeks Old Offspring

Treatment	Offspring		Average Body wt. (Gm)	Organ Weight			
	Sex	No.		Testis (Gm)	Ovary (Mg)	Adrenal (Mg)	Pituitary (Mg)
Control	male	12	185	2.2 ± 0.04	--	28.6 ± 1.2	5.5 ± 0.3
	female	10	164	--	41.3 ± 9.7	30.6 ± 1.6	5.2 ± 0.2
X-rays and AET	male	6	209	2.4 ± 0.18	--	35.4 ± 1.6	9.9 ± 0.9
	female	12	143	--	49.5 ± 5.2	43.9 ± 2.9	9.6 ± 0.8
X-rays and MEA	male	9	201	2.2 ± 0.11	--	36.9 ± 1.1	9.4 ± 0.3
	female	14	151	--	53.5 ± 0.3	51.2 ± 1.7	10.3 ± 0.5
X-rays and AET and MEA	male	9	167	2.2 ± 0.07	--	31.3 ± 1.8	7.3 ± 0.3
	female	14	145	--	50.0 ± 3.5	42.7 ± 1.6	8.9 ± 0.5

Table 7. C. Reproductive Capacity of Offspring at 8 Weeks of Age
(Fertility test: A period of 5 consecutive days)

Reproductive Capacity	Controls (120 days old)	X-irradiation (200 r)	
		AET (50 mg)	MEA (25 mg)
Percent female mated	--	50 (8)	50 (10)
Ave. implantation	13.0	10.5	11.2
Ave. litter size	12.8	10.0	10.0
Ave. birth weight (gm)	6.3	6.4	6.2

Table 7. D. Brain Weight of 8 Weeks Old Offspring

Treatment	Offspring		Average Body wt. (gm)	Brain Weight (gm/100 gm body wt.)	
	Sex	No.		Sex Average	Group Average
Control	male	4	174	0.91 ± 0.03	1.08 ± 0.09
	female	4	145	1.24 ± 0.04	
X-rays & AET	male	6	209	0.75 ± 0.04	0.95 ± 0.04
	female	12	143	1.04 ± 0.03	
X-rays & MEA	male	9	201	0.82 ± 0.02	0.95 ± 0.03
	female	14	151	1.04 ± 0.02	
X-rays and AET & MEA	male	9	167	0.91 ± 0.03	0.98 ± 0.02
	female	14	145	1.04 ± 0.02	

Table 8. The effect of $CaNa_3DTPA$ on rat fetuses. Studies at 19 days of gestation[1]

	Saline Control	$CaNa_3DTPA$ (mg/rat)			
		62.5	125	187.5	250
No. of pregnancies	4	2	7	4	4
Ave. implantation	13	8.5	10.9	12.8	12.3
Dead fetuses (%)	0	0	0	0	0
Resorption (%)	1.9	5.9	6.6	5.9	8.2
Live fetuses (%)	98.1	94.1	93.4	94.1	81.8
Ave. fetal wt. (gm)	2.7	2.7	2.5	2.7	2.6
Eye defects (%)	0	12.5	25.8	29.2	28.9
Other defects (%)	0	0	0	0	2.2[2]

(1) See Table 2A.

(2) Exencephalia

Table 9. The effects of X-irradiation on fetuses in rats receiving $CaNa_3DTPA$ prior to irradiation

A. Studies at 19-days of Gestation[1]

	Saline Control	X-rays Control	$CaNa_3DTPA$ (mg/rat) With and Without X-irradiation							
			62.5 mg		125 mg		187.5 mg		250 mg	
			0	200r	0	200r	0	200r	0	200r
No. of pregnancies	4	7	2	4	7	7	4	4	4	2
Ave. implantation	13.0	12.1	8.5	12.3	10.9	12.4	12.8	12.0	12.3	12.5
Dead fetuses (%)	0	0	0	2.0	0	1.2	0	6.3	0	0
Resorption (%)	1.9	49.4	5.9	53.1	6.6	66.7	5.9	79.2	8.2	96.0
Live fetuses (%)	98.1	50.6	94.1	44.9	93.4	32.2	94.1	14.6	91.8	4.0
Ave. fetal wt. (gm)	2.6	2.2	2.7	2.1	2.5	1.8	2.7	1.9	2.6	1.9
Eye defects (%)	0	95.5	12.5	90.9	25.8	100.0	29.2	100.0	28.9	100.0
Other defects (%)	0	(2) 8.7	0	(2) 27.3	0	(3) 46.4	0	(4) 57.1	(2) 2.2	0

(1) See Table 2A.

(2) Exencephalia

(3) Absence of fore limb's toes and exencephalia

(4) Short-tail and exencephalia

Table 9. B. Studies after Parturition[1]

	Saline Control	X-rays Control	$CaNa_3DTPA$ (mg/rat) With and Without X-irradiation							
			62.5 mg		125 mg		187.5 mg		250 mg	
			0	200r	0	200r	0	200r	0	200r
No. of pregnancies	5	5	5	3	5	3	4		3	
Stillborn (%)	0	9.1	0	21.4	1.7	0	0		23.1	
Ave. litter size	9.8	2.4	12.2	(2) 9.3	11.8	0	9.0		(4) 4.3	
Ave. birth wt.(gm)	6.3	5.5	5.9	5.1	5.5	0	5.6		4.8	
Eye defects (%)	0	100.0	9.8	92.9	46.6	0	50.0		27.3	
Other defects (%)	0	0	0	(3) 3.6	0	0	0		(5) 15.4	

(1) See Table 2B.

(2) 22.73% lived through weaming

(3) Harelip cleft palate

(4) Died within one week after birth

(5) Anemia and absence of left fore-limb's toes

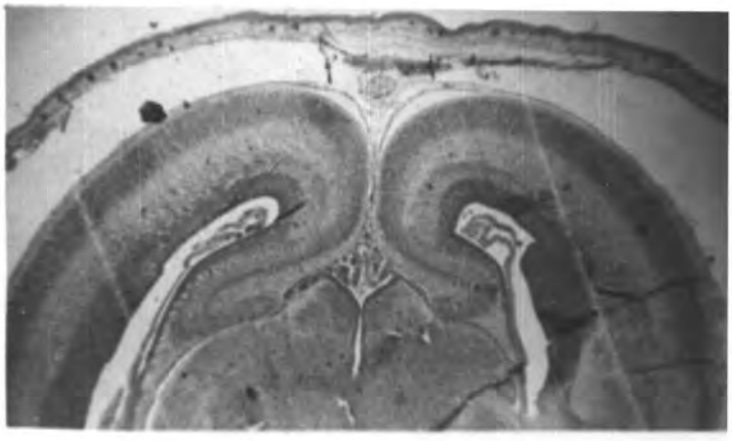


Figure 7. Cross section of brain of 19-day fetus from non-irradiated rat (X 3.5, H & E).

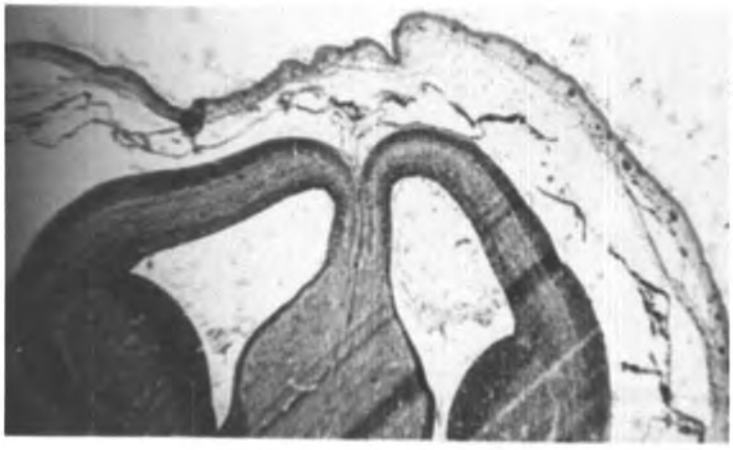


Figure 8. Cross section of brain of 19-day fetus from rat exposed to 200 r X-irradiation at 9.5 days of gestation showing the condition of hydrocephalus(X 3.5, H & E).

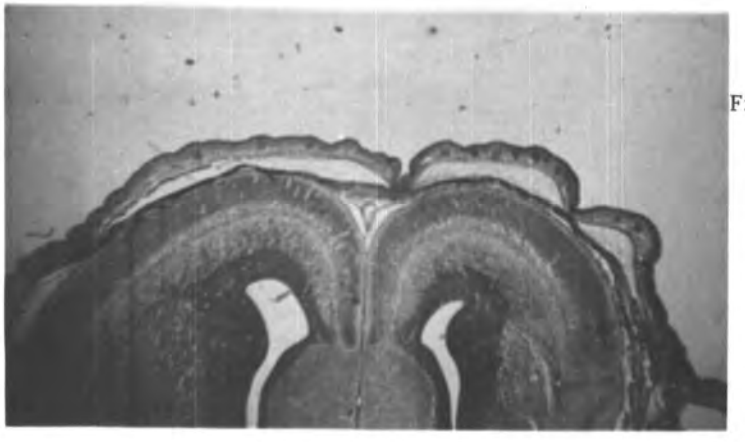


Figure 9. Cross section of brain of 19-day fetus from rat exposed to 200 r X-irradiation at 9.5 days of gestation showing the protective effect of AET. No apparent anomalies in the brain section (X 3.5, H & E).

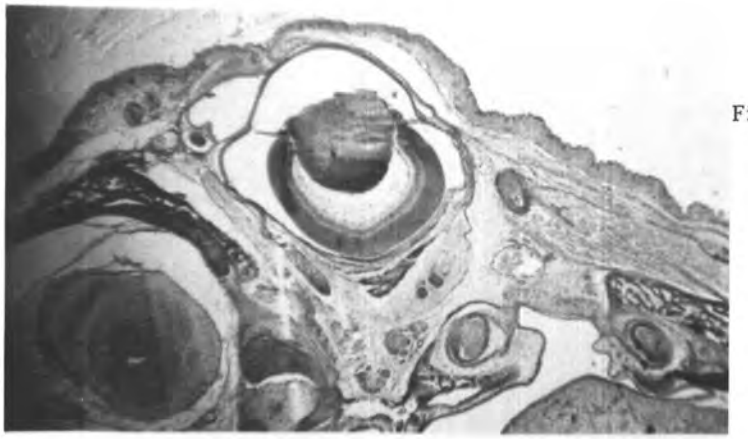


Figure 10. Cross section of eye of 19-day fetus from rat receiving no irradiation (X 3.5, H & E).

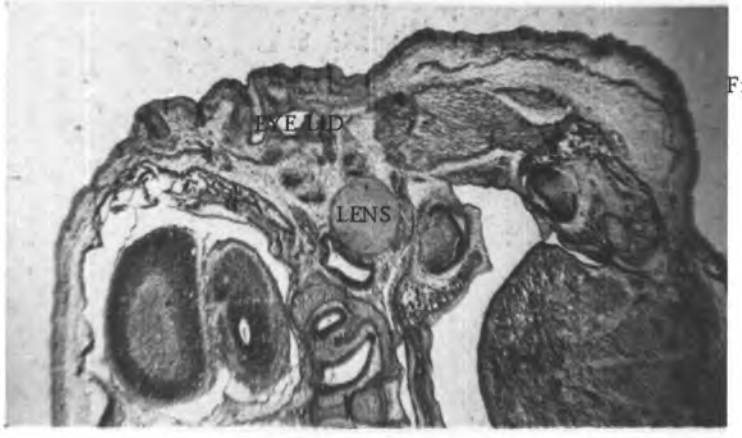


Figure 11. Cross section of eye of 19-day fetus from rat receiving 200 r X-irradiation at 9.5 days of gestation. Note the malformation of the eye. No central nerve part developed, but lens and eye lids are presented (X 3.5, H & E).

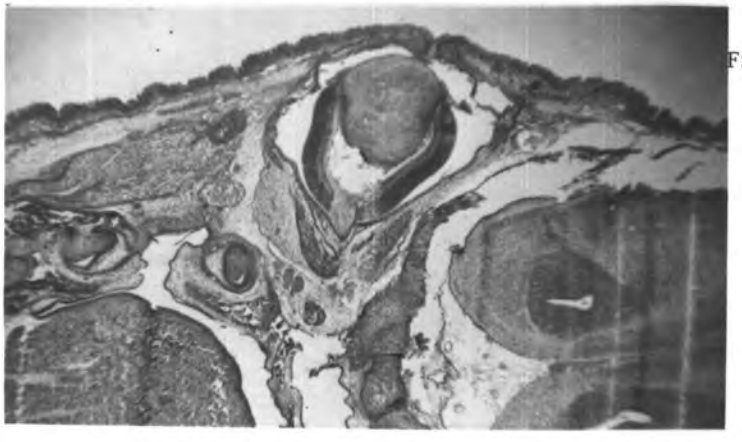
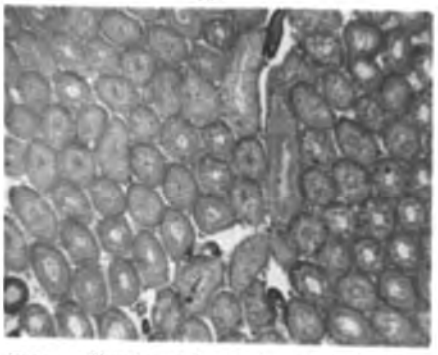


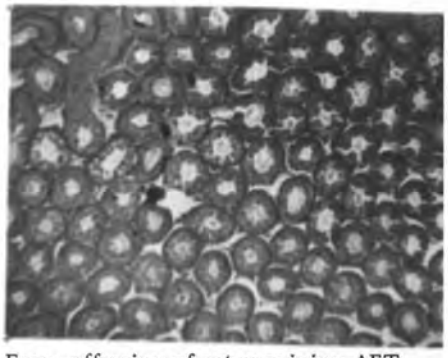
Figure 12. Cross section of eye of 19-day fetus from rat receiving AET (50 mg) and MEA (25 mg) before 200 r X-irradiation on 9.5 days of gestation, showing protective effect of the agents. No apparent anomalies were observed (X 3.5, H & E).

A



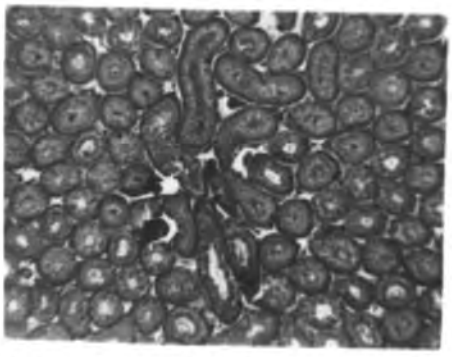
From offspring of non-irradiated rat.

B



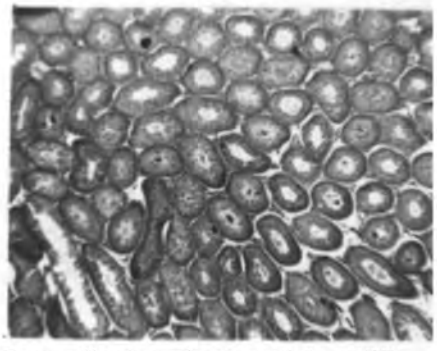
From offspring of rat receiving AET (50 mg) prior to X-irradiation.

C



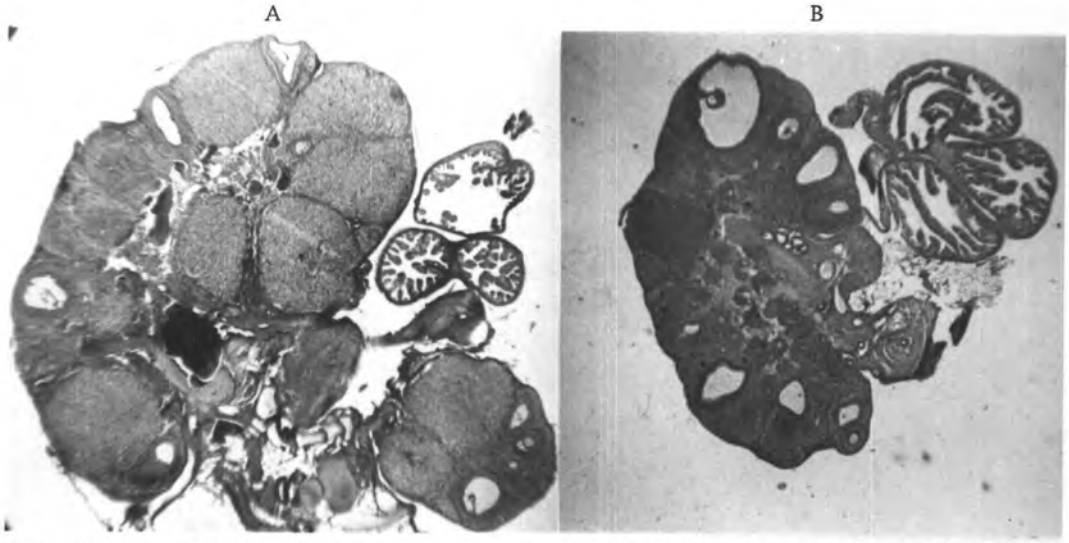
From offspring of rat receiving MEA (25 mg) prior to X-irradiation.

D



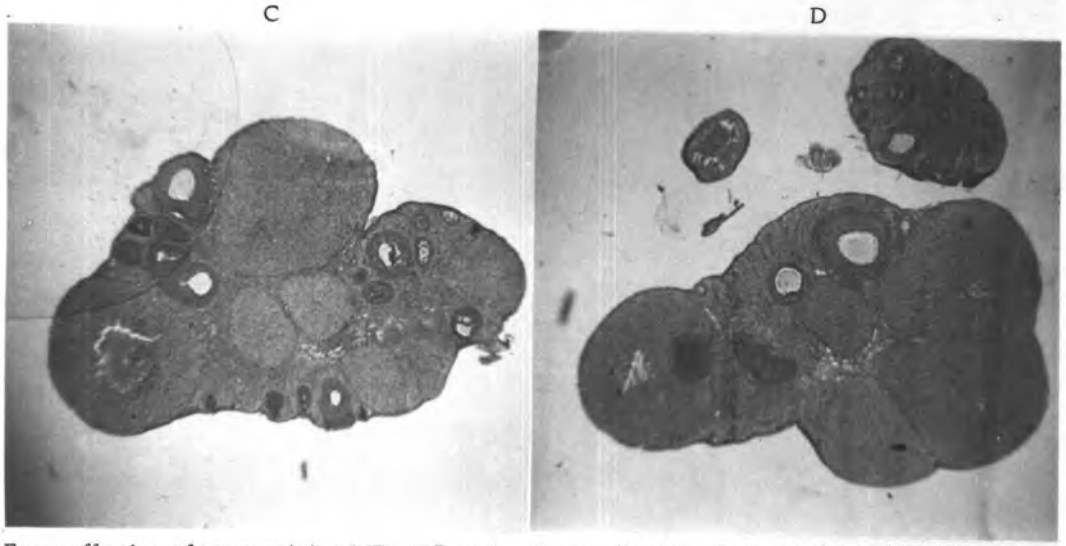
From offspring of rat receiving AET (50 mg) and MEA (25 mg) prior to X-irradiation.

Figure 13. Cross section of testes of eight week old offspring from non-irradiated rats and rats receiving AET and MEA prior to 200 r whole body X-irradiation at 9.5 days of gestation. Note active spermatogenesis apparent in all cases, indicating the protective effect of AET and MEA (X 3.5, H & E).



From offspring of non-irradiated rat.

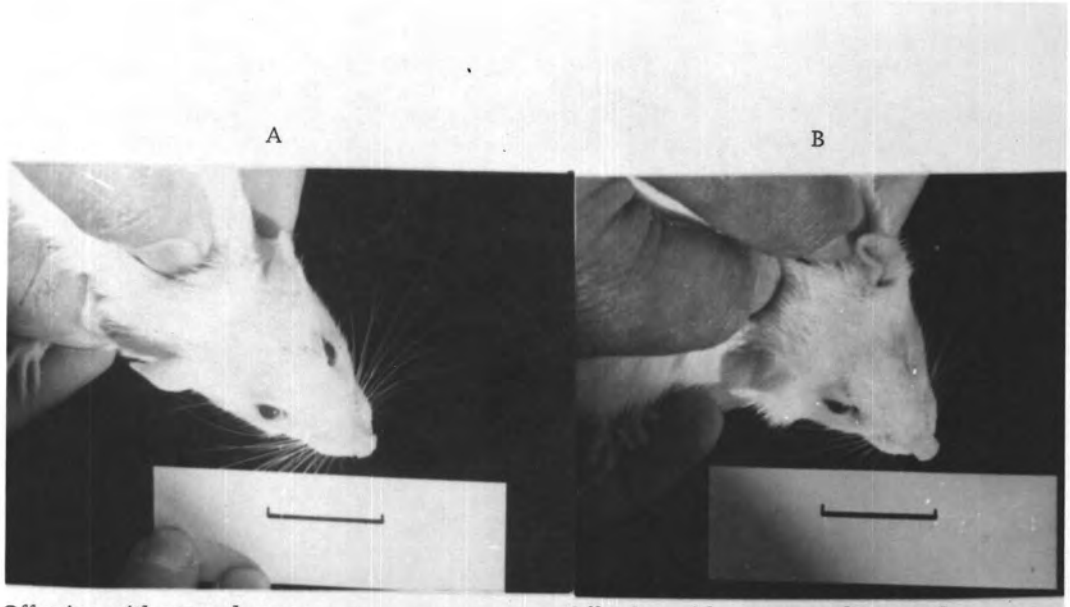
From offspring of rat receiving AET (50 mg) prior to X-irradiation.



From offspring of rat receiving MEA (25 mg) prior to X-irradiation.

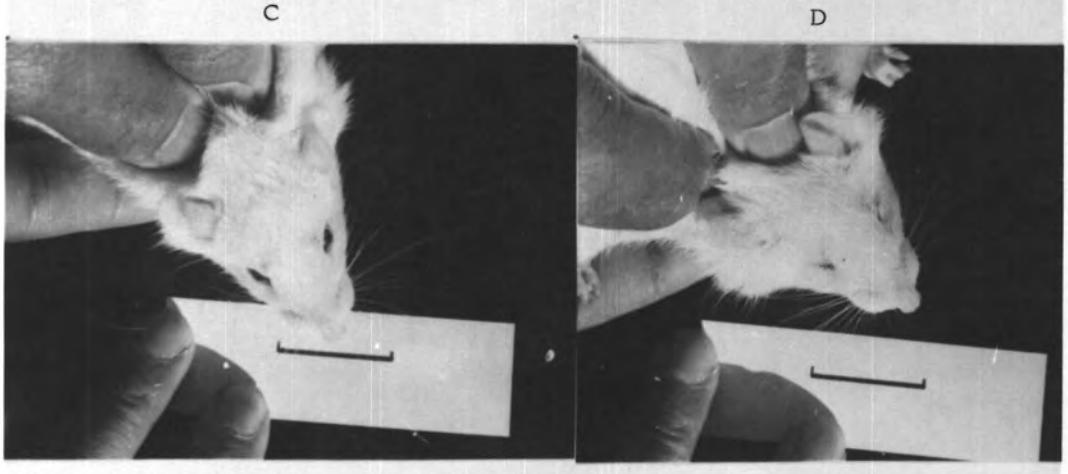
From offspring of rat receiving AET (50 mg) and MEA (25 mg) prior to X-irradiation.

Figure 14. Cross section of ovaries of eight weeks old offspring from non-irradiated rat and rats receiving AET and MEA prior to 200 r whole body X-irradiation at 9.5 days of gestation. Note active oogenesis apparent in all cases, indicating the protective effect of AET and MEA (X 3.5, H & E).



Offspring with normal eyes.

Offspring with one normal eye and one eye suffering from anophthalmia.



Offspring suffering from microphthalmia, (bilateral).

Offspring suffering from anophthalmia (bilateral).

Figure 15. Offspring from non-irradiated rat (A) and rats receiving $CaNa_3DTPA$ (B, C and D) at 9.5 days of gestation showing eye anomalies induced in rat fetuses by the administration of DTPA.