Effects of Radioprotective Agents on Foot Deformities and Gait Defects in the Prenatally X-Irradiated Rat.* (27802)

BENJAMIN H. ERSHOFF, CECIL W. STEERS, JR., AND LAWRENCE KRUGER
Western Biological Labs., Culver City, and Department of Anatomy, University of California School of Medicine, Los Angeles

Numerous reports have indicated that x-irradiation during certain stages of pregnancy may induce abnormalities in the young with effects dependent on the embryonic stage at which x-irradiation occurs and the dosage employed(1-7). However, there are few studies concerning to what extent such effects can be prevented or ameliorated by administration of radioprotective agents. Maisin *et al.*(8) found that cysteamine gave some protection to the fetus when administered to rats 15-18 days pregnant before exposure to 300 r x-irradiation. Rugh and Clugston(9) reported that cysteamine administered before x-irradiation at 14.5 or 17.5 days of gestation in mice increased the percentage survival and weight increment of the young during the first month after birth. Similar effects were obtained with cystamine(10). A reduction in abnormalities in the fetuses of pregnant mice treated with cysteamine before radiation exposure has also been reported by Woolam and Millen(11). Further data are presented here on the deleterious effects of prenatal x-irradiation in the rat and the protective effects of administering radioprotective agents thereon.

Procedure and results. Eighty-four female rats of the Long-Evans strain which had been raised from weaning on a natural food stock ration were selected at 3 to 4 months of age and an average body weight of 212 g. Animals were divided at random into 7 equal groups of 12 rats each and were mated to males of proven fertility. Pregnancy was dated by the finding of sperm in the vaginal smear.† Animals in Group I served as non-irradiated controls; those in the other groups received a single exposure of 150 r total body x-irradiation on the 14th day of pregnancy. The irradiated groups differed as follows: Group II served as non-injected irradiated controls; Group III was administered an intraperitoneal injection of saline solution at a level of 0.33 cc per 100 g body weight 20 minutes prior to x-irradiation exposure; Group IV was similarly treated except that it received a pH 7.5 phosphate buffer solution; Groups V, VI and VII received a single intraperitoneal injection of AET (S-(2-aminoethyl) thiuronium bromide hydrobromide), cysteamine (beta-mercaptoethylamine) and MEG (2-mercaptoethylguanidine) respectively 20 minutes prior to x-irradiation exposure. These radioprotective agents were administered as a 3% solution at a level of 100 mg per kg of body weight.‡ Radiation factors were as follows: GE Model Maximar 250; 250 kv; 15 ma; 0.5 mm Cu and 1 mm Al filters plus a Cu parabolic filter;§ HVL, 2.15 mm Cu; target distance to top of box, 78 cm; and exposure dose rate, 17.92 r per minute (measured in air, at top of box). The animals to be irradiated were placed in a

* This investigation was supported by a research grant from Division of Radiological Health, P.H.S., N.I.H.

† Start of pregnancy was dated from the time sperm were actually observed in the vaginal smear. As mating may have occurred at any time from 5 p.m. of the previous day to 8 a.m. of the day sperm were found, fertilization may have occurred as much as 15 hours before sperm were observed. First day of pregnancy covered the first 24 hours following finding of sperm. It is estimated that the actual time of x-irradiation was 13½ days post fertilization.

‡ The AET and cysteamine were dissolved in saline solution. MEG was prepared by dissolving weighed amount of S-(2-aminoethyl) thiuronium bromide hydrobromide in pH 10.5 phosphate buffer (0.06M) and rapidly adding NaOH until pH 7.5 was reached. The solution was brought to a final volume (30 mg MEG per cc) by diluting with pH 7.5 phosphate buffer. The solution was covered and used within 2 hours of preparation.

§ A non-uniform filter which produces a flat isodose surface of x-ray intensity constructed by the method of Greenfield and Hand (12). The center of the filter had a thickness of 1.7 mm Cu; the edge, 0.5 mm Cu.

wooden box divided into 14 compartments 7 cm wide, 16 cm long and 10 cm deep. Partitions and top were made of 1/8" cellulose acetate sheeting; the top, one side and bottom of each compartment were perforated with holes for ventilation. The container was rotated slowly on an electrically driven turntable to insure equivalent irradiation. Throughout pregnancy and lactation rats were fed a natural food stock ration.‖ Food and water were provided *ad libitum*. On the 20th day of pregnancy rats were placed in individual cages with solid bottom covered with wood shavings in which they remained during the balance of the pregnancy and lactation period. At least 8 rats in each of the groups cast litters. With the exception of the findings indicated below these young appeared grossly normal. At 3 days of age litters of 9 or more young were reduced to 8 each. No significant differences in body weight of the young at birth or at 5, 10 or 21 days of age were observed between the various groups, nor did the groups differ as to percentage of young surviving until weaned at 21 days of age. Marked differences were observed, however, at the time of weaning between offspring of the various groups in respect to deformities of foot and defects in gait.

Approximately 70% of the offspring of non-injected irradiated rats (Group II) exhibited deformities of the hind or fore feet. These were grossly manifest as the absence of one or more digits or by the fusion of digits to give a club-foot appearance (Fig. 1a and 1b) in contrast to that of the offspring of non-irradiated rats (Fig. 1c). In general the hind feet when affected were more severely deformed than the fore feet although the incidence of deformities was somewhat greater in the latter. Similar changes were observed in the offspring of irradiated rats injected with saline or buffer solution (Groups III and IV). The incidence of deformities (particularly those of the hind feet) was greater in these groups than in the offspring of rats of Group II. A highly significant reduction

‖ Rockland Rat Diet, A. E. Staley Manufacturing Co., Decatur, Ill.

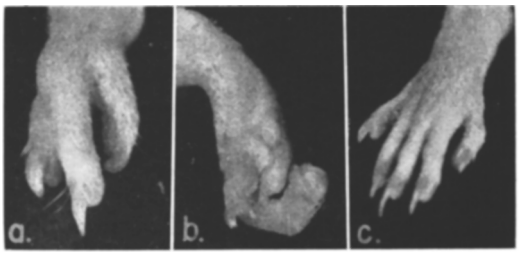

FIG. 1. Absence of digits (a) and club-foot appearance (b) of hind feet of offspring of irradiated rats (Group II). (c) Normal appearing hind foot in offspring of non-irradiated rat (Group I).

in incidence of foot deformities occurred in the offspring of irradiated rats injected with AET, cysteamine or MEG (Groups V, VI and VII). The protective effect of administering a radioprotective agent was particularly marked in offspring of rats treated with MEG. A significant proportion (*i.e.*, 24%) of the offspring of non-injected irradiated rats (Group II) also displayed a defect in gait which resembled hopping in the hindlimbs. This was not correlated with the foot deformities indicated above inasmuch as some rats with gait defects did not have foot deformities and some rats with foot deformities had no defect in gait.¶ The neurological disturbance appeared to be restricted to the hindlimbs. A sensory defect was evidenced by the loss of the non-visual tactile placing reaction and a larger angular displacement required for eliciting the hopping reaction. There was also a bilaterally symmetrical marked increase in resistance to passive manipulation and an increase in palpable tone. The motor defect was readily apparent in the progression of the animals. Similar findings were observed in 27% of the offspring of irradiated rats injected with buffer solution (Group IV) and in 73% of the offspring of irradiated rats injected with saline solution (Group III). A significant reduction in incidence of the gait defect indicated above occurred in offspring of irradiated rats injected with AET, cysteamine or

¶ Subsequent studies indicate that in contrast to the effects obtained following x-irradiation on the 14th day of pregnancy, foot deformities or a defect in gait were not observed in any of the offspring of rats exposed to a single dose of 150 r total body x-irradiation on the 13th or 15th day of pregnancy.

TABLE I. Effects of Radioprotective Agents on Incidence of Foot and Gait Defects in Prenatally X-irradiated Rats.

Group	No. of animals	% of rats with		
		Front foot defects	Hind foot defects	Gait defects
Offspring of non-irradiated rats (Group I)	68	0	0	0
Offspring of irradiated rats				
Non-injected (Group II)	63	70	35	24
Saline injected (Group III)	55	91	100	73
Buffer injected (Group IV)	56	70	70	27
AET injected (Group V)	49	33	24	12
Cysteamine injected (Group VI)	51	25	10	0
MEG injected (Group VII)	62	16	3	2

MEG. The protective effect of administering a radioprotective agent was particularly marked in the offspring of rats of Groups VI and VII (Table I).

Three males and 3 females from each group selected at random at weaning were maintained on the stock diet for an additional 90 days, then were sacrificed and the brains removed and weighed. The male rats at time of sacrifice ranged from 307 to 372 g in body weight; female rats from 179 to 226 g. The brain weights of the above rats expressed as g per 100 g body weight were .574 for male offspring of Group I and .440, .410, .428, .430, .445, and .422 for male offspring of Groups II-VII respectively. Brain weights of the female rats expressed as g per 100 g body weight were .932 for the offspring of rats of Group I and .668, .625, .630, .647, .703, and .663 for the offspring of Groups II to VII respectively. It is apparent from these findings that the ratio of brain weight to body weight was reduced in both male and female offspring of irradiated rats and that administration of the radioprotective agents, AET, cysteamine and MEG, was without significant effect in counteracting the reduction in brain weight. Both male and female offspring of Groups II, III and IV also displayed a distinct change in the gross morphology of the brain as compared to the offspring of the other groups. The abnormality was readily apparent in respect to the dimensions of the cerebral hemispheres. The most severe change in contour was evident in the posterior medial portion of the cerebral cortex so that the superior colliculus appeared exposed rather than being overlapped by the caudal pole of the cortex.** The latter is the normal appearance and was exhibited by both the male and female offspring of non-irradiated rats (Group I) (Fig. 2). The above abnormalities in offspring of rats irradiated on the 14th day of pregnancy confirm findings previously reported by Hicks(7). No significant difference was observed between the offspring of Groups II, III and IV in this regard. Also the brains of rats in these groups which had a defect in gait did not differ grossly in appearance from those in the same group which did not exhibit this defect. Both male and female offspring of irradiated rats injected with AET, cysteamine and MEG (Groups V, VI and VII) were intermediate in appearance between the male and female offspring of non-irradiated rats (Group I) and those of non-injected or saline or buffer injected irradiated rats (Groups II, III and IV). These animals displayed an intermediate condition of development of the caudal portion of the cerebal cortex with more of the superior colliculus exposed than in the offspring of Group I, but less than in the offspring of Groups II, III and IV (Fig. 2).

Present findings indicate that the radioprotective agent AET and, to a greater degree, cysteamine and MEG largely prevented occurrence of foot deformities and gait defects in the young of rats exposed to a single

** Microscopic examination of a complete series of 12 μ sections of the brain (Nissl stained) from 2 animals in Group II which had both foot deformities and a defect in gait did not reveal any lesion or failure of development in any specific structure of the brain, although the relative size of various structures may have been affected.

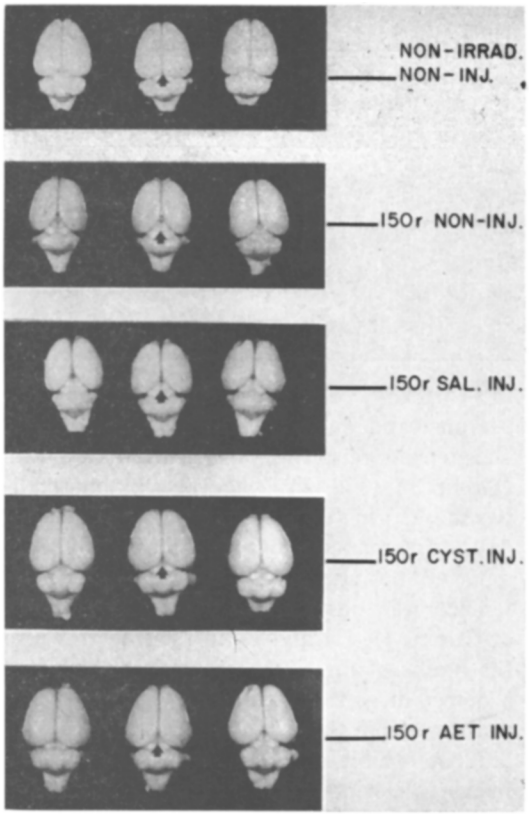

FIG. 2. Photograph of brains of 3 male rats per group demonstrating distortion of development of cerebral hemispheres and effect of radioprotective agents in offspring of rats exposed to 150 r total body x-irradiation on 14th day of pregnancy. The arrow indicates the caudal border of the superior colliculus which is exposed to varying degrees in offspring of irradiated groups. Brains of offspring of MEG-treated rats (Group VII) were similar to those of offspring of cysteamine and AET treated animals (Groups V & VI); brains of offspring of buffer treated rats (Group IV) were similar to those in offspring of saline injected rats (Group III). Gross appearance of the brains of female rats in the various groups was similar to that of male rats of the same group.

dose of 150 r total body x-irradiation on the 14th day of pregnancy. These radioprotective agents also counteracted in part the occurrence of an abnormality in dimensions of the cerebral hemispheres of offspring of non-injected or saline or buffer injected irradiated rats but were without significant effect in altering the reduced ratio of brain to body weight in such rats. Unpublished studies from this laboratory indicate that AET, cysteamine and MEG when administered at the above dosage were also without effect in reducing the high incidence of anopthalmia and microphthalmia observed in the young of rats exposed to a single dose of 150 r total body x-irradiation on the 10th day of pregnancy; these agents were active, however, in preventing the testicular degeneration and failure of spermatogenesis in the young of rats exposed to the above dose of x-irradiation on the 18th day of pregnancy. It would appear from the above findings that radioprotective agents differ in capacity to protect against various manifestations of radiation injury in the prenatally x-irradiated rat, although the possibility that findings may reflect differences in the dose of radioprotective agent required by different organ systems has not been excluded.

Summary. The radioprotective agents AET, cysteamine and MEG largely prevented the occurrence of foot deformities and a defect in gait (which was unrelated to the foot abnormalities) in the young of rats exposed to a single dose of 150 r total body x-irradiation on the 14th day of pregnancy. These substances also prevented in part the occurrence of an abnormality in development of the cerebral hemispheres of such young but were without significant effect in altering an attendant reduction in the ratio of brain to body weight.

1. Warkany, J., Schraffenberger, E., *Am. J. Roentgenol.,* 1947, v57, 455.

2. Russell, L. B., *Radiation Biology,* Edit. by A. Hollaender, McGraw-Hill Book Co., N. Y., 1954, vI, Part 2, Chap. 13.

3. ———, *J. Exp. Zool.,* 1950, v114, 545.

4. ———, *ibid.,* 1956, v131, 329.

5. Russell, L. B., Russell, W. L., *J. Cell. and Comp. Physiol.,* Suppl. 1, 1954, v43, 103.

6. Wilson, J. G., *ibid.,* Suppl. 1, 1954, v43, 11.

7. Hicks, S. P., *ibid.,* Suppl. 1, 1954, v43, 151.

8. Maisin, H., Dunjic, A., Maldague, P., and Maisin, J., *Compt. rend. soc. biol.,* 1955, v149, 1687.

9. Rugh, R., Clugston, H., *Science,* 1956, v123, 28.

10. Rugh, R., *Am. J. Physiol.,* 1957, v189, 31.

11. Woolam, D. H. M., Millen, J. W., *Nature,* 1958, v182, 1801.

12. Greenfield, M. A., Hand, K., *Am. J. Roentgenol. Rad. Ther.,* 1952, v68, 960.

Received September 11, 1962. P.S.E.B.M., 1962, v111.