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Effects of CE^{144} Administered to Pregnant Rats.* (29351)

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Although numerous reports concerning the responses of embryos to external irradiation have been published(1), the consequences of administering radioisotopes to pregnant females have received only occasional attention. The LD_{50} dose for the rat embryo of maternally administered P^{32} increases from 0.46 mc per gram of embryonic tissue on day 6 of gestation to 1.29 mc on the tenth day(2). Injections of 0.2, 0.4, or 0.6 mc of P^{32} on day 14 or 17 of gestation have caused pronounced interference with spermatogenesis in the resulting progeny(3). Body size and growth rates were reduced in litters of females injected with 5 to 17 μc of P^{32} per

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gram of body weight 2 to 6 days prepartum (4). Increased mortality and growth retardation of embryos followed maternal injections of 191 μc of Sr^{90} on the second day of gestation; 382 μc doses on day 10 caused growth retardation only(5).

Cerium^{144} is very poorly absorbed by the digestive system(6) and would therefore be expected to gain entry into the embryo only in insignificant quantities. This report delineates some of the effects on the embryo of the passage of fairly large quantities of this material through the system of the pregnant rat at various times during the gestation period.

Materials and methods. Female rats of the Sprague-Dawley strain were caged with fertile males in the afternoon. Those females whose vaginal smears contained sperm the following morning were considered to be in the first day of gestation. Ten randomly assigned females were dosed on each of days 4 through 9 of gestation and 6 were dosed on each of days 10 through 14. Doses of carrier-free Ce^{144} in water solution were administered by stomach tube late in the afternoon of the appropriate day. Half the females on each day received 1.0 mc of the isotope (in 0.5 ml of solution), the remainder were given 0.5 mc. Stock ration and fresh water were freely available to animals throughout the experiment.

As soon as possible after the birth of each litter the numbers of live and dead young were recorded. Litters were checked daily for additional deaths until they were weaned at 21 days of age. Body weights were recorded at 21, 35 and 49 days of age. Gonads were taken for histological examination at 49 days of age from 2 males and 2 females representing the higher dose level of each of the gestation days.

Those females which failed to litter or which lost all their young within 7 days after parturition were euthanized; the number of implantation sites was compared to the number of young to which they had given birth as an indication of embryonic death rates. Forty-three females were bred a second time one week after weaning their litters to ascertain whether or not dosing affected the size of a subsequent litter. These were killed when estimated by palpation to be 12-15 days pregnant and fetuses and implantation sites were counted. Twelve control females were killed 2-3 days after littering to determine normal litter size and embryonic resorption rates. Ten control litters were reared to establish expected growth rates and post-natal death rates. Four females were dosed with 1.0 mc on day 13 of gestation and killed 5 days later. Liver samples and femurs as well as whole embryos from these females were counted in a scintillation counter to determine retention of radioactive materials in the animal's body.

Results. Although no detailed measurements were attempted on rate of passage of the dose through the animals, on the sixth day after dosing the 24-hour feces and urine accumulations registered less than 0.5 mr/hr on a survey meter. In females sacrificed 5 days after a 1.0 mc dose, total contents of less than 1.0 μc were present in both liver and femur while no activity could be detected in whole-body counts of embryos.

The data of Table I summarize the average number of young born to females dosed on various days of gestation. Of the 60 females dosed on days 4 through 9, 34 failed to litter or their litters died within 7 days

TABLE I. Young Born to Female Rats Given Oral Doses of Ce^{144} on Various Days of Gestation.*

Day gestation	Dose (mc)	No. of litters	Avg young born: Alive	Dead	% born alive living to 21 days
4	1.0	1	2.0	1.0	0
	.5	4	6.3	.5	96
5	1.0	4	2.8	.8	64
	.5	4	3.5	2.5	71
6	1.0	4	1.3	.3	0
	.5	4	3.0	1.3	100
7	1.0	1	8.0	1.0	63
	.5	4	5.8	1.0	61
8	1.0	3	9.3	.7	86
	.5	4	7.0	1.0	82
9	1.0	1	4.0	1.0	50
	.5	4	7.5	1.3	80
10-14 (av.)	1.0	15	10.8	1.3	90
	.5	15	13.0	1.0	94
Control	—	10	12.4	.7	98

* Five females at each level on days 4-9; 3 per level on days 10-14.

TABLE II. Ce^{144} Dosed Female Rats Autopsied Following Failure to Litter or Loss of Litter Within 1 Week After Parturition.*

Day gestation	Dose (mc)	No. females examined	Avg young born	Avg implantation sites at autopsy
4	1.0	4	.8	13.5
	.5	1	1.0	10.0
5	1.0	3	1.3	15.3
	.5	2	5.5	12.0
6	1.0	4	1.3	11.5
	.5	4	2.0	9.0
7	1.0	2	0	9.0
	.5	1	5.0	15.0
8	1.0	1	0	11.0
	.5	1	2.0	15.0
9	1.0	1	0	15.0
	.5	0		
Control	—	12	13.5	15.0

* Excludes those females showing no evidence of having conceived.

after parturition. Autopsy of these females showed only 10 which had not conceived. The performance of the remaining 24 is summarized in Table II. A very high fetal resorption rate among these females is shown by the small number of young born although the total number of implantation sites remained near normal. The low incidence of stillborn young and dead fetuses indicate that most deaths occurred fairly early in gestation. Post-natal mortality rates were also noticeably higher among the progeny of females dosed in early gestation. A sharp increase occurred in survival rate of embryos when dosing was done later than day 9 of gestation; of the 30 females dosed on days 10 through 14, none failed to litter. Average litter size as well as the percent surviving were definitely higher in these latter groups. Neither weaning weight nor weight gains from 21 to 49 days of age deviated greatly from similar values for untreated animals.

Physical defects among the young were rare except for those affecting the eyes. One member of a litter from a female dosed on day 7 was born with a 5 mm hole in the center of the skull within which no brain had developed. One offspring of a 14-day female had a moderately deformed tail. A total of 9 young were seen with grossly visible eye defects ranging from one small, weak eye to complete absence of both organs. Six of these rats were from 3 litters dosed on day 9, while one occurred following dosing on day 8, and 2 following day 7 doses.

The histological characteristics of the gonads taken from 49-day old rats were not unusual except in the 13- and 14-day testes. The 14-day samples contained sterile tubules as well as many in which the general arrangement of cells seemed disrupted. Roughly half the tubules in one sample had been affected compared to only a few in the other. The 13-day samples showed occasional deranged tubules.

No reduction could be detected in the size of the next litter of females which had been dosed. The 43 rats which were bred after dosing carried an average of 13.7 fetuses and 14.9 implantation sites compared to 13.5 young and 15.0 sites for the control females.

Discussion. The unusually small number of young born to female rats dosed between the 4th and 9th days of pregnancy generally support the findings of Rugh(7) that exposures in the first trimester of pregnancy result in higher embryonic death rates than those administered at later dates. When Sikov and Noonan(8) injected rats with P^{32}, they found that those embryos which were killed died within a short time after injection. The definite implantation sites in females dosed on the 4th day indicate an appreciable time lapse between dosing and death of the embryo.

The reduction in litter size among females dosed on days 4 through 9, indicates that many individual embryonic deaths occurred without loss of the entire litter. Part of this variation in sensitivity between members of a litter was probably due to differences in the dose received by embryos as a result of their location within the uterus. This is not the complete answer, however, since variations in embryonic susceptibility have also been noted following whole-body exposures of pregnant females, in which case the dose to the embryos should be fairly constant(9,10). Although live births occurred following dosing during the first week of pregnancy, an effect on the survivors of these early doses is indicated by the lower per-

centage which lived until 21 days of age. The internal changes produced in the embryo which cause this increase in early post-natal death rates are as yet undetermined, although similar results have been observed by others (11,12).

An inherent problem in studies of this nature is that of determining the irradiation dose actually received by the embryo. Brent (9) calculated a 29% fetal resorption rate following 150 r whole-body exposures on day 6, while Sikov and Lofstrom(13) killed about 10% by protracting the same 150 r dose over days 9 to 9½ or 10 to 10½ of gestation. Ershoff(11) obtained no young from 10 females given a 300 r exposure on day 10 of gestation while 7 of 10 exposed on day 14 had litters. Comparisons of the present results with those cited indicate that the 1.0 mc dose subjected the embryos to more damage than a 150 r whole-body exposure of the dam but less than a 300 r exposure. The protraction of the total dose from an ingested isotope compared to an acute whole-body exposure is not overlooked, but its effect is impossible to calculate.

The number of grossly discernible defects noted among the progeny of dosed females was surprisingly low considering that whole-body exposures have produced from 10 to almost 100% defective young(7,11,13). The only physical defects seen in significant numbers of young were those affecting the eyes following dosing on days 7, 8 and especially day 9 of gestation. The timing of these defects is in reasonable agreement with the results of Sikov and Lofstrom(13) although they noted a peak incidence of defective eyes following acute exposures on the 10th day of gestation. The protraction of dose in the present study probably contributed to the low numbers of deformed young since fractionation has been shown to lower the incidence of abnormalities(13,14).

The sensitive period of the embryonic rat testis seems to begin at about 14 days of gestation, depending somewhat on the exposure dose(11,15). This probably explains the fact that histological changes were seen in the gonads only in the 13- and 14-day young and then were not very serious. While damage could be seen in some tubules of these two groups, the testes were definitely producing sperm and the males would not have been suspected of sterility.

Summary. Single oral doses of 0.5 and 1.0 mc of Ce^{144} were delivered to female rats on days 4 through 14 of gestation. High embryonic resorption rates resulted in markedly reduced numbers of young among those dosed between 4 and 9 days. Doses of 0.5 mc were damaging in this early period although less so than the higher level. Doses administered later than day 9, caused fewer embryonic deaths than those given earlier in pregnancy. The only physical defects noted in significant numbers among surviving progeny were those affecting the eyes in litters dosed on days 7, 8 or 9. No definite effect on growth rate of the offspring could be ascertained and only moderate changes in testis histology following 13- or 14-day doses.

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