mixture was acetylated and chromatographed on alumina and then Magnesol: Celite, using a hexane-acetone system. Details of the chromatographic procedures will be published subsequently. The compound was crystallized from acetone: pentane, and the final purification was achieved by sublimation in high vacuum.

The white crystalline solid finally obtained melted at 124°–125°; mixed mp with progesterone, 123.5-5-125° (progesterone mp 123°–125°). The derivative made with dinitrophenylhydrazine melted at 288°–294° as compared with known progesterone *bis*-dinitrophenylhydrazone, which melted at 287°–297°. The amount of isolated progesterone was roughly 1 mg/kg fresh tissue and seemed sufficient to account for the known progestational activity of the starting material.

From the biological point of view, the isolation of progesterone from placentae provides evidence in support of the concept that this organ secretes progesterone during the latter part of pregnancy. Since progesterone has been isolated from tissues of the whale, sow, and steer, it also suggests that the human has adopted the same chemical structure as other mammals for progestational action.

References

1. NEWTON, W. H. In E. Allen, C. Danforth, and E. Doisy (Eds.), *Sex and Internal Secretions.* Baltimore: Williams & Wilkins (1939).
2. DE FREMERY, P., LUCHS, A., and TAUSK, M. *Arch. ges. Physiol.,* **231**, 341 (1931).
3. FORBES, T. R. *Am. J. Obstet. Gynecol.,* **60**, 180 (1950).
4. ———. *Endocrinology,* **49**, 218 (1951).
5. PEARLMAN, W. H. In G. Pincus and K. V. Thimann (Eds.), *The Hormones.* New York: Academic Press (1948).
6. HASKINS, A. L., JR. *Proc. Soc. Exptl. Biol. Med.,* **73**, 439 (1950).
7. BUTT, W. R., *et al. Biochem. J.,* **49**, 434 (1951).
8. PEARLMAN, W. H. *Federation Proc.,* **10**, 232 (1951).
9. PRELOG, V., and MEISTER, P. *Helv. Chim. Acta,* **32**, 2435 (1949).
10. HOOKER, C. W., and FORBES, T. R. *Endocrinology,* **41**, 158 (1947).
11. RUZICKA, L., and PRELOG, V. *Helv. Chim. Acta,* **26**, 986 (1943).

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Effects of Whole Body Exposure to Irradiation upon Subsequent Fertility of Male Rabbits[1]

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The biological action of ionizing radiation on the functional activity of the sperm has been the subject of a number of studies in recent years. Most of the interest, however, has been centered on the effects of direct irradiation of the sperm *in vitro* (*1–3*) or of localized radiation of the testes (*4–9*), with the major parts of the body protected by a lead shield. Few studies have dealt with the effects of whole body exposure to relatively low levels of radiation, prior to mating, upon the subsequent fertility of the exposed male (*10, 11*). Most of the observations concerning the effects of whole body exposure to radiation on fertility have been incidental to other studies.

The data in this report are the results of a preliminary investigation of the effects of whole body exposure of male rabbits to x-rays upon their subsequent fertility when mated to normal females.

The mature male and female rabbits used in this study were purchased from commercial rabbit breeders. All males were test-mated prior to treatment to insure the use of potentially fertile males.

An estimate of the effects of irradiation of the male on his potential fertility was obtained by comparison of the data obtained from normal (test) females bred to irradiated males with similar data obtained from normal (control) females bred to nonirradiated males. The evaluation of the potential fertility of each male was based on (1) the initial fertilization rate of ova as determined 28–72 hr after mating, (2) the fetal death rate as determined between the 9th and 24th day of pregnancy, and (3) the litter size and the viability of the young.

The initial fertilization rate of ova was studied only in matings made within the first 2 weeks of the male post-treatment period, and the data on fetal mortality, litter size, and viability of young born were obtained from matings made throughout the 108-day experimental period. Three females, in which none of the eggs were fertilized, were excluded from the summary of data on the initial fertilization rate.

Abdominal palpations of all females, not sacrificed within 72 hr after mating, were done on the 10th day after mating and at weekly intervals thereafter for evidence of pregnancy (*12*). No females diagnosed as not pregnant on the 10th day were sacrificed for the determination of the fetal mortality rate. Although this may have introduced a slight error, every effort was made to prevent unduly penalizing the male by charging him with the failure of fertilization when, in fact, the female may have been at fault.

Eight males were bilaterally exposed to whole body irradiation administered with a General Electric Maxitron x-ray machine. Three levels of radiation, measured in air, were given: 2 males received 100 r, 4 received 200 r, and 2 received 300 r. The factors of radiation were 250 kvp, 30 ma, hvl 0.4 mm copper, inherent filtration 1 mm aluminum, added filtration 3 mm aluminum, TSD 93.7 cm, rate 48 r/min.

Experimental matings to test females (nonirradiated) were started as soon after irradiation as possible, and all males were mated at least one time during the first week after exposure. The frequency of postirradiation matings was determined by the availability of estrous females. Matings of 3 nonirradiated males provided the control data.

The chi-square test for homogeneity was used as the test of significance (*13*).

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[2] From the Veterinary Corps, U. S. Army.

TABLE 1

FETAL MORTALITY IN NORMAL FEMALE RABBITS MATED TO CONTROL OR IRRADIATED MALES

Treatment of males	No. of matings	No. of corpora lutea*	No. of fetuses†		Percentage of eggs missing	Percentage fetal loss
			Alive	Dead		
Control	7	74 (10.6)	59 (8.4)	0	20.3	0.0
All irradiated males	26	318 (12.2)	146 (5.6)	71	31.8	32.7
100 r	6	92 (15.3)	46 (7.7)	18	30.4	28.1
200 r	14	163 (11.6)	74 (5.3)	34	33.8	31.5
300 r	6	63 (10.5)	26 (4.3)	19	28.6	42.2

* Numbers in parentheses are the average number of corpora lutea per female.
† Numbers in parentheses represent the average potential litter size if all live to birth.

TABLE 2

FERTILITY IN NORMAL FEMALE RABBITS MATED TO CONTROL OR IRRADIATED MALES

Treatment of males	No. of matings	No. of matings fertile	No. of litters born*	No. of young born†	Av litter size†	Av litter size born alive per mating
Control	34	31 (91.2%)	30 (1)	233 (223)	7.7 (7.4)	6.6
All irradiated males	57	47 (82.5%)	38 (9)	244 (218)	6.4 (5.7)	3.8
100 r	15	12 (80%)	10 (2)	60 (51)	6.0 (5.1)	3.4
200 r	26	23 (88.5%)	19 (4)	122 (116)	6.4 (6.1)	4.5
300 r	16	12 (75%)	9 (3)	62 (51)	6.9 (5.7)	3.2

* Figures in parentheses in this column are the numbers of litters resorbed.
† Figures in parentheses in these columns are the young born alive.

The initial fertilization rate was 78.3 and 77.0%, respectively, in the control and test female groups. A total of 115 ova, of which 90 were fertilized, was recovered from 10 control females; a total of 74 ova, of which 57 were fertilized, was recovered from 9 test females. The percentage recovery of ova in the two groups, based on the number of ovulation points counted in the ovaries, was 91.3 and 86.1, respectively. These data failed to demonstrate that irradiation, within the levels used in this study, significantly affected the ability of the sperm to fertilize the ova in normal females during the first 2 weeks of the postirradiation period.

The fetal mortality rate was zero and 32.7% for the control and all test female groups, respectively (Table 1). The differences between the control and each of the test groups are highly significant ($P < .01$). The failure to find any fetal deaths in the control group is not in agreement with the work of Hammond (*14*) in which it was found that approximately 9% of the eggs ovulated were subsequently represented by atrophic fetuses. However, if one assumes that a 10% fetal death loss is to be expected, the differences between the control and test groups are still highly significant. The differences between the three test female groups are not significant.

The differences between the number of corpora lutea and the total number of fetuses are expressed as "percentage of eggs missing" in Table 1. The 20.3% of eggs missing in the control female group is in close agreement with the 23.7% of eggs missing found by Hammond (*14*). However, the 31.8% of eggs missing in the test female group is definitely higher and approaches a level of significance ($P = .052$). The fact that there was a greater loss of eggs in each of the test groups is suggestive either of some interference with fertilization or of such early fetal death that the decidual reaction resulting in development of the placentoma was not initiated. The authors are inclined to accept the latter possibility at the present time.

This interpretation seems to be in accord with the finding of Amoroso and Parkes (*1*) that, after insemination of female rabbits with sperm exposed *in vitro* to 250, 500, or 1000 r by x-rays, an increasing proportion of the tubal ova obtained about 40 hr after ovulation showed arrest of segmentation. A similar phenomenon has been observed in females mated to male rabbits exposed to 400, 500, or 600 r (unpublished data in this laboratory). However, it should be noted that in this study there is no trend toward a higher percentage loss of eggs associated with the higher dose of irradiation received by the male parent.

The difference in percentage of matings fertile, 91.2 and 82.5, respectively, between the control and test female group is not significant. One male that received 100 r was sterile at 51 days, one that received 200 r was sterile at 50 days, and one that received 300 r was sterile at 95 days after irradiation. All other treated males continued to produce some pregnancies throughout the experimental period. No control males became sterile during the study.

The data on the number of whole litters resorbed (Table 2) were obtained from females which were diagnosed as pregnant on the 10th day after mating and which subsequently failed to produce litters. Although the difference between the control and test

group is not significant, it is interpreted as an indication that irradiation of the male prior to mating may increase the frequency of resorption of whole litters in rabbits.

The difference in numbers of young stillborn, 4.3 and 11.1%, respectively, in the two groups is highly significant ($P < .01$). The litters of 2 control and 3 test females which did not prepare a nest prior to parturition were excluded from the summary, because of the possibility that the young may have died from exposure. The observed difference in viability of the young at birth is believed to be due to the exposure of the male parent to irradiation prior to mating.

The average number of young born was 7.7 and 6.4, respectively, in the control and test groups (Table 2). The over-all reduction in fertility is further emphasized in the average number born alive per mating (Table 2).

Since it has been suggested that genetic damage would more likely be apparent in offspring conceived immediately after irradiation of the parent than in later births (*15*), the data on fetal mortality (Table 1) and numbers of young born (Table 2) for all females mated to irradiated males were tabulated so that any differences in fertility, zero to 35 days and 36 to 108 days post-treatment, could be studied. The data fail to show any difference; consequently, the data for the two periods were pooled for analysis. Admittedly the two intervals were somewhat arbitrarily selected, but it has been reported that in rabbits, after ligation of the epididymis, the sperm contained in the epididymis were in no case capable of effecting fertilization longer than 38 days (*16*).

No information regarding the viability or growth of the young which were born alive was obtained from this study. It is conceivable that not all the defective offspring died before or at the time of birth. A study of the growth rates and other criteria of viability in such young would seem desirable.

The pooled fertility data for all treated males indicate that deleterious effects on the prenatal viability of the offspring occur when the male has been exposed to the relatively low levels of radiation (100, 200, or 300 r) used in this study. In view of this, extreme caution should be exercised in the voluntary exposure of humans to ionizing radiation approaching this order of magnitude until further experimental evidence is available.

References

1. AMOROSO, E. C., and PARKES, A. S. *Proc. Roy. Soc. (London)*, B, **134**, 57 (1947).
2. KOSIN, I. L. *Physiol. Zoöl.*, **17**, 289 (1944).
3. RUGH, R. *Proc. Am. Phil. Soc.*, **81**, 447 (1939).
4. HENSON, M. *J. Exptl. Zoöl.*, **91**, 405 (1942).
5. HOOKER, D. R. *Am. J. Roentgenol. Radium Therapy*, **14**, 327 (1925).
6. HU, C. K., and FRAZIER, C. N. *Proc. Soc. Exptl. Biol. Med.*, **44**, 44 (1941).
7. SNELL, G. D. *J. Exptl. Zoöl.*, **65**, 421 (1933).
8. STRANDSKOV, H. H. *Ibid.*, **63**, 175 (1932).
9. STURKIE, P. D., *et al. Radiology*, **52**, 112, (1949).
10. BOCHE, R. D., RUST, J. H., and BUDY, A. Univ. Chicago Toxicity Lab. Quart. Rept. (Oct. 1950) and further unpublished data.
11. FOSTER, R. F., *et al. Growth*, **13**, 119 (1949).
12. SUITOR, A. E. *USDA Leaflet 245* (1946).
13. SNEDECOR, G. W. *Statistical Methods*. Ames: Iowa State Coll. Press (1946).
14. HAMMOND, J. *J. Agr. Sci.*, **11**, 337 (1921).
15. CHARLES, D. R. *NEPA 1019-IER-17* (1949).
16. HAMMOND, J., and ASDELL, S. A. *Brit. J. Exptl. Biol.*, **4**, 155 (1926).

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Electron Microscopy of Isolated Chromosomes[1]

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Electron microscopical studies of isolated chromosomes have been made by a number of investigators (*1–3*). In the present work, the organization of chromosomes isolated from turkey and chicken erythrocytes and from calf thymus by the methods of Mirsky and Ris (*4, 5*) has been studied in the electron microscope, which was used in conjunction with certain chemical and enzyme treatments. Special emphasis has been placed on the use of an electron staining procedure for desoxyribose nucleic acid, which has been described by Bretschneider (*6*).

Since the procedure used for the isolation of fowl erythrocyte chromosomes has been slightly modified from that described by Mirsky and Ris (*4*), it is outlined briefly here. Oxalated turkey or chicken blood was frozen at −40° C immediately upon withdrawal from the fowl. The thawed blood was then used for the isolation of erythrocyte nuclei, which were washed with 0.14 *M* NaCl until practically colorless. Four successive 5-min treatments of dilute suspensions of the nuclei in the Waring blendor produced a chromosome suspension almost free of unbroken nuclei (microscopic examination of aceto-orcein stained specimens). The chromosomes, washed twice by dispersion in saline in the blendor, were stored in plastic tubes in a cold room at 4° C. The use of blood that had been frozen prior to use for isolation of nuclei was found to greatly facilitate their rupture in the subsequent Waring blendor treatment.

The staining procedure described by Bretschneider (*6*) for the demonstration of desoxyribose nucleic acid in bull sperm has been applied to whole chromosomes from fowl erythrocytes and from calf thymus. "Residual chromosomes" were prepared from fowl erythrocyte chromosomes by 1 *M* NaCl extraction (*2*), in order to remove the outer nucleohistone layer, and were similarly stained. The staining procedure applied to concentrated suspensions of chromosome preparations in saline comprised mercuric chloride fixation,

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