Fabrizio, D. P. A., Virology, 1964, v22, 286.
14. Toolan, H. W., Proc. Am. Assn. Cancer Res., 1964, v5, 64.
15. Moore, A. E., Virology, 1962, v18, 182.
16. Dawe, C. J., Kilham. L., Morgan, W. D., J. Nat. Cancer Inst., 1961, v27, 221.
17. Cochran, K. W., Payne, F. E., Proc. Soc. Exp. Biol. and Med., 1964, v115, 471.
18. Hampton, E. G., Cancer Res., 1964, v24, 1534.
19. Whalley, J. M., Biochem. J., 1965, v94, 10.

Received April 26, 1965. P.S.E.B.M., 1965, v119.

Reduction in Life Expectancy as a Measure of Radiation-Induced Genetic Damage in Mice.* (30337)

J. F. SPALDING AND M. R. BROOKS
Los Alamos Scientific Laboratory, University of California, Los Alamos, N. Mex.

A general assumption concerning the genetic consequence of exposure to ionizing radiation is that most mutations are deleterious and will in one way or another affect the fitness of an organism. One logical effect of a genetic decrement in fitness could be a reduction in life span through early mortality. The Report of the United Nations Scientific Committee on the Effects of Atomic Radiation(1) states than an increase in mutation rates can be expected to give rise to a substantial reduction in viability even though the mutations produced are not responsible for visible harmful traits. The report also states, "It has, in fact, been shown in mice that the offspring of irradiated parents have a higher mortality than control animals during the early part of life." The hope was expressed (1) that more work would be done in this field of endeavor. The following is a report on one of a series of life span studies being done at this Laboratory.

Experimental methods. One sib pair of RFM strain mice from a line with a history of more than 25 generations of inbreeding propagated the mice used in this study. The experimental approach used to obtain mice for comparative life span studies is illustrated in Fig. 1. Two sib pair littermates from the parent pair were used to start 2 lines. Male mice used to propagate the irradiated line were given whole body exposures of 200 rads of X-rays at 25 $\pm$ 2 days of age and were caged with their sisters. The germ cells used for breeding had reached the spermatid or earlier stages of maturation at time of exposure. The mean time-interval between X-irradiation of irradiated males used to propagate the irradiated line and productive breeding was 27 days. Irradiated line mice reported here were unirradiated second litter male and female offspring from 20 generations of X-irradiated sires (F^{I}_{21}) and their second litter virgin daughters (F^{I}_{22}).

Following 10 generations of X-irradiation, a line designated as the irradiated sub-line (Fig. 1) was started. This line has been continued with no further X-ray exposure. Irradiated sub-line mice reported here were unirradiated second litter male and female offspring from 10 generations of X-irradiated males followed by 10 generations with no X-ray exposure (F^{Is}_{21}) and their second litter virgin daughters (F^{Is}_{22}).

Control line mice were second litter male and female offspring from 20 generations of sib-mated unirradiated males (F^{C}_{21}) and their second litter virgin daughters (F^{C}_{22}).

The following X-ray factors were used to provide X-irradiation to males in the experimental lines: 250 KVP, 30 ma, Thoreaus II filter, 2.6 mm Cu HVL, 60 cm target-to-specimen distance, and 50 rads/minute dose rate.

Results and discussion. Mean life span for the 9 groups of mice in question is tabulated in Table I. Breeding males of this strain have a longer life expectancy than do breed-

* Work performed under the auspices of U. S. Atomic Energy Commission.

TABLE I. Mean Life Span of Mice from Irradiated and Unirradiated Ancestral Lines.

	Offspring observed (No.)		Mean life span (days)	
	Females	Males	Females	Males
F_{21} (breeders)				
Control line	91	96	596 ± 16.0	652 ± 16.1
Irradiated line*	96	96	580 ± 13.9	690 ± 13.1
Irradiated sub-line†	96	99	613 ± 12.1	680 ± 11.8
F_{22} (virgin females)				
Control line	74		676 ± 18.0	
Irradiated line*	74		663 ± 16.6	
Irradiated sub-line†	71		660 ± 14.4	

* From 20 generations of X-irradiated sires.
† From 10 generations of X-irradiated sires plus 10 generations with no further X-ray exposure.

ing females, and the sex difference in Table I is obvious. Life spans for F_{21} females in the 3 groups tested were not different from one another. The mean life span value of F^{C}_{21} males was somewhat smaller than either of the irradiated lines, but the small difference was not significant at the 5% level. Mean life span values for F_{22} virgin female groups were not significantly different from one another. These results are in agreement with earlier studies and failed to show any decrement in life span attributable to ancestral exposure to ionizing radiation.

Gowen(2,3) has pointed out some of the genetic reasoning for an expected radiation-induced life shortening, and Russell(4) and McGregor *et al*(5) have observed increased infant mortality in offspring of irradiated sires. The number of stillbirths from irradiated line mice at this Laboratory has been consistently higher than in a control line with no history of radiation exposure(6-8). However, births-to-weaning ratios indicate no increase in infant mortality in irradiated line mice above that of control line mice(8,9). That radiation to the gonads of the mammal

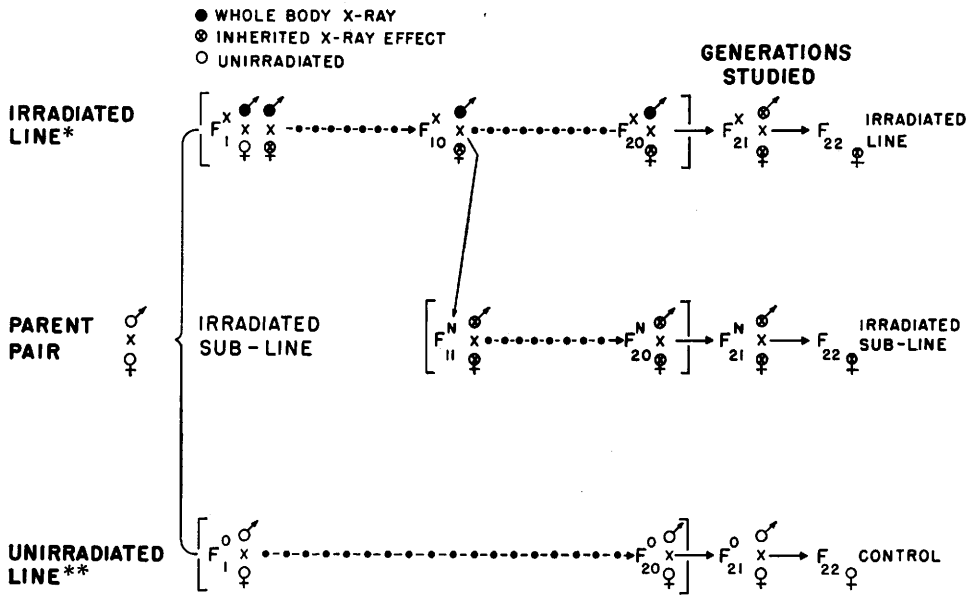

FIG. 1. Schematic diagram of experimental design.

can produce detrimental mutations is a known fact, but the question at test is whether or not mutations will cause reduced viability which is measurable in terms of increased mortality in early life. The strongest evidence in support of this thesis is a report by Russell(4) in which the statement is made that, "length of life in the offspring of male mice exposed to moderate doses of neutron radiation from a nuclear detonation is shortened by 0.61 day for each rep received by the father over the dose range tested," which was 31 to 186 rep. However, the sample size in Russell's study was extremely small with 3 of the 5 doses tested having but 8, 5, and 2 animals each for mean life span values. Kohn and Bailey(10), using paternal X-ray doses of from 248 to 720 rads, observed a tendency for female progeny to show a life lengthening and male progeny to show a life shortening. However, the effect of life shortening in male progeny was admittedly small. Lindop and Rotblat(11) found no evidence of decreased longevity of offspring from sires given 350 r of X-rays at 4 weeks of age. In addition, no cumulative effect of 3 generations of paternal exposure could be detected.

Perhaps the largest study and one which was designed specifically to attempt verification of Russell's early report(4) tested life span of first and second generation offspring from male mice exposed to fission neutrons (9). In this study both early and late matings were used, and both neutron and gamma-ray exposures were made. The results failed to confirm the findings of Russell (4).

Mice used in the present study (from a continuing program) were from inbred lines; therefore, in the irradiated line one might expect to find a greater nonlethal gene frequency of recessive mutations than in a randomly bred population. If this, in practice, did occur, it was not reflected in the life span of second litter, first and second generation offspring of the paternally irradiated lines. The United Nations report(1) specified that this effect (higher mortality rate in offspring from irradiated parents) was apparent during the early part of life. This was obviously not a reference to Russell's report (4), since he made a point of the fact that only one of his mice died before reaching one year of age.

Death distributions in this study (Fig. 2-4) fail to show early death groupings in irradiated line mice as compared to control mice. Litter data from the parents of F_{22}

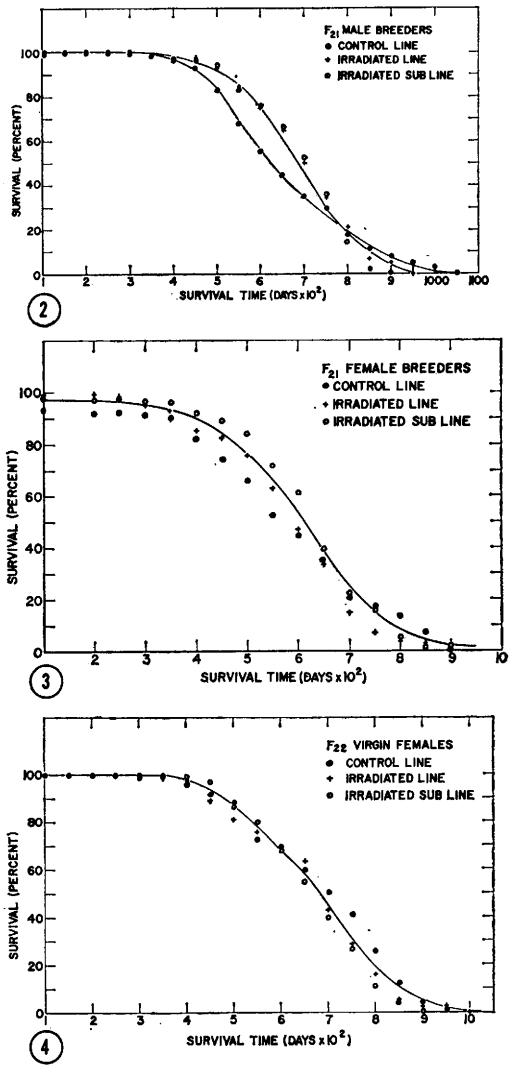

FIG. 2. Survivorship curves for control male mice and first generation male mice from 20 generations of paternal X-ray exposure (F_{21} breeders).

FIG. 3. Survivorship curves for control mice and first generation female mice from 20 generations of paternal X-ray exposure (F_{21} breeders).

FIG. 4. Survivorship curves for control female mice and female mice 2 generations removed from 20 generations of X-irradiated sires (virgin females).

mice used in this study(7) show no increase in mortality in irradiated line mice between live birth and weaning. It would appear from the results of this and other studies reported here that, although the theory of reduced viability (life span shortening) as a measure of increased mutation rate is a logical one, factual statements to this effect may be premature. Studies continue, however, and offspring and grandoffspring from 25, 30, and 35 generations of paternal X-ray exposures are now being studied.

Summary. Life span studies on first generation male and female breeders and on second generation virgin females from 20 generations of X-irradiated sires were done. The results of this and other studies strongly suggest that factual statements crediting reduction of life span as being a measure of a radiation-induced genetic decrement in viability are premature and inconsistent with experimental evidence at this date.

1. United Nations, Report of the Scientific Committee on the Effects of Atomic Radiation, 1962.

2. Gowen, J. W., Fed. Proc., 1961, suppl. 8, 35.

3. ———, J. Am. Geriatrics Soc., 1962, v10, 107.

4. Russell, W. L., Proc. Nat'l. Acad. Sci., 1957, v43, 324.

5. McGregor, J. F., James, A. P., Newcombe, H. B., Radiation Res., 1960, v12, 61.

6. Spaulding, J. F., Strang, V. G., Le Stourgeon, W. L., Genetics, 1963, v48, 342.

7. Spalding, J. F., Brooks, M., McWilliams, P., ibid., 1964, v50, Part 2, 1179

8. Spalding, J. F., Brooks, M. R., Archuleta, R. F., Health Phys., 1964, v10, 293.

9. Spalding, J. F., Proceedings of the International Symposium on the Effects of Ionizing Radiation in the Reproductive System, Fort Collins, Colorado, 1962 (Pergamon Press), p147.

10. Kohn, H. I., Bailey, D. W., Proceedings of the Second International Congress of Radiation Research, 1962, p396.

11. Lindop, P. J., Rotblat, J., Proceedings of the Second International Congress of Radiation Research, 1692, p417.

Received April 13, 1965. P.S.E.B.M., 1965, v119.

Plasma Levels of Ovarian Ascorbic Acid Depleting Activity (Luteinizing Hormone?) in Pregnant Rabbits.* (30338)

J. DAVIES, J. FALCK LARSEN,† G. R. DAVENPORT AND B. SCHMELLING
Department of Anatomy, Vanderbilt University Medical School, Nashville, Tenn.

Recent interest in the action of pituitary luteinizing hormone (LH) in enhancing the *in vitro* synthesis of progesterone in slices of luteal tissue(1) and its possible luteolytic role under certain conditions(2) make a systematic study of plasma LH levels in pregnant animals especially timely. The rabbit, in which ovulation is not spontaneous but induced(3) and in which luteal function is essential at all stages of pregnancy(4), presents particularly favorable conditions for such a study. It is well known that ovulation in the rabbit occurs about 10 hours after the coital stimulus and that the changes leading up to ovulation are probably dependent on the release of LH into the circulation, though it is not known to what extent other gonadotrophic hormones are involved. Hypophysectomy later than 60 minutes(5) after coitus will not prevent ovulation, indicating that enough gonadotrophic hormone is released from the pituitary gland within a short period to ensure ovulation. There is a coincident depletion of anterior pituitary gonadotrophic material(6) and a rise in plasma gonadotrophic activity(7). Other experiments have indicated that about twice the necessary amount of ovulating hormone is present within the first hour after coitus(8). There is a degranulation of pituitary basophiles and the appearance within a few hours of characteristic modified acidophile or carminophile cells of pregnancy, tentatively identified as

* This investigation was supported by USPHS Research Grant, HD 0097, and National Science Foundation Grant 1639.

† Present address: Dept. of Electron Microscopy, Univ. of Aarhus, Denmark.