Lifetime Activity Studies in Mice from Ten to Thirty Generations of X-Irradiated Progenitors.* (31864)

J. F. SPALDING
Los Alamos Scientific Laboratory, University of California, Los Alamos, N. Mex.

Experience with *Drosophila* and mice has demonstrated that gross physical abnormalities resulting from radiation-induced mutations are rare events. However, less obvious genetic degradations resulting in decrements to body functions, fertility, disease resistance, life expectancy, and an increase in lassitude with advancing age might be expected in mammals if the genetic load of radiation-induced mutants accumulates with successive generations of X irradiation. Investigations of life expectancy in offspring of irradiated mouse progenitors(1,2,3,4) and recent studies of survival in children of parents exposed to atomic bombings(5) have not disclosed decrements reflected in reduced life span. Lüning(6,7) has demonstrated increases in rate of recessive lethals in offspring from one or more generations of X-irradiated progenitors, and other studies(8, 9,10) have revealed decrements in reproductive fitness in offspring from irradiated ancestry. Behavioral effects in rats from several generations of irradiated progenitors have been reported by Brown(10) and Newcombe(11). The following is an account of the use of voluntary activity as a measure of the possible load of radiation-induced mutations leading to a decrement in general body function in nonirradiated mice from up to 30 generations of X-irradiated progenitors.

Materials and methods. The experimental design of the program from which mice used in this investigation were obtained has been published(9). One sib pair of mice from a highly inbred colony of RFM strain mice was used to propagate the population lines being studied. Population I was the result of 30 generations of sib mating with no history of progenitor irradiation. In population II all male mice in 30 generations of progenitors were exposed to 200 rads of X rays (250 kv, 30 ma, Thoraeus II filter, 2.6 mm Cu HVL, target-to-specimen distance 60 cm, dose rate 50 rads/min) at 26 $\pm$ 2 days of age and were allowed to procreate the next generation at the earliest time possible. Population III originated from line I after 20 generations of sib matings and for the following 10 generations were treated as described for line II. Mice in population IV originated from line II after 10 generations of irradiated male progenitors. From the 10th through the 32nd generations, mice in this line were treated as was line I. Thus, all mice in this investigation originated from one sib pair, Group I being F_{32} mice from 31 generations of sib matings, Group II being F_{32} mice from 30 generations of X-irradiated progenitors, Group III being F_{32} mice originating from the 20th filial generation of line I followed by 10 generations of X irradiation to progenitor males, and Group IV being F_{32} mice originating from the 10th filial generation of line II followed by 21 generations of no irradiation. Mice used in this study were 40 virgin females randomly selected from each of the 4 populations described.

Activity wheels connected to a single mouse cage provided with water and food were used to measure voluntary activity for 24-hour periods at 4-12 week age-intervals throughout the mean life span of the mice (Table I). Mice were allowed free access to either the cage or activity wheel during 24-hour tests, and performance was measured by recorded wheel revolutions. Activity data for the 4 groups were analyzed by the analysis of variance method, and age *versus* activity curves were made by fitting quadratics to the data using the usual least-squares technique.

Results and discussion. Mean values for activity in each of the 4 groups tested at 12 age levels are tabulated in Table I. At ages 2.0, 9.5, and 21.5 months, there were no significant differences in activity among the 4 groups. At no age studied did voluntary

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

TABLE I. Voluntary Activity of Nonirradiated Mice from Different Histories of Progenitor X-Ray Exposure.

Age (mo)	Mean activity and standard error of mean for 4 lines studied				Test of significance (.05)		
	Group I	Group II	Group III	Group IV	I-II	I-III	I-IV
2.0	13,881 ± 575	12,386 ± 815	12,779 ± 913	13,973 ± 753	No	No	No
3.5	11,730 ± 875	13,284 ± 728	10,728 ± 802	15,841 ± 478	No	No	Yes
5.5	11,011 ± 631	11,716 ± 776	11,925 ± 788	15,205 ± 860	No	No	Yes
7.0	10,242 ± 632	13,271 ±1014	10,138 ± 473	15,843 ± 514	Yes	No	Yes
8.5	11,223 ± 470	15,687 ±1160	11,413 ± 534	17,550 ± 501	Yes	No	Yes
10.0	10,990 ± 553	12,955 ± 778	11,150 ± 586	14,579 ± 729	No	No	Yes
11.5	10,248 ± 418	11,860 ±1005	9,977 ± 619	14,614 ± 791	No	No	Yes
14.5	8,536 ± 634	10,474 ± 975	8,675 ± 392	13,441 ± 783	No	No	Yes
16.0	9,915 ± 813	9,986 ± 869	7,864 ± 506	11,685 ±1001	No	No	No
18.0	8,083 ± 706	8,601 ± 817	7,078 ± 727	9,665 ± 627	No	No	No
19.5	6,485 ± 542	7,320 ± 684	5,922 ± 307	7,493 ± 803	No	No	No
21.5	3,575 ± 595	5,296 ±1158	3,490 ± 487	5,222 ±1042	No	No	No

Group I = F_{32} mice from 31 generations of inbreeding.
Group II = F_{32} mice from 30 generations of X-irradiated male progenitors.
Group III = F_{32} mice from F_{20} of Group I followed by 10 generations of X-irradiated male progenitors.
Group IV = F_{32} mice from F_{10} of Group II followed by 21 generations free of X irradiation.

activity in Group I differ significantly from that of Group III (Table I). Group III originated from line I (control) following 20 generations of sib matings and was then given X irradiation for 10 consecutive generations. Thus, one may conclude that 10 generations of X-irradiated male progenitors totaling 2000 rads failed to yield a viable mutant load which affected general body function as measured by voluntary activity. Group I differed significantly from Group II only at 2 ages tested: 7.0 and 8.5 months. In both instances activity in Group II was greater than that of the control group (I). It may be concluded from this comparison that 30 generations of X irradiation to male progenitors produced no genetic decrement which could be demonstrated in voluntary activity. Group IV showed significantly greater activity than the control group (I) at the 7 most active age levels (Table I). This group (IV) originated from line II following 10 generations of X-ray exposure to the male ancestry, and all male mice of the following 21 generations received no X irradiation. Again, 10 generations of X irradiation to male progenitors (followed by 21 generations of control-like sib matings) failed to yield genetic damage in terms of a decrement in voluntary activity and, in fact, this line showed significantly more vigor than any of the lines tested.

Age *versus* activity curves were plotted for each line and are shown in Fig. 1-4. It is interesting to note the similarity between the curve fits of lines I and III (Fig. 1 and 3) and of lines II and IV (Fig. 2 and 4). Although all 4 lines have ancestry common to one parent pair, lines I and II have been separated since F_1, and Groups III and IV were offshoots of lines I (at F_{20}) and II (at F_{10}, respectively.

Voluntary activity investigations have failed to demonstrate a radiation-induced genetic decrement; however, curve fits of the data (Figs. 1-4) do indicate an inherent difference in the two basic lines: I and II. Earlier studies on radioresistance(12) have also shown a basic genetic difference between these two lines which could not be attributed to a radiation-induced genetic shift. An H-2 analysis revealed that line I was primarily AKR strain in response, while II was predominantly RFM for this genetic characteristic. The two basic lines are being selectively bred to standardize this characteristic and to determine to what extent the H-2 locus is involved in controlling voluntary activity and radioresistance.

Summary. Lifetime voluntary activity studies on non-irradiated mice from 10-30 generations of X-irradiated progenitor males were done. Mice from irradiated lines were equally active to nonirradiated line mice. It was apparent from the results of these in-

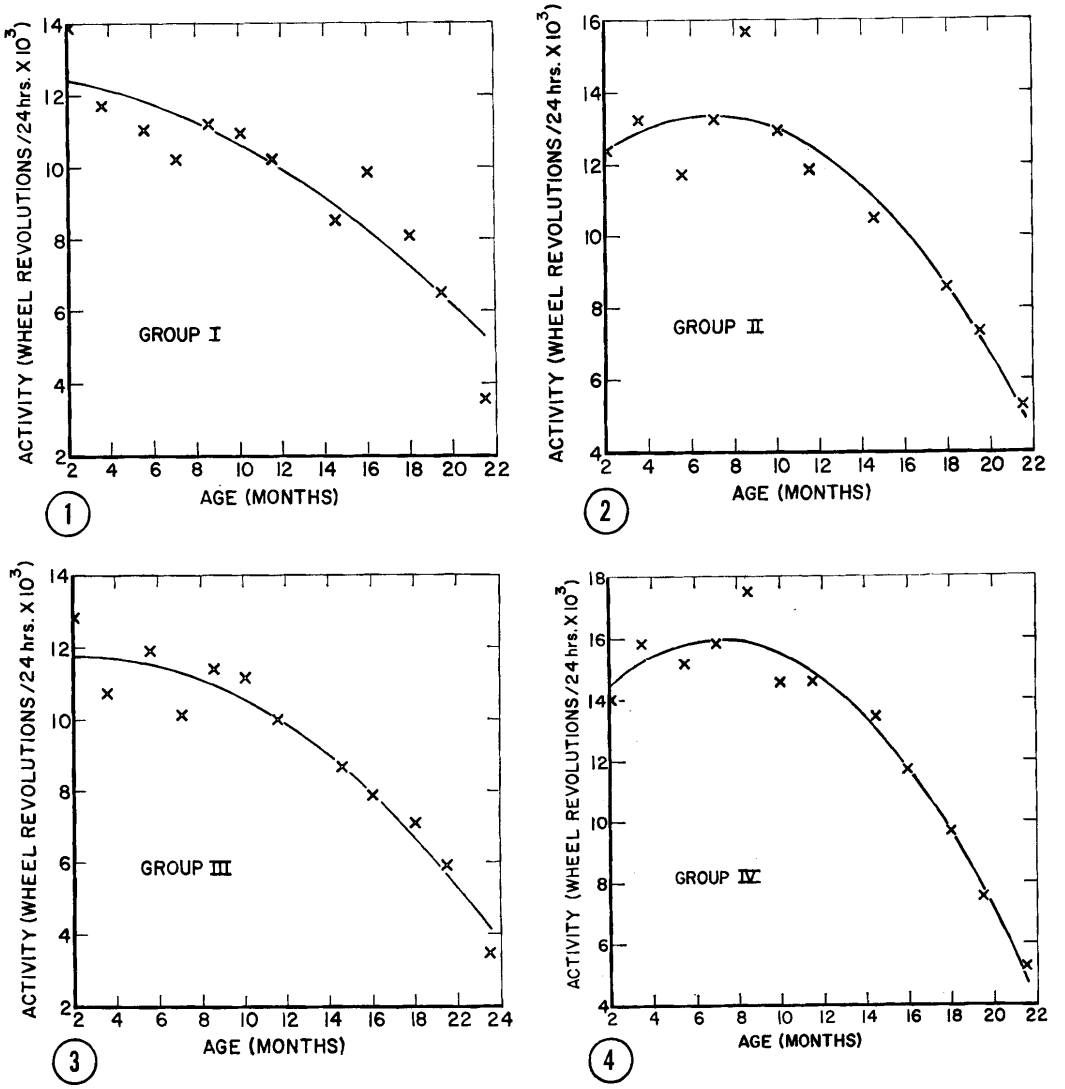

Fig. 1. Quadratic curve fit of activity *vs* age for F_{32} mice from 31 generations of sib matings.
Fig. 2. Quadratic curve fit of activity *vs* age for F_{32} mice from 30 generations of X-irradiated male progenitors.
Fig. 3. Quadratic curve fit of activity *vs* age for F_{32} mice from 20 generations of sib matings followed by 10 generations of X-irradiated progenitor males.
Fig. 4. Quadratic curve fit of activity *vs* age for F_{32} mice from 10 generations of irradiated progenitor males followed by 21 generations of control sib matings.

vestigations that 30 generations (the equivalent of 900 years in man) of X irradiation totaling 6000 rads failed to produce a genetic decrement measurable in terms of reduced vigor.

The author gratefully acknowledges P. C. McWilliams of the Theoretical Division (Group T-1) for data analysis and R. F. Archuleta for technical assistance. The author also gratefully acknowledges Mrs. Diana M. Popp and the Oak Ridge National Laboratory for providing her services to this Laboratory for two weeks. During her visit, Mrs. Popp made H-2 analyses on the LASL RFM and RF stock colony mice and on the RFM experimental colony mice.

1. Frölen, H., Mutation Res., 1965, v2, 287.
2. Kohn, H. I., Epling, M. L., Guttman, P. H., Bailey, D. W., Radiation Res., 1965, v25, 423.

3. Spalding, J. F., Effects of Ionizing Radiation on the Reproductive System, W. D. Carlson, F. X. Gassner, ed., Macmillan Co., New York, 1964.
4. Spalding, J. F., Brooks, M. R., Proc. Soc. Exp. Biol. and Med., 1965, v119, 922.
5. Kato, H., Schull, W. J., Neel, J. V., Am. J. Human Genetics, 1966, v18, 339.
6. Lüning, K. G., Mutation Res., 1964, v1, 86.
7. Lüning, K. G., Sheridan, W., Frölen, H., ibid., 1965, v2, 60.
8. Green, E. L., Genetics, 1964, v50, 423.
9. Spalding, J. F., Brooks, M. R., McWilliams, P., ibid., 1966, v54, 755.
10. Brown, S. O., ibid., 1964, v50, 1101.
11. Newcombe, H. B., McGregor, J. F., ibid., 1964, v50, 1065.
12. Spalding, J. F., Brooks, M., McWilliams, P., ibid., 1964, v50, 1179.

Received November 15, 1966. P.S.E.B.M., 1967, v124.

Isolation of an Unclassified Enterovirus from Healthy Children.* (31865)

ANDREW S. ABRAHAM† (Introduced by F. S. Cheever)
Department of Microbiology, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.

During a long term epidemiological study of viral infection in a semiclosed community (Detention Home of the Juvenile Court of Allegheny County) in Pittsburgh, Pa., carried out during 1960-61, an antigenically distinct enterovirus apparently unrelated to any of the recognized human types was isolated from 2 healthy children by the inoculation of primary monkey kidney cell cultures. This paper describes the circumstances of isolation, and the attempts to characterize and classify this virus.

Materials and methods. The sources of prototype viruses and immune sera, assay of viruses, the technique of neutralization tests, preparation of viruses for electron microscopy, and the method of preparation of immune sera and cell cultures have been described(1). The Caldwell virus(2) and immune serum were received from Dr. P. S. Kamitsuka, and the Bryant virus from Dr. G. S. Hsiung(3).

Experimental results. Isolation of the virus: The first virus of the Hu 2080 group (Hu 1967) was isolated in primary rhesus monkey kidney cultures from a rectal swab taken from a child during a routine weekly sampling. He had been a resident for many months in this institution, and left a few days after the last swab (from which the virus Hu 1967 was isolated) was taken. Another child, also a resident for many months, yielded the second virus of this group (Hu 2080) in April, 1961, a week after the release of the first mentioned child. This child remained a resident for 2 more months in this institution, but none of the rectal swabs taken subsequently at weekly intervals yielded this virus again.

Identification of the virus: Both the Hu 1967 and Hu 2080 agents were propagated in primary rhesus monkey kidney cell cultures, and carried through fourteen terminal dilution passages. After both the fourth and the fourteenth passages neutralization tests were carried out employing immune sera prepared against the recognized human enterovirus types with the exception of ECHO 28 and 29 for which no antisera were available. The results were uniformly negative. Potent immune sera were prepared against both viruses in rhesus monkeys. The two agents proved to be serologically identical and Hu 2080 was arbitrarily designated as the prototype. No immunological relationship was detected when these two sera were tested against all human prototype enteroviruses. Several other enterovirus-like agents, isolated in this laboratory and elsewhere, were also tested in cross-neutralization tests. These

* This investigation was carried out under the sponsorship of the Commission on the Enteric Diseases, Armed Forces Epidemiological Board, and was supported by Office of the Surgeon General, Dept. of the Army.

† Present address: Peter Bent Brigham Hospital, Boston, Mass.