July 25, 1972

To the Graduate Council:

I am submitting herewith a dissertation written by Paul Bruce Selby entitled "X-Ray-Induced Specific-Locus Mutation Rates in Newborn and Young Mice." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Biomedical Sciences.

William L. Russell
Major Professor

We have read this dissertation
and recommend its acceptance:

R. C. Foster

E. F. Oakberg

E. H. Grell

Leon B. Russell

Accepted for the Council:

Hatton A. Smith
Vice Chancellor for
Graduate Studies and Research

X-RAY-INDUCED SPECIFIC-LOCUS MUTATION RATES IN NEWBORN AND YOUNG MICE

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Paul Bruce Selby

August 1972

ACKNOWLEDGMENTS

This doctoral research was conducted under the direction of Dr. W. L. Russell at the Biology Division, Oak Ridge National Laboratory.*

The author wishes to express his deepest gratitude to Dr. W. L. Russell for his valuable advice and many inspiring contributions to his training.

For their helpful suggestions and contributions to his training, the author is indebted to Drs. R. C. Fuller, W. M. Generoso, D. G. Gosslee, E. H. Grell, T. J. Mitchell, E. F. Oakberg, L. B. Russell, Miss E. M. Kelly, and many other employees of the Biology Division.

In addition, the author wishes to express his deep appreciation to his wife, Patricia, for her assistance, encouragement, and understanding.

*Research supported by U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

ABSTRACT

The specific-locus mutation frequency resulting from 300 R of acute X irradiation has been determined for the germ cells present in male mice at 0, 2, 4, 6, 8, 10, 14, 21, 28, and 35 days of age and also for female mice at 0 days of age. Sample size was much larger for the males irradiated on day 0 than for other age groups but in all groups it was large enough to insure that an extremely high rate would be noticed. At 35 days of age the testis is histologically similar to that of the adult. It was important to know if the germ cells present in immature mice yield the same mutation frequency as those in the adult.

The mutation frequency is only 1/2 as high in the day-0 male as it is in the similarly irradiated adult male, this difference being statistically significant. Taken together, the remaining nine groups of males have an average mutation frequency similar to that of the adult. None of the nine groups has a mutation rate statistically significantly higher than the adult.

There is a statistically significantly higher incidence of both permanent sterility and clusters of mutations following irradiation of day-0 males than is found for similarly irradiated adults. Both of these results would be expected if there are relatively fewer surviving germ cells following irradiation of the day-0 testis.

The mutation frequency per R is only 1/5 as high for the females irradiated with 300 R on day 0 as it is for adult females exposed to 400 R, the nearest dose used, this difference in rate being highly statistically significant. The reproductive capacity of the females irradiated on day 0 is about 1/3 that of control females.

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

CHAPTER I

INTRODUCTION

Between birth and adulthood the mouse testis and ovary undergo great changes both in size and in germ-cell stages present. The purpose of this research was to explore the possibility that some germ-cell stages present in newborn and young mice might have a much higher frequency of induction of specific-locus mutations by X irradiation than is found in the adult. If the mutation frequency in the young animals turned out to be much higher, this would be of considerable concern in the estimation of genetic hazards in man.

The specific-locus method, first used in the mouse by Russell (47), permits the detection of mutational events specifically located at seven loci in the mouse genome. F_1 hybrids between inbred strains 101 and C3Hf are exposed to the desired treatment, which was X irradiation in these experiments. These F_1 hybrids are homozygous for the wild-type allele at each of the seven test loci. Following treatment, the F_1 hybrids are mated with mice of a test stock which is homozygous for a recessive marker allele at each of the seven test loci. Unless a mutation occurs at one of the seven genetically-marked loci, all progeny from this cross express the wild-type phenotype.

The seven genetically marked loci are nonagouti (*a*), brown (*b*), chinchilla (c^{ch}), Maltese dilution (*d*), pink-eyed dilution (*p*), piebald spotting (*s*), and short-ear (*se*). The first six of these are coat color mutations, and the last one is a mutation which decreases the size of

the outer ear. These seven loci are distributed among five autosomes. The $\underline{c}^{ch}$ and $\underline{p}$ loci are loosely linked, and the $\underline{d}$ and $\underline{se}$ loci are very tightly linked, being only 0.16 cM apart on linkage-group II (42).

The specific-locus mutations are thought to be of two main types, either gene mutations or small deficiencies. The induction of intermediate alleles (57) supports the view that some specific-locus mutations may result from intra-genic changes instead of larger changes or losses of genetic material.

It is known from previous X-ray mutagenesis studies in the mouse that different germ-cell stages in the adult can have different specific-locus mutation rates. For example, following a moderate dose of acute X irradiation, the mutation frequency of spermatogonia is only 1/2 that of a sample of postspermatogonial stages (58) and 3/5 that of oocytes in maturing follicles (53). These changes are not only quantitative, but also qualitative. All three of these stages differ in the distribution of mutations among the seven loci (52). Furthermore, the proportion of $\underline{d}$ $\underline{se}$ region mutations involving more than one functional unit is a function of the germ-cell stage irradiated (42).

Extensive experimentation has been performed in the past to determine the X-ray-induced specific-locus mutation rates of the germ cells of adult mice under a multitude of different irradiation conditions. By contrast, only a small number of studies have been done to determine the X-ray-induced specific-locus mutation rates of the germ cells present in male and female fetuses at various ages. Until this doctoral research was undertaken, no attempt had been made to determine the specific-locus

mutation rates resulting from X irradiation of mice during the period from birth to adulthood.

The experiments on males were divided into two groups according to the goals desired and the sample sizes planned to achieve those objectives. An experiment on newborns was designed so that a large enough sample of offspring (about 50,000) would be observed for specific-locus mutations to provide a reliable estimate of the actual mutation frequency, providing, of course, that the mutation frequency was not so low that few mutations would be obtained.

The objective of the remaining experiments, on males ranging from 2 to 35 days of age, was to test whether or not there might be a greatly increased mutation frequency during some particular stages of development. While a slightly increased mutation rate in some transitional stage of development might have little effect on an estimate of genetic hazard to man, a greatly increased mutation rate in some stage would warrant concern. In order to explore this possibility, smaller samples of offspring were observed for mutations at many different stages of development. Although smaller, these samples were, nevertheless, large enough to reveal, through calculation of the upper 95 percent confidence limit of the mutation frequency at each stage, whether any of the stages tested had a much higher specific-locus mutation rate.

The specific-locus mutation frequency was also determined for newborn females. No specific-locus mutation experiments were attempted on young females older than newborns because of the extreme radiosensitivity to killing of oocytes that prevails during much of the time from shortly after birth to adulthood (38, 61).

CHAPTER II

SPECIFIC-LOCUS MUTATION-RATE STUDY ON NEWBORN MALE MICE

A. INTRODUCTION

Gametogenesis in the Male Mouse

The primordial germ cells of the mouse have been observed to originate in eight-day embryos in the yolk-sac splanchnopleure, the caudal end of the primitive streak, and the root of the allantoic mesoderm. They can be stained selectively because of their high alkaline phosphatase activity. Chiquoine (15) has traced their migration via the germ-tract into the germinal ridges of twelve-day embryos.

The smallest number of germ cells seen in any eight-day embryo is ten. By the end of migration the number has increased to 5000 or more (27).

At eleven days of embryonic life the sex of the embryo is histologically discernible. In the male, the distribution of those germ cells which have already reached the germinal ridges is distinctly central. This results in the medullary dominance characteristic of the male (27). The further development of the male mouse is as described below.

According to Mintz (27), the primordial germ cells become enclosed in developing medullary cords. These germ cells continue to multiply, and they occupy the center of the tubules (or sex cords) in the fetus. The primordial germ cells (or gonocytes) may enter a transitional stage in late fetal life. At this time mitotic figures are rare.

Widmaier (66) reports that some gonocytes begin to degenerate on the day of birth, which occurs about 19 days after conception. According to observations on different strains of mice by Mintz (27) and Widmaier, the first type A spermatogonia appear on the day of birth (66) or three days after birth (27). The gonocytes are all gone five to seven days after birth, either because of degeneration or differentiation into type A spermatogonia (27, 66).

The discrepancy between Mintz and Widmaier concerning the first appearance of type A spermatogonia in mice could be due to many factors such as strain differences in the rate of development of the germ cells and variations in the gestation period. The gestation period varies between mouse strains from 18 to 22 days, and litter size influences the length of the gestation period in a single strain (66).

Mintz (27) has clearly shown that the primordial germ cells are the only precursors of the type A spermatogonia. Genetic abnormalities that greatly decrease the number of primordial germ cells cause sterility. Furthermore, destruction of primordial germ cells by irradiation prevents the formation of type A spermatogonia. The germ line is therefore continuous from the embryo to the adult. The first type A spermatogonia eventually become the A_s spermatogonia, which Oakberg (35) has shown to be the true stem cells of the adult testis.

Widmaier found that primary spermatocytes were not commonly observed until seven to eight days after birth. The lumen was open in most tubules by 20 days after birth, and at this time some spermatids were also found. At 25 days almost all tubules contained secondary spermatocytes and spermatids. At 30 days the germinal epithelium was

fully developed. Widmaier concluded that the first cycle of the seminiferous epithelium begins on the day of birth in the mouse and that the cycles proceed essentially as described by Oakberg (28) for the adult mouse. Oakberg (29) has shown that the time required for the type A spermatogonia to develop into mature spermatozoa averages 34 1/2 days in the adult male.

Details of Gametogenesis in the Rat near the Time of Birth

Although gametogenesis has been less extensively studied near the time of birth in the mouse than in the rat, the sequence of events during early gametogenesis is thought to be quite similar for these two species. However, the time-scale is probably advanced by several days in the mouse.

The sex of a rat can be distinguished histologically 14.5 days after conception, although the primordial germ cells in the two sexes appear essentially similar until about the "seventeenth" day of gestation. (Where an author has used ordinal numbers to describe the developmental stage, such numbers have been placed in quotation marks.) Development of the male rat proceeds as follows. Between 14.5 and 18.5 days of gestation the large gonocytes frequently undergo mitotic divisions. The gonocytes continue to increase in size as development proceeds, and they are closely packed in the sex cords (7). Huckins and Clermont (20) report that no mitotic figures are detected in the gonocytes of Wistar rats between the "eighteenth" embryonic and "seventh" post-natal day. (Birth occurs after about 22 days of gestation.) Beaumont and Mandl (7) report mitoses as being very rare in the gonocytes of the Birmingham

strain of rats between 18.5 days of gestation and 3.5 days after birth.

At birth there is a dramatic lengthening of the sex cords, and the gonocytes become uniformly diluted along the entire cord (20). A few days after birth extensive degeneration of gonocytes begins (7, 20). Huckins and Clermont (20) find that between five and seven days after birth just a scattering of cross-sections of tubules are populated by gonocytes, while many more cross-sections contain no germinal elements at all. The surviving gonocytes are found to be in isolated clusters of one to sixteen cells. Each sex cord contains about 30 such clusters. (The average number of sex cords per rat testis is 30.) Beaumont and Mandl (7) report that in their rats the gonocytes undergo cytological transformation to "transitional" cells between four and six days after birth. Mitotic activity resumes and transforms the "transitional" elements into type A spermatogonia, which are found in appreciable numbers at five to five-and-one-half days after birth. Huckins and Clermont (20) find that in the Wistar rat gonocytes resume mitotic activity one week after birth to produce pre-type A spermatogonia. This is not preceded by a cytological transformation into "transitional" cells. The pre-type A spermatogonia later become the A_s spermatogonia, which are the true stem cells of the adult testis (19). The pre-type A spermatogonia divide more frequently than their adult counterparts (20).

Huckins and Clermont (20) have shown conclusively that gonocytes in the rat are the precursors of pre-type A spermatogonia. Embryonic gonocytes were labeled with tritiated thymidine, and this label was detected later in early pre-type A spermatogonia.

Two groups of authors, each studying a different rat strain, estimate that 29 percent or 71 percent of the gonocytes degenerate spontaneously. Approximately 100,000 or 22,000, respectively, survive to resume division and differentiate into type A spermatogonia (7, 20).

Radiosensitivity to Killing of Mouse Germ Cells

L. B. Russell et al. (45) tested the fertility of ten males which were irradiated with 200 R of acute X irradiation at 11 1/2 days post-conception. All were completely sterile.

Carter (10) irradiated (C3H X 101)F_1 male mice at 13 1/2 days of gestation with 300 rads of X irradiation delivered at 70 rads/minute. He found that 30 percent of these males never became fertile.

Rugh and Jackson (40) exposed CF1 mouse fetuses to various doses of 101 R/minute X irradiation at 15 1/2, 16 1/2, 17 1/2, or 18 1/2 days of fetal life. The peak sensitivity for the males occurred on days 15 1/2 and 16 1/2. All of the males irradiated with 200 R on days 17 1/2 and 18 1/2 (samples of 17 and 8, respectively) became fertile. In contrast, 36 percent (4/11) and 37 percent (6/16) of mice given the same dose on days 15 1/2 and 16 1/2, respectively, never became fertile up to nine months of age, at which time they were sacrificed. Rugh and Jackson also found that while body weights (measured at nine months) of the mice irradiated on 15 1/2 and 16 1/2 days of gestation were reduced by 14 percent and 20 percent, respectively, compared to unirradiated controls, their testis weights (measured at nine months) were reduced by 68 percent and 70 percent. None of the fetuses exposed to doses of 100 R or less was sterilized.

Carter et al. (12) exposed (C3H X 101)F_1 mice at 17 1/2 days of gestation to 200 R of 72 R/minute X irradiation. They found that 7.6 percent of these males were sterilized, and a few sired fewer young than normal.

It was not known what effect irradiation between birth and adulthood would have upon the fertility of a male mouse.

Although 300 R of acute X irradiation does not kill spermatozoa, spermatids, and all spermatocytes in the adult testis, it does destroy all spermatogonia, with the important exception of the A_s stem-cell stage (34, 36). An idea of the radiosensitivity of A_s spermatogonia was given by Oakberg (34) who showed that five days after acute X irradiation with 100 R or 500 R only 39 percent or 13 percent survive, respectively. The surviving A_s cells repopulate the germinal epithelium.

Radiosensitivity to Killing of Rat Germ Cells

In contrast to the mouse, the gonocytes of the Birmingham rat undergo a consistent increase in radiosensitivity from the "thirteenth" to the "nineteenth" day of gestation (4). On the nineteenth day, 150 R causes all tubules to be sterile upon histological examination 25 days after birth (4). Hughes (21) has shown that the period of high radiosensitivity in the Birmingham strain lasts until one day after birth. The sensitivity then gradually decreases. The decline in radiosensitivity coincides with the increase of germinal mitoses leading to the formation of the first spermatogonia (21).

Shaver (64) irradiated rats at various ages after birth with X irradiation delivered at 68 R/minute and then studied sections of the

seminiferous tubules after various periods of elapsed time. In rats irradiated with 300 R when three, four, or five days old, regeneration was seen in only a small number of tubules. Most tubules appeared to be completely sterilized and incapable of regeneration. On the other hand, rats that were irradiated with 400 R 10 to 15 days after birth exhibited complete regeneration of the germinal epithelium.

Earlier Specific-locus Mutation-Rate Studies in Mice

Before discussing the present experiments, it is of interest to know how the mutation frequencies previously reported for fetal stages of the mouse compare with the mutation frequency for the similarly irradiated adult.

Russell (53) irradiated adult F_1 hybrid males from the cross of the 101 and C3H inbred strains with 300 roentgens of X irradiation delivered at 90 R/minute. Forty specific-locus mutations were found in 65,548 offspring conceived long enough after irradiation to insure that they represented the mutation rate in spermatogonia. This is a mutation frequency of 29.1×10^{-8} mutations/locus/R. The 95 percent confidence limits of this rate are 21.0×10^{-8} and 39.0×10^{-8}.

Carter (10) irradiated (C3H X 101)F_1 hybrid males 13 1/2 days after their conception with 300 rads of X irradiation delivered at 70 rads/minute. He found one specific-locus mutation in 10,155 progeny. This is a mutation frequency of 4.7×10^{-8} mutations/locus/R with 95 percent confidence limits of 0.2×10^{-8} and 25.0×10^{-8}. This rate is significantly lower than that of similarly irradiated adults, $P = 0.02$ (for a one-tailed test).

Carter et al. (12) also exposed (C3H X 101)F_1 males 17 1/2 days after their conception to 200 R of X irradiation delivered at 72 R/minute. Eight specific-locus mutations were found in 31,253 offspring. The original report of this experiment stated that nine specific-locus mutations had been found, one of which was a d se double mutation. This mutation was later shown to be viable in homozygous condition (personal communication, R. J. S. Phillips). L. B. Russell and Russell (44) have shown that d se double mutations which are viable in homozygous condition are almost certainly homozygous for the test-stock linkage-group II, as a result of nondisjunction in both parents. All the d se double mutations found in controls at Oak Ridge have been of this type (42). The d se double mutation reported in the experiment by Carter et al. has therefore been omitted in all analyses made in this dissertation.

Of the four mutations at the s locus, three occurred in two litters from the same male, indicating that they arose from a single mutational event. The occurrence of this cluster argues that all of the offspring observed cannot be considered as independent occurrences. Therefore only six of his eight mutations have been considered as such, and the appropriate correction has been made in statistical comparisons of the mutation frequency (55). Based on these corrections, the mutation frequency following a 200 R irradiation of the 17 1/2-day-old male fetuses is 18.3×10^{-8} mutations/locus/R. The 95 percent confidence limits of this rate are 8.0×10^{-8} and 39.1×10^{-8}. This mutation frequency is not significantly lower than that of adults, P = 0.19 (for a one-tailed test).

Purpose of this Experiment

No knowledge existed concerning the mutational response of the germ cells in the testis between birth and adulthood. This experiment was designed to be large enough to provide a good chance of obtaining a reliable estimate of the specific-locus mutation rate resulting from the X irradiation of newborn male mice. The dose of 300 roentgens of acute X irradiation was chosen to permit a direct comparison of the mutation rate in newborn male mice with the mutation rate in adult male mice.

B. MATERIALS AND METHODS

Newborn (101 X C3Hf)F_1 males were exposed to 300 roentgens of whole-body X irradiation in a single exposure. The irradiation conditions were: 250 kVp; 15 mA; inherent filtration 3 mm. Al; H.V.L. 0.4 mm Cu; dose rate range from 73 R/minute to 83 R/minute; animals in lucite containers on turntable rotating above masonite scatter block. The same X-ray machine was used as the one in which Russell (53) irradiated adult mice, and irradiation conditions were identical with the exception of the dose rate. Whereas Russell's dose rate was 90 R/minute, the dose rate delivered by this machine had decreased to 83 R/minute at the start of the present experiment.

The irradiations of newborn males were carried out at various times over a period of 16 months. During this time the dose rate of the X-ray machine decreased further to 73 R/minute. The time of exposure was correspondingly increased to insure the same total dose. This slight decrease in dose rate would not be expected to influence the results.

All newborn mice were obtained from a large number of breeding pens set up for the production of (101 X C3Hf)F_1 hybrids. The 101 dams varied in age and in the number of previous litters. Other factors that would be expected to influence length of the gestation period were litter size and whether or not the dam was lactating (66). Pens containing pregnant females were examined periodically for the appearance of newborn mice. Males in newborn litters were either irradiated, sham-irradiated, or left undisturbed to be used in experiments described later. The males were distributed at random by litter to these different groups.

Newborn males were only irradiated or sham-irradiated if this could be accomplished within nine hours after birth. This tight restriction on the number of hours was instituted in order to avoid adding unduly to the degree of variation in morphological development which already exists between individuals and litters at the time of birth. The sham-irradiated males were treated identically to those irradiated in all ways, except for the fact that the X-ray machine was not turned on.

All males were paired with test-stock females at some time after weaning and almost always, but not exclusively, before they became fertile. They were kept with females of breeding age throughout the remainder of their lives. From these matings, only offspring living until weaning were counted. Litters were usually weaned sometime during the week following the time of full development of the external ears. Animals carrying presumed specific-locus mutations were genetically tested to determine if the mutation really was an allele of the expected test locus.

C. RESULTS AND CONCLUSIONS

Mutation Rates

Sixteen specific-locus mutations were found in 55,456 offspring of males irradiated with 300 R within nine hours after birth. The 16 mutations included three clusters of two mutations each, and appropriate corrections have been made in the statistical analyses using Russell's approach (55). The mutation frequency on day 0 is therefore 13.7×10^{-8} mutations/locus/R. The 95 percent confidence limits of this frequency are 7.1×10^{-8} and 22.6×10^{-8}. This rate is statistically significantly lower than the rate of 29.1×10^{-8} mutations/locus/R found for similarly irradiated adult males, $P = 0.011$ (for a one-tailed test).

No specific-locus mutations were found in the 38,448 progeny of the sham-irradiated control males. The mutation frequency was therefore highly significantly greater following the 300 R X irradiation, $P = 0.0003$ (for a one-tailed test). Russell (53) reported finding 28 mutations in 531,500 offspring of unirradiated control males. This rate is not significantly higher than that of the sham-irradiated newborn males, $P = 0.14$ (for a one-tailed test).

As was mentioned earlier, Carter's (12) relatively small experiment on males irradiated at 17 1/2 days of gestation yielded a mutation rate that is not statistically significantly lower than that of the adult. Furthermore, this frequency is not statistically significantly different from that found in the present experiment for newborn males. The point estimate derived from Carter's data, 18.3×10^{-8} mutations/locus/R, is closer to the rate found for newborns than it is to that found for the

adult. The mice irradiated in the present experiment were only about two days older than those irradiated by Carter. The work of Mintz (27) and Widmaier (66) suggests that most of the germ cells present at both of these times are probably gonocytes. (It must be stressed, however, that an exact timing of these developmental changes has not been made on the C3H X 101 cross used by Carter (10) and Carter et al. (12) or on the 101 X C3Hf cross used in the present experiment.) On the assumption that all gonocytes have the same mutational sensitivity, which may not be true, the present results and those of Carter's could be regarded as two estimates of the same mutation rate, and could, therefore, be combined to make a comparison with the adult rate. When this is done, it is found that the mutation rate from fetal day 17 1/2 and day 0 combined is still significantly lower than that of the adult, $P = 0.010$ (for a one-tailed test).

There is no statistically significant difference between the mutation rate for fetal day 17 1/2 and day 0 combined and that of 4.7×10^{-8} mutations/locus/R found by Carter (10) for males irradiated at 13 1/2 days of gestation. However, the 13 1/2-day fetal experiment was too small to permit a meaningful comparison.

Delayed Fertility

Male mice irradiated on day 0 exhibited considerable variability in age at the onset of fertility. For example, of the 664 males that eventually became fertile, 26 or 3.9 percent did not sire their first litter until they were more than 300 days of age. This is in sharp contrast to the sham-irradiated controls. Among these, only 2 or 0.6 percent

of the 341 males that eventually became fertile had not sired their first litter by 300 days of age. The onset of fertility by males irradiated as newborns is therefore significantly delayed, P = 0.0009 (Fisher's exact test).

Among the controls, only 11 or 3.2 percent of the 341 that became fertile were delayed as long as 100 days. This is smaller than the percentage of males irradiated as newborns that was delayed as long as 300 days. The slowest irradiated male sired his first litter at about 601 days of age (assuming a gestation period of 19 days), and the slowest control male sired his first litter at about 358 days of age.

Permanent Sterility

The delay in onset of fertility complicates the calculation of the percentage of newborn males that was rendered permanently sterile by the irradiation. Obviously, a male that died at 200 days of age without siring any offspring should not be considered permanently sterile. Had he lived, he might not have sired his first litter for many months.

An estimate of the percentage of males that was permanently sterilized can be obtained by dividing the number of males alive at any given age that would never produce any offspring by the total number of males alive at that age. Table I clearly shows how these estimates depend upon the age chosen.

The estimates are higher if made at earlier ages because of the relatively early deaths of males that might have become fertile. Because of the delayed fertility, a much more meaningful estimate of the percentage of males that was actually permanently sterilized is obtained by

TABLE I

ESTIMATES MADE AT VARIOUS AGES OF THE INCIDENCE OF PERMANENT STERILITY FOLLOWING X IRRADIATION OF NEWBORN MALE MICE

	Age in Days						
Incidence	50	100	200	300	400	600	800
Ratio	38/702	36/699	32/685	31/674	30/656	26/559	14/223
Percentage	5.4	5.2	4.7	4.6	4.6	4.7	6.3

only considering those that lived to an age at which most had had sufficient time to become fertile, if they ever would.

Following this line of reasoning, 30/656 or 4.6 percent is considered the best estimate of the percentage of newborn males which was permanently sterilized by irradiation on day 0. This estimate is based on the number of males alive at 400 days because this age is late enough so that all but about one percent of the eventually fertile males had sired their first litter, and it is early enough so that the sample size has not been decreased much by death. Unless otherwise mentioned, this is the estimate that will be used in comparisons with other experiments.

Among the 342 sham-irradiated males that were mated, only one produced no young. To be conservative, it is counted as sterile. However, it had the disadvantage (shared by no other male in these experiments) of losing one of its hind legs between birth and weaning. Furthermore, it died at an age of only 177 days. The incidence of permanent sterility among males irradiated with 300 R on day 0 is significantly higher than among sham-irradiated controls, $P = 0.00003$ (Fisher's exact test).

In contrast, Russell (48) found no increase over controls in the incidence of permanent sterility among adult males exposed to 600 R of whole-body or 1000 R of partial-body acute X irradiation. These mice were also F_1 hybrids of the same cross used in the present experiments. Among males receiving 600 R he found 4/1717 or 0.2 percent to be permanently sterile. The classification of two of these four was considered questionable because they died early. Among males exposed to 1000 R, 3/414 or 0.7 percent were permanently sterilized. None of these three was considered adequately tested.

It is apparent that newborn males exposed to 300 R of whole-body X irradiation experience a higher incidence of permanent sterility than adult males exposed to 600 R of whole-body or 1000 R of partial-body irradiation, $P < 0.000001$ and $P = 0.0001$, respectively (Fisher's exact test). It is not known whether this increased incidence of permanent sterility is due entirely to greater radiosensitivity to killing of the germ cells present in the newborn mouse. However, it seems likely, in view of the fact that the tubules in the rat testis of comparable age undergo such extreme and permanent damage (21, 64). Furthermore, the finding of clusters supports the view that there has been relatively more killing of germ cells, as will be discussed in more detail later.

As would be expected from the obviously extensive damage caused by irradiation of the testis of the newborn male, there is a dramatic effect upon the weight of the testis. Fazylov and Pomerantseva (16) found that three months following the irradiation of newborn male mice with 200 R, 400 R, or 600 R, their testes weighed only 63 percent, 24

percent, and 16 percent, respectively, those of controls. In comparison, after the same time interval, the testes of adults that had been irradiated with 400 R weighed the same as those of controls.

The newborn male differs greatly from the adult male in the susceptibility to radiation-induced permanent sterility. However, as has already been outlined in the introduction of this chapter, day 0 is evidently not the most sensitive stage in the mouse. More detailed comparisons, discussed below, show that at 17 1/2 days post-conception there appears to be slightly increased sensitivity to sterilization, and that at 13 1/2 and 11 1/2 days of fetal life the susceptibility is very high indeed. Carter's two experiments (10, 12) and the present experiments utilized the same strain of mice.

Carter et al. (12) reported that 47/621 or 7.6 percent of the males mated when about eight weeks old proved to be sterile following 200 R of X irradiation at 17 1/2 days of gestation. They mentioned that "a few sired fewer young than normal," which probably means that some of their males also exhibited delayed fertility. Since they based their estimate of permanent sterility on the number of males alive at eight weeks, the corresponding figure from the present experiment, 38/702 or 5.4 percent, must be used in a comparison. This difference is not statistically significant; however, the males in the present experiment on newborns received a 50 percent higher dose of X irradiation. This result certainly suggests that the 17 1/2-day-old male fetuses are even more susceptible to being sterilized than are newborn males.

There is no question that the males irradiated at day 13 1/2 of embryonic life are more susceptible to sterilization than those

irradiated on the day of birth. Carter (10) irradiated these fetal mice with 300 R, and they can therefore be compared directly with the day-0 experiment. Of these males, 62/206 or 30 percent proved infertile. Since it is not known how Carter estimated permanent sterility, this is conservatively compared to the too high estimate of 5.4 percent on the day of birth. The incidence of permanent sterility on day 13 1/2 is highly significantly greater than that of newborn males, $P << 0.001$ (Chi-square = 99).

L. B. Russell et al. (45) found that all ten males irradiated with 200 R of acute X irradiation on day 11 1/2 post-conception were completely sterilized. The incidence of permanent sterility is highly significantly greater on fetal-day 11 1/2 than on fetal-day 13 1/2, $P << 0.001$ (Chi-square = 21). A few males were similarly irradiated at 1/2, 1 1/2, 4 1/2, 7 1/2, and 9 1/2 days of embryonic life. All five of these groups were much less susceptible to sterilization than day 11 1/2. The samples are too small to permit meaningful comparisons with the other days discussed.

Comparing the results of L. B. Russell et al. (45), Carter (10), Carter et al. (12), Rugh and Jackson (40), Russell (48), and the present experiment, it appears that between 11 1/2 days after conception and birth the male mouse is much more susceptible to being permanently sterilized by X irradiation than it is as an adult. The susceptibility is greater during the first half of this eight-day period. Although it appears that there may be a continuous decrease in susceptibility during this eight-day period, this has not been conclusively shown.

One observation from the present experiment fits in with the possibility of a decreasing sensitivity to sterilization during the last few days before birth. There is evidence that some litters of males may be more susceptible to sterilization than others. The 30 permanently sterile males that lived at least 400 days came from only 25 of the 320 litters that contributed males to the experiment on newborns. In three of these litters, both of two males were permanently sterile. In a third litter, three of four males were sterile. If there were no similarity in susceptibility to sterilization among sibs, the probability of having both of two males in one litter permanently sterilized would be $(.046)^2$ or 0.002. This means that only about three such litters would be expected out of 1500. However, in this experiment there were three such litters out of a total of only 107 that contributed exactly two males each. This strongly suggests that some litters may have a higher sensitivity to the sterilizing effect of X irradiation than others. Normal fluctuations in the length of the gestation period operating in the presence of a decreasing sensitivity to sterilization during the days preceding the average time of birth would easily explain a similarity in susceptibility to sterilization among sibs.

Clusters

As was previously mentioned, only 13 of the 16 specific-locus mutations observed in the progeny of males irradiated as newborns represent independent occurrences. There were two clusters of two each at the *b* locus and one cluster of two at the *d* locus. In none of these three clusters were both mutations found within a single litter.

Therefore, twinning could not be the explanation for these clusters. Excluding the highly unlikely occurrence of two independent mutations at the same locus in the offspring of one irradiated male, each cluster presumably resulted from fertilizations by sperm descended from the same irradiated germ cell.

In contrast, there were no clusters among the 40 specific-locus mutations sired by males similarly irradiated as adults (57). The frequency of clusters following irradiation of newborn males is significantly higher than that found following similar irradiation of adult males, P = 0.012 (Fisher's exact test). The fact that the males irradiated on day 0 produced only about 85 percent as many total young, on the average, as the males irradiated as adults (personal communication, W. L. Russell) would have slightly decreased the chances of finding a cluster in the experiment on newborn males. The conclusion that the frequency of clusters is higher following irradiation of newborn males is therefore strengthened.

Clusters are thought to be indicative of relatively decreased survival of germ cells following irradiation. If the testis is repopulated by fewer germ cells, there is a higher chance that some of the gametes sampled will be descended from the same irradiated germ cell. In this regard, it is interesting to note the corresponding significantly higher incidence of permanent sterility following irradiation of newborn males as compared to irradiated adults. The occurrence of clusters on day 0 certainly is consistent with the view that the permanent sterility is the actual result of the decreased survival of germ cells. It can be

speculated that a certain critical number of germ cells must survive irradiation if a given tubule is going to become functional. If the germinal epithelium is repopulated in enough tubules, the male becomes fertile.

Of the six independent specific-locus mutations found by Carter et al. (12) following the irradiation of 17 1/2-day-old fetuses with 200 R, one occurred as a cluster of three. Two of these s mutations occurred in the same litter. This opens the possibility that one of these could have resulted from twinning; however, it is clear that a cluster was involved and presumably was the basis for all three s mutations.

One cluster in six independent events does not differ significantly from the three clusters in thirteen independent events found for day 0. Also, it does not differ significantly from the zero frequency of clusters found in the adult irradiated with 300 R, P = 0.13 (Fisher's exact test). However, it should be noted that the dose of irradiation was 50 percent higher in the experiments on newborns and adults. No attempt has been made to correct for this.

Again it could be argued that the experiments on day-17 1/2 fetuses and newborn males are actually two estimates of the frequency of clusters following the irradiation of immature testes populated essentially entirely with gonocytes. Combining the results from these two experiments gives a cluster frequency of 4/19 which is also highly statistically significantly greater than that found for adults receiving a dose of 300 R, P = 0.0085 (Fisher's exact test). This is the case even though no correction has been made for the lower dose given to the 17 1/2-day fetuses.

Russell (57) found six clusters of two mutations each in 105 independent mutational events among the offspring of adult males exposed to 600 R of acute X irradiation and one cluster of three mutations in 21 independent events following the irradiation of adult males with 1000 R of acute X irradiation. Even when combined, these frequencies of clusters are not significantly greater than the zero frequency observed for adult males that received 300 R. However, the finding of these seven clusters when none was found after 300 R indicates that there may be relatively fewer spermatogonia surviving after higher doses are administered to adult males. Oakberg's histological studies (33, 34) have clearly shown that this is the case.

The frequency of clusters on day 0 is on the borderline of significance of being higher than that found in adults irradiated with 600 R, $P = 0.06$ (Fisher's exact test), and the combined frequency of clusters on fetal day 17 1/2 and on day 0 is significantly higher than that of the adult irradiated with 600 R, $P = 0.046$ (Fisher's exact test). Since this last comparison does not take into account the fact that the 17 1/2-day fetuses were exposed to only 200 R, the evidence is strong that the cluster frequency is higher among the immature males than it is among adults exposed to a two-fold higher dose.

Distribution among the Loci of Mutations

Table II shows the distribution among the loci for mutations induced in the males irradiated as newborns. Only independent mutations are recorded in this table. For comparison, other distributions and expected distributions are also shown in this table. In addition to

TABLE II

DISTRIBUTION AMONG THE LOCI OF SPECIFIC-LOCUS MUTATIONS INDUCED BY X IRRADIATION OF GERM CELLS IN PRENATAL, NEWBORN, AND ADULT MICE

Age at Irradiation	Locus								Total
	a	b	c	d	p	s	se	d se	
Adult[a]	2	32	15	24	22	69	2	0	166
Day 0 (observed)[b]	0	6	0	2	1	3	0	1	13
Day 0 (expected)[b]	0.2	2.8	1.1	1.9	1.7	5.2	0.2	0.1	13
Fetal day 17 1/2[c] + Day 0[b] (observed)	0	7	2	2	2	5	0	1	19
Fetal day 17 1/2[c] + Day 0[b] (expected)	0.2	4.0	1.8	2.7	2.5	7.6	0.2	0.1	19

[a] Russell, W. L., and L. B. Russell. The genetic and phenotypic characteristics of radiation-induced mutations in mice, Radiat. Res., Suppl. 1 (1959) 296-305.

[b] Present report.

[c] Carter, T. C., M. F. Lyon and R. J. S. Phillips, The genetic sensitivity to X-rays of mouse foetal gonads, Genet. Res., Camb., 1 (1960) 351-355.

single mutational events at each locus, double mutations can occur encompassing both of the very closely-linked d and se loci.

Russell (53, 57) has performed three single-dose 90 R/minute specific-locus experiments on spermatogonia in male mice with total doses of 300 R, 600 R, and 1000 R of X irradiation. He found no statistically significant difference between the distributions among the loci in these three experiments (57). The combined distribution of 166 mutations from these three experiments is therefore shown in Table II as a basis for comparison.

The expected distribution for day 0 is calculated from the combined distributions recorded for day 0 and the adult. Expected values in the 2 X 8 contingency table are too small to permit a valid Chi-square test of heterogeneity between the entire distributions in the adult and newborn male.

The types of mutations that gave the greatest suggestion of a possible change in proportion between day 0 and the adult were analyzed individually by means of Fisher's exact test for a 2 X 2 contingency table. The proportions of mutations found at a single locus in the newborn and adult were compared. The sample on day 0 suggests that there may be a higher proportion of d se and b mutations and a lower proportion of s mutations following irradiation on day 0. However, none of these differs significantly from the proportion in the adult. The one that comes closest is b, for which P = 0.034 (Fisher's exact test). Because of the fact that b was selected for this comparison from among the eight categories solely because it gave a suggestion of a higher proportion, this P-value would have to be less than an individual comparison

error rate of 0.0064 in order for this difference to be declared statistically significant. This individual comparison error rate is equivalent to an experimentwise error rate of 0.05 (65). Because the P-value, 0.034, is greater than the individual comparison error rate of 0.0064, the null hypothesis must be accepted; that is, the proportion of b mutations on day 0 is not significantly higher, at the 5 percent level.

Although, at first glance, it is striking that one d se mutation occurred in this sample of 13 mutations while none occurred in the 166 mutations in the 90 R/minute experiments in the adult, it should be emphasized that three other d se mutations have been found in three smaller studies at different dose rates in the adult (42). One occurred following a 600 R exposure of Co^{60} gamma irradiation delivered at 48 R/minute. Another was from the experiment in which 600 R of X irradiation was delivered at 9 R/minute (51), and the third came from an experiment in which the dose rate of 800 R/minute was administered for a total exposure of 300 R (personal communication, W. L. Russell). Should further experiments indicate that d se mutations constitute a significant fraction of the specific-locus mutations induced in the newborn male, this would be an important qualitative difference between the mutations induced in the newborn male and in spermatogonia of the adult.

The argument could again be made that the data should really be pooled from fetal day 17 1/2 and day 0. Table II (page 25) shows the observed and expected distributions for the combined data. The expected distribution is calculated from the combined distributions from all three ages. Again, the Chi-square test of heterogeneity of the entire distributions cannot be used validly. There is still an indication that

the b, s, and d se mutations may occur in different proportions following the irradiation of these different ages; however, as before, none of these differs significantly from the proportion in the adult.

Allelism Testing

When possible, each presumed specific-locus mutation is tested for allelism at the locus predicted on the basis of phenotype, although past experience has shown that almost all specific-locus mutations can be correctly classified on the basis of phenotype alone (57). Allelism has to date been conclusively demonstrated for 11 of the 16 specific-locus mutations reported in this experiment. Three others give strong evidence of being allelic. Of the remaining two, one was lost before testing and the other was sterile.

The one that was lost was phenotypically b in appearance, and it was therefore almost certainly correctly classified. The sterile mutation was a presumed s mutation. This locus occasionally presents a problem in classification from phenotype alone, in that s mutants, although generally extensively spotted, occasionally have a decreased amount of spotting that could be confused with mutations at a number of dominant spotting loci.

The sterile presumed s female was less extensively spotted than the average s mutant, but she was within the range of expected variation. She was smaller than her sibs at the time of weaning, which is characteristic of many s mutants. Her eyes were only 1/2 the usual diameter, and smaller eyes are characteristic of dominant spotting mutants at the microphthalmia locus (62). The inclusion of this somewhat questionable

mutation makes very little difference in the comparisons, not enough to affect the validity of conclusions.

The d se mutation was found to be lethal in homozygous condition. This confirms that it should be counted as a specific-locus mutation (44).

Of the 15 mutants that lived long enough to be mated, all but the s mutant had apparently normal fertility.

Sex Ratio

The percentage of males among the offspring of males irradiated as newborns was 50.75 percent. The sex ratio among the progeny of the sham-irradiated control males was 50.96 percent. This slight difference in the sex ratio does not approach statistical significance.

Analysis of Possible Decrease in Mutation Rate with Time

The present experiment gives a slight suggestion of a decrease in the mutation rate with time. If the data are divided at that point in time that maximizes the difference, 14 mutations are found in the 33,761 offspring born before the division and 2 in 21,695 after it. Following the appropriate correction for independent occurrences (55), the mutation frequency in the latter interval is not statistically significantly lower, by a one-tailed test.

No decrease in the mutation frequency with time has been found in adults following irradiation of spermatogonia with a single exposure of X irradiation (60). Should the mutation frequency decrease with time following irradiation of the newborn, it might result from the induction of some specific-locus mutations that caused slightly decreased viability of the germ cells. Slight selection against A_s spermatogonia carrying

such mutations would gradually remove them from the testis. Although the present experiment in no way proves that the mutation frequency decreases with time, the deviation of the data from expectation is large enough to warrant a check of this if further experiments are performed.

D. DISCUSSION

At least four different suggestions can be made to account for the difference in mutation frequencies found in newborn and adult male mice. Firstly, gonocytes may have an intrinsically lower mutational response than A_s spermatogonia, and the presence of gonocytes in the newborn mouse could then be the entire explanation for the lower mutation frequency. The gonocytes may have a more efficient repair system, they may be less susceptible to mutational damage, or the mutations induced in them may decrease the viability of the germ cells they are in, thereby resulting in elimination of some of them by selection (23, 54). It is not unknown for a germ cell with a higher radiosensitivity to killing to yield a lower mutation rate. Russell has demonstrated that oocytes in intermediate stages of follicular development have both an extremely low mutation frequency and a high radiosensitivity to killing (54).

Secondly, should there be a positive correlation between radiosensitivity to killing and mutational response among the germ cells present in the newborn male, the mutation frequency per R could be lower after 300 R than after a lower dose having less of an effect upon fertility. Such a positive correlation was suggested by Russell (49) as the possible explanation for the departure from linearity with dose found

after a dose of 1000 R to adult male mice. Russell (59) had found a mutation rate of 13.3 X 10^{-5} mutations/locus after a dose of 600 R and a mutation frequency of only 10.3 X 10^{-5} mutations/locus following 1000 R. The mutation rate at 1000 R was only about 1/2 as high as was expected assuming a linear relationship of mutation rate and dose. If a positive correlation between mutational response and radiosensitivity to killing is the explanation for the lower mutation frequency on day 0, an exposure of newborn males to a lower total dose would be expected to yield a higher mutation frequency per R. A determination of the dose-versus-response curve on day 0 would resolve this question.

Thirdly, the mutation frequency on day 0 could be lower than that of the adult because of a decrease in the mutation rate with time. As discussed earlier, this could result from a slight selection against A_s spermatogonia containing some of the specific-locus mutations induced in the germ cells present at birth. This slight selection would account for the decrease in mutation frequency being delayed until late in life. If the data in this experiment are divided at that point in time that maximizes the mutation frequency before the possible drop in the rate occurred, the mutation frequency in the earlier interval is 19.7 X 10^{-8} mutations/locus/R. This is still lower than the adult rate of 29.1 X 10^{-8} mutations/locus/R. It thus appears that if there really were a decrease in the mutation frequency with time, it would not be entirely responsible for the lower mutation frequency following the irradiation of newborn males.

Lastly, it is quite likely that there is extremely little mitotic activity, if any, in the germ cells present at birth (7, 20, 27).

Kimball (22) has shown for G1 in Paramecium that the longer the time between irradiation and S, the lower the mutation frequency, regardless of whether one irradiates in log phase or in stationary phase. In the short G1 of normal log phase there is only a moderate decline; however, in the long G1 of a prolonged stationary phase the decline is more extensive. The decreased mutation frequency in the newborn male mouse may result from an increase in time for repair during this period. In the adult there would always be some A_s spermatogonia undergoing DNA synthesis shortly after exposure to irradiation. Presumably there would be much less time in which mutational damage could be repaired.

Whatever the explanation for the difference from the adult turns out to be, this difference may be a useful tool for increasing knowledge about the biological factors affecting mutagenesis in mammals.

It is of interest to compare the present findings with some recently reported for a very different type of genetic damage. Fazylov and Pomerantseva (16) studied the comparative sensitivity to X-ray-induced reciprocal translocations of gonocytes in 16-day-old fetal and newborn males and of spermatogonia in adult males. The yield of reciprocal translocations was determined by analysis of spermatocytes in the diakinesis-first-metaphase stage of meiosis.

Fazylov and Pomerantseva reported that the dose-versus-response curve was linear following irradiation of newborns and adults within the range of 20 R to 400 R and that the mutational response of the newborn male was only 1/3 that of the adult. There were very few data at doses below 100 R, so the shape of the dose-versus-response curve is certainly not reliably known. With all doses of 50 R or more, the frequency of

reciprocal translocations was considerably lower following the X irradiation of 16-day fetal and newborn males than of adults, thereby suggesting a clearly lower rate of induction of reciprocal translocations in the immature males.

Fazylov and Pomerantseva discussed only one explanation for their results. On the basis of a high frequency of translocations in the 16-day fetus relative to the adult following a dose of 20 R, they concluded that the reason for the lower mutation frequency in the newborn and fetal males was probably a positive correlation between mutational response and radiosensitivity to killing. Because of this correlation, only gonocytes with a lower mutational response were thought to survive at doses of 50 R and above.

The authors, however, do not take full account of an important feature of their data, namely the likely occurrence of clusters. Clusters of translocations in a few males could have explained the higher frequency of translocations they found following the dose of 20 R administered to fetal males, thereby invalidating their statistical analysis (24) and their conclusion. Although Fazylov and Pomerantseva also attempted a comparison of the sensitivities of 16-day fetal, newborn, and adult males to the induction of dominant lethal mutations, their results were inconclusive.

The parallelism between the frequencies of induction of these two different types of genetic damage -- specific-locus mutations and reciprocal translocations -- is of interest: in both cases, the mutation rate is lower in newborn males than in adults. It will be of great interest

to learn if the explanation for the decrease in the frequency of specific-locus mutations, whatever it is, is also the reason for the lower yield of reciprocal translocations.

CHAPTER III

SPECIFIC-LOCUS MUTATION-RATE STUDY ON YOUNG MALE MICE

A. INTRODUCTION

Between birth and adulthood, the mouse testis is known to undergo many changes both in size and cell stages present. Not only do the gonocytes transform into type A spermatogonia (27, 66), but also many new cell types appear during the first cycles of the seminiferous epithelium (66). Spontaneous death of many gonocytes (7, 20), rapid changes in tubule size (20), and differing mitotic rates (7, 20, 27) are among the factors that make this a time of rapid and unique transitions in the development of the testis. The experiments discussed in this chapter were designed to explore the possibility that the germ cells present in the testes of young male mice might have a much higher specific-locus mutation frequency than the A_s spermatogonia of the adult.

Rather than looking for a possible slight change in the mutation frequency at just a few stages of development, which was the approach used for newborn males, nine different stages were tested for the possibility of having a much higher specific-locus mutation rate than is found in adults. The rationale for this approach was as follows: while a slightly increased mutation frequency in a transitional stage of development might have little effect upon overall estimates of genetic hazard to man, a greatly increased mutation rate in some stage would warrant concern. By collecting much smaller samples than those collected in the

experiment on newborns, it was possible to test young male mice at nine stages of development. Although smaller, these samples were, nevertheless, large enough to reveal, through calculation of the upper 95 percent confidence limit of the mutation rate at each stage, whether any of the stages tested were highly responsive to the induction of specific-locus mutations by X irradiation. Large enough samples were collected so that there was a good chance that the upper 95 percent confidence limit would be less than four times the point estimate of the adult spermatogonial mutation rate if the mutation frequency were not, in fact, higher than that of the adult.

It was not known what effect X irradiation would have upon the future fertility of these young male mice. The effect of irradiation on the seminiferous tubules of young rats had been studied histologically by Hughes (21), Shaver (64), and Léonard et al. (25). The extreme sensitivity found in the rat near the time of birth (4, 21) decreased when type A spermatogonia first appeared (21). Shaver (64) reported that in rats irradiated with 300 R three to five days after birth, regeneration of the germinal epithelium was seen in a small number of seminiferous tubules; however, the vast majority were "absolutely sterilized and incapable of regeneration." Tubules of rats irradiated with 400 R at 10 to 15 days of age experienced considerable damage to the germinal epithelium; however, recovery was complete. Rats exposed at 26 to 34 days experienced only slightly more damage than the adult rat. In rats exposed to 500 R at 35 to 50 days of age, the damage to the testis was reduced to a level very similar to that seen in the adult (64).

Léonard et al. (25) studied histologically the damage to tubules in the testes of rats irradiated with 250 R of acute X rays at 8 and 17 days of age, neither of which age was studied by Shaver (64). Surprisingly, they found greater damage on day 17. Seventy days after irradiation, 85 percent of the tubules in the former group contained spermatozoa in contrast to only 55 percent in the latter group. The tubules in both of these groups eventually recovered completely. Rats irradiated at both of these ages became fertile. The rat, therefore, appears not to have a steady decrease in radiosensitivity to cell killing between birth and adulthood.

Russell had some unpublished data showing that a few male mice that had been irradiated on the day of birth had become fertile. The fact that these newborn mice could become fertile at a time when the testis of the rat is extremely radiosensitive to killing suggested that it was quite likely that enough irradiated young mice would become fertile to permit an investigation of their mutational response.

Oakberg observed sections of testes from mice sacrificed at 0, 1, 3, 6, 10, 14, 21, and 35 days of age. No detailed study was made; however, these observations showed that very rapid changes occur in the testis of the (101 X C3Hf)F_1 mouse during the first ten days after birth. After that, changes are more gradual, until at 35 days of age the testis is histologically similar to that of the adult.

B. MATERIALS AND METHODS

On the basis of Oakberg's findings, (101 X C3Hf)F_1 male mice were irradiated with 300 R at 2, 4, 6, 8, 10, 14, 21, 28, or 35 days of age.

The closer intervals at the earlier ages were chosen because of the more rapid histological changes that had been observed during that time. Although the testis appears histologically mature at 35 days of age, it was not known whether it would have the same mutational sensitivity as the older males used in the earlier specific-locus experiments.

The method of determining the time of birth, irradiation conditions, and time of pairing with a test-stock female were identical to those described in Chapter II. All males were irradiated within plus-or-minus seven hours of the age stated. This restriction on the number of hours was established in order to avoid adding unduly to the degree of variation in morphological development that naturally exists between and within litters. As an example, those said to be irradiated on day 2 were irradiated 48 plus-or-minus 7 hours after their exact time of birth. The lucite containers that held the mice in place during the irradiation were modified for the individual ages so that the testes of all mice passed through the same portion of the X-ray field.

Males were distributed at random by litter to the different age groups. The rest of the procedure was the same as that described in the previous chapter.

C. RESULTS AND CONCLUSIONS

Mutation Rates

Table III shows the results of these nine experiments. For comparison, the results from day 0 and the adult have been included in this table.

TABLE III

SPECIFIC-LOCUS MUTATION FREQUENCIES RESULTING FROM IRRADIATION OF MALE MICE AT VARIOUS AGES WITH 300 ROENTGENS OF X RAYS

Age at Irradiation in Days	Number		Mutation Frequency [X 10^{-8}] (Locus^{-1} R^{-1})	95% Confidence Limits of Mutation Frequency (X 10^{-8} Locus^{-1} R^{-1})		Ratio of Upper Limit to Mutation Frequency of Adult
	Specific-Locus Mutations Found	Offspring Observed		Lower	Upper	
0	16[a]	55,456	13.7	7.1	22.6	0.78
2	2	8,127	11.7	2.1	39.2	1.35
4	3	8,784	16.3	4.4	43.9	1.51
6	4	7,559	25.2	8.6	60.5	2.08
8	7	8,463	39.4	18.5	77.5	2.66
10	3	8,580	16.6	4.5	45.0	1.55
14	9	8,397	51.0	25.3	95.1	3.27
21	5[b]	8,920	26.7	7.3	72.1	2.48
28	6	10,009	28.5	12.4	61.0	2.10
35	4	9,056	21.0	7.2	50.5	1.74
Adult[c]	40	65,548	29.1	21.0	39.0	1.34

[a] Includes three clusters with two mutations each.

[b] Includes two clusters with two mutations each.

[c] Russell, W. L., Studies in mammalian radiation genetics, Nucleonics, 23 (1965) 53-56, 62.

Of the nine ages, seven have point estimates of the mutation frequency that are lower than that of the adult. The point estimate of the highest is 1.8 times the rate of the adult. However, neither this age nor the other age with a higher point estimate has a mutation rate statistically significantly higher than the adult mutation rate. The upper 95 percent confidence limit of only one of the nine age groups exceeds three times the adult mutation frequency, and even that age group's upper 99 percent confidence limit is less than four times the adult mutation frequency. It therefore appears highly unlikely that the mouse testis passes through any stages of development between birth and adulthood during which the germ cells in it are very much more responsive to the induction of specific-locus mutations than those of the adult.

If the data from all nine ages are combined, there are 43 specific-locus mutations in 77,895 offspring. This is a specific-locus mutation frequency of 26.3×10^{-8} mutations/locus/R. The average mutation frequency for young male mice of all age groups combined is therefore very similar to the spermatogonial mutation frequency of 29.1×10^{-8} mutations/locus/R found for the adult. This does not exclude the possibility that a few of the ages tested may have a somewhat different mutation rate.

Permanent Sterility

The estimate of the frequency of permanent sterility for each of these nine ages is shown in Table IV. Since the estimate would have been distorted upward by including the males that died early, it is

TABLE IV

INCIDENCE OF PERMANENT STERILITY
FOLLOWING X IRRADIATION
OF YOUNG MALE MICE

Age at Irradiation in Days	Incidence	
	Ratio	Percentage
2	0/81	0
4	1/81	1
6	1/78	1
8	0/76	0
10	0/78	0
14	0/85	0
21	0/84	0
28	0/91	0
35	0/78	0

based only upon those males that lived at least 100 days. One hundred days was a sufficient interval in these nine experiments because very few males experienced a long delay in becoming fertile. Again, the incidence is determined by dividing the number of males alive at 100 days that never produced any offspring by the total number of males that lived 100 days.

All comparisons with the frequency of permanent sterility following irradiation on day 0 are made with the estimate at 400 days of age (i.e., 4.6 percent). The frequency of permanent sterility following irradiation on day 2 is significantly lower than for day 0, P = 0.028 (Fisher's exact test). Furthermore, the incidence of permanent sterility in males irradiated during the week after birth (i. e., days 2, 4, and 6 combined) is significantly lower than for day 0, P = 0.003 (Fisher's exact test). The nine ages, singly or grouped, do not have a significantly higher proportion of permanently sterile males than do adults irradiated with 600 or 1000 R (48) or unirradiated controls.

Clusters

The only one of the nine age groups that yielded a cluster was day 21. That group yielded two clusters in three independent events. One cluster was of two mutations at the p locus and the other, also of two mutations, at the s locus. This is a statistically significantly higher proportion of clusters than the 0 in 40 found for the similarly irradiated adults by Russell (57), P = 0.0033 (Fisher's exact test). Because of the fact that day 21 was selected out of the ten ages of irradiated males for comparison with the adult solely because it had the

highest frequency of clusters, the P-value shown was compared to an individual comparison error rate of 0.0051. This error rate is equivalent to an experimentwise error rate of 0.05 (65). Because the P-value, 0.0033, was less than the individual comparison error rate of 0.0051, the null hypothesis was rejected. That is, the frequency of clusters on day 21 is significantly higher, at the 5 percent level.

The average number of offspring observed per fertile male was about 7 percent higher in this experiment than in the comparable experiment on the adult (personal communication, W. L. Russell). This would have slightly increased the chances of finding clusters in the day-21 experiment.

Except for the fact that the germ cells of the 17-day-old rat have increased radiosensitivity to killing, as shown by Léonard et al. (25), there is no biological reason to expect clusters on day 21. The developmental stage of the testis in the 17-day-old rat and the 21-day-old mouse should be quite similar.

The frequency of clusters on day 21 was two in three independent events, or 0.67. The 95 percent confidence limits of this are 0.04 and 0.99. A specific-locus experiment utilizing a high total dose could easily determine if the incidence of clusters on this day was very high. If it should prove to be, day 21 would be especially suited for studies on the possible positive correlation between mutational response and radiosensitivity to killing, and also for experiments exploring the details of repopulation of the testis from small numbers of germ cells.

It would be interesting to know if males irradiated at 21 days of age are delayed longer in reaching fertility than immature males

irradiated at other ages. This would be expected if they really do experience relatively more killing of A_s spermatogonia. Unfortunately, too few males irradiated on day 21 were paired with females early enough to provide a clear answer. There was, however, some suggestion that they might be delayed about one week.

Distribution among the Loci of Mutations

The distribution among the loci of the specific-locus mutations found in these nine experiments is shown in Table V. Only independent mutations are listed. The number of mutations is too small to permit a meaningful comparison between the distribution found for each of these age groups and that for the adult.

In order to give some indication of the relationship of the distribution in the young male mouse to that in the adult male mouse, the data have been arbitrarily combined into three groups as follows: days 2 to 6 combined, days 8 to 14 combined, and days 21 to 35 combined. Table VI shows the observed distribution and the expected distribution for each of these groups and for all nine experiments combined. For comparison, the distribution Russell (57) found in the adult male is also given. The expected distributions are calculated from the combined distribution reported for the category in question and the adult.

The *se* locus is the only one that shows any indication of a difference in proportions between age groups, and this slight discrepancy is due to results for the 8-14 day group. No biological reason is known that justifies selecting only ages 8 to 14 for a comparison with the adult proportion of *se* mutations. However, this comparison was made to

TABLE V

DISTRIBUTIONS AMONG THE LOCI OF SPECIFIC-LOCUS MUTATIONS INDUCED BY X IRRADIATION OF GERM CELLS IN YOUNG MICE OF VARIOUS AGES

Age at Irradiation in Days	Locus								Total
	a	b	c	d	p	s	se	d se	
2	0	0	0	0	2	0	0	0	2
4	0	2	0	0	0	1	0	0	3
6	0	1	1	0	1	1	0	0	4
8	0	0	0	1	1	4	1	0	7
10	0	0	0	0	1	1	1	0	3
14	0	3	0	1	1	3	1	0	9
21	0	1	0	0	1	1	0	0	3
28	0	1	0	0	2	3	0	0	6
35	0	0	1	1	0	2	0	0	4

TABLE VI

COMPARISONS OF THE DISTRIBUTIONS AMONG THE LOCI OF SPECIFIC-LOCUS MUTATIONS INDUCED BY X IRRADIATION OF YOUNG AND ADULT MALE MICE

Age at Irradiation	Locus								Total
	a	b	c	d	p	s	se	d se	
Adult[a]	2	32	15	24	22	69	2	0	166
Days 2-6 Combined (Observed)[b]	0	3	1	0	3	2	0	0	9
Days 2-6 Combined (Expected)	0.1	1.8	0.8	1.2	1.3	3.6	0.1	0	9
Days 8-14 Combined (Observed)[b]	0	3	0	2	3	8	3	0	19
Days 8-14 Combined (Expected)	0.2	3.6	1.5	2.7	2.6	7.9	0.5	0	19
Days 21-35 Combined (Observed)[b]	0	2	1	1	3	6	0	0	13
Days 21-35 Combined (Expected)	0.2	2.5	1.2	1.8	1.8	5.4	0.2	0	13
Days 2-35 Combined (Observed)[b]	0	8	2	3	9	16	3	0	41
Days 2-35 Combined (Expected)	0.4	7.9	3.4	5.4	6.1	16.8	1.0	0	41

[a] Russell, W. L., and L. B. Russell, The genetic and phenotypic characteristics of radiation-induced mutations in mice, Radiat. Res., Suppl. 1 (1959) 296-305.

[b] Present report.

determine if there was any chance that this might be a meaningful difference. The heterogeneity of the proportion of se mutations in these two groups was tested using Fisher's exact test, and P = 0.008. Since this comparison selected se mutations from among the eight possible categories of specific-locus mutations solely because of its high proportion, the individual comparison error rate of 0.0064 must be used (65). This error rate, equivalent to an experimentwise error rate of 0.05, is smaller than the calculated P-value, and, therefore, the null hypothesis is accepted. Thus the distribution of se mutations is not significantly different, even after restricting the comparison to the only ages on which they occurred, and these experiments cannot be interpreted as indicating any change in the proportion of se mutations.

The distribution of specific-locus mutations induced in the combined data from young males is clearly very similar to that induced in the adult, but the data are too limited to exclude the possibility that some particular age might have a different distribution.

Allelism Testing

Of the 43 specific-locus mutations reported in these nine experiments, allelism at the locus predicted on the basis of phenotype has been proved for 35. Two presumed s mutants died before testing. Six other presumed s mutants have been only partially tested; however, based on the present results, there is no indication that any of these are not allelic. All presumed s mutants reported, with the exception of the one from day 10, had a very extensive spotting pattern. This virtually insures that they are actually allelic, even without complete testing.

The one presumed s mutant from day 10 was less extensively spotted than the average s mutant, but was well within the range of expected variation. Before becoming sterile, it produced enough spotted offspring to reduce the probability that it is not allelic to only 0.13.

Two mottled animals were found in these experiments, one in the day-10 experiment and the other from day 21. Each one was sufficiently tested to show that it carried no dominant or X-linked mottling factor and no allele that caused mottling when in combination with one of the specific-locus test alleles. These mice were clearly somatic mosaics; however, neither one has been conclusively proved to be a germinal mosaic. One died giving inconclusive results, and the other is still being tested. There is some indication that the latter mouse may be a germinal mosaic.

L. B. Russell (41) reported on the testing of 40 certain and probable specific-locus mosaics. Twenty-one of these had one irradiated parent, whereas 19 came from a contemporary unirradiated control population of only slightly smaller size. She concluded that there is no evidence against all of the specific-locus mosaics being of spontaneous origin. The specific-locus mosaics apparently result from spontaneous mutation during development, in most cases soon after fertilization. Since the two mottled animals found in these experiments were thus presumably not the result of radiation-induced mutations, they were not counted as specific-locus mutations.

Sex Ratio

Table VII shows the percentage of males obtained in each of these

TABLE VII

SEX RATIO AMONG OFFSPRING OF CONTROL MALES AND YOUNG MALES IRRADIATED AT VARIOUS AGES

Age at Irradiation in Days	Sex Ratio
Unirradiated Control	50.96
2	50.31
4	50.83
6	50.37
8	50.94
10	50.94
14	50.49
21	49.97
28	51.20
35	50.57

nine experiments and in the sham-irradiated control described in Chapter II. The sex ratio is not statistically significantly different from the control following irradiation on any of these days. Even combining all ten experimental groups, days 0-35, no significant difference is seen from the control.

Time of Occurrence of Mutations

As was shown in the section in this chapter entitled Mutation Rates, the average mutation frequency for all of the young males combined is very similar to that of the adult and considerably higher than that found for the newborn males. Spermatocytes and spermatids are present at many of the ages that were irradiated (66). In the adult, the post-spermatogonial stages have a higher specific-locus mutation frequency than spermatogonia (58). This raises the possibility that the mutation frequency found for these ages might be inflated by the inclusion of mutations induced in postspermatogonial stages.

Oakberg (30, 37) has shown that, in the adult, type A spermatogonia present at the time of irradiation are not utilized for matings until 42 days later, at the earliest. Irradiated postspermatogonial stages are utilized exclusively in matings occurring within 34 days of irradiation, and to a decreasing extent thereafter. For most of the nine age groups, the mean age of the male at the time of the first impregnation was between 50 and 60 days. Very few males impregnated a female before 40 days of age. If the timing of utilization of postspermatogonial stages is similar in the adult and the young male, it is likely that the first offspring sired by many males resulted from sperm

that had been irradiated in a postspermatogonial stage. Although the total number of such offspring would be very small relative to the total number of offspring produced by these males during their entire lifetime, the possibility clearly did exist that mutations induced in postspermatogonial stages could have been recovered.

Analysis of the data, however, indicates that it is very unlikely that any of the mutations observed were induced in a postspermatogonial stage. Assuming a gestation period of 19 days, the earliest mutant was not conceived until 22 days after type A spermatogonia present at the time of irradiation would have first been utilized for matings. It seems very unlikely that any postspermatogonial stages at the time of irradiation would have persisted that long.

The mutations found in these nine experiments were well distributed throughout the entire breeding period, which is also the case in the adult (60). There was no suggestion whatsoever of a decrease in the mutation frequency with time.

D. DISCUSSION

The average mutation frequency for the nine age groups of young males combined, 26.3 X 10^{-8} mutations/locus/R, is significantly higher than that of the newborn males, 13.7 X 10^{-8} mutations/locus/R, P = 0.024 (for a one-tailed test). Furthermore, the average mutation rate for the nine age groups combined is almost the same as that of similarly irradiated adults, 29.1 X 10^{-8} mutations/locus/R, thereby suggesting that most of these ages may have a mutation frequency much like that of the adult.

It is of considerable interest to know when the transition in mutation frequency, from lower in the newborn testis to higher in the adult, occurs.

Within the data for these nine age groups are a number of indications of a possible trend that suggest that the change in mutational response may have occurred by day 8. Day 8 has the next to the highest mutation frequency of all nine groups. All three ages before it have lower mutation frequencies than the adult. The mutation frequencies on days 2 and 4 were the lowest found among these nine groups, both being very similar to the rate found for newborn males. The point estimates for the data are arbitrarily grouped in Table VIII. For comparison, the point estimates for day 0 and the adult are also shown.

Chapter II discusses some of the possible explanations for the decreased mutation frequency on day 0. The finding that the mutational response appears to increase during the week after birth is consistent with the explanations presented.

These experiments also have demonstrated that a decrease in radiosensitivity to killing occurs in the germ cells of the testis almost immediately after birth. Although the similar change in the rat has been shown to be correlated with the transformation of gonocytes into type A spermatogonia (21), this remains to be examined in the mouse.

This study of X-ray-induced specific-locus mutation rates in newborn and young male mice has explored the possibility that some stage during the development of the testis between birth and adulthood might have a much higher mutation frequency than the adult. No evidence has been found of any greatly increased mutational response. On the other

TABLE VIII

ARBITRARY GROUPING OF MUTATION-RATE DATA FOR YOUNG MICE TO ILLUSTRATE LIKELY TIME OF TRANSITION IN MUTATIONAL RESPONSE

Age at Irradiation in Days	Point Estimate of Mutation Frequency ($\times 10^{-8}$ Locus^{-1} R^{-1})
0[a]	13.7
2-6 Combined[a]	17.5
8-35 Combined[a]	30.3
Adult[b]	29.1

[a] Present report.

[b] Russell, W. L., Studies in mammalian radiation genetics, Nucleonics, 23 (1965) 53-56, 62.

hand, in at least one stage, the newborn, the testis clearly has a lower specific-locus mutation rate than the testis of the adult mouse.

CHAPTER IV

SPECIFIC-LOCUS MUTATION-RATE STUDY ON NEWBORN FEMALE MICE

A. INTRODUCTION

Gametogenesis in the Female Mouse

The sex of a mouse fetus can be determined histologically at about 11 days of embryonic life. In the female, the distribution of the primordial germ cells that have already migrated into the germinal ridges is distinctly peripheral. The progressive cortical development of the immature ovary follows. The primordial germ cells themselves look identical in the two sexes of the mouse until meiosis begins in the female (27).

The rapid mitotic activity of the primordial germ cells ceases in the female on days 13 and 14 of embryonic life (8). Borum (8) has studied the early development of the oocytes in albino mice of the Street strain. She reported that by day 14 most of the germ cells were in leptotene and zygotene stages. On day 15, all germ cells were in meiosis, most of them either in the zygotene or pachytene stage. Almost all oocytes were in pachytene on day 16; however, some of these were degenerating.

The quantitative portion of Borum's study illustrates the asynchrony of the oocytes during this period of development, and it also indicates the rate at which oocytes pass through the various stages of meiotic prophase. On day 17 of embryonic development she found the

following approximate distribution of oocyte stages: 5 percent leptotene, 5 percent zygotene, 55 percent pachytene, and 5 percent diplotene. The remaining 30 percent were degenerating pachytene oocytes. In contrast to this, during the first 1/3 day after birth approximately 10 percent of the oocytes are in pachytene, 30 percent in early diplotene, and 10 percent in an early dictyate (diffuse or late diplotene) stage of meiosis. At this time approximately 50 percent are degenerating pachytene oocytes

Brambell (9) also reported finding only pachytene, diplotene, and dictyate oocytes in newborn female mice. Pachytene oocytes were scarce, diplotene oocytes plentiful, and the majority had reached the dictyate stage.

Four days after birth all oocytes are in the dictyate stage of nuclear development (8, 9). Nuclear development is arrested in the dictyate stage during follicular development. In the fertile adult female, oocytes are found in all stages of follicular development ranging from primordial follicles, in which oocytes are surrounded by a single layer of flattened or cuboidal granulosa cells, to mature Graafian follicles (2, 32).

Meiosis resumes at diakinesis about one day before ovulation (2), and the first meiotic division occurs in the ovary about six hours before ovulation. At the time of ovulation, the oocytes are in metaphase of the second meiotic division, and they remain in this stage until sperm entry (1).

The major distinction between gametogenesis in the male and female is the complete absence of stem cells in the female after birth.

All oocytes that will ever exist are present before birth (32). By demonstrating that genetic abnormalities which cause a pronounced deficiency of primordial germ cells in embryonic life lead to a sterile ovary in the adult, Mintz (27) has shown that the primordial germ cells are precursors of the oocytes in the adult female.

Radiosensitivity to Killing of Germ Cells in the Mouse and the Rat

L. B. Russell et al. (45) reported a severe decrease in reproductive capacity in female mice exposed to 200 R of acute X irradiation at 11 1/2 or 13 1/2 days of gestation. Rugh and Jackson (40) found that the total number of young produced between two and nine months of age was approximately halved in female mice irradiated with 200 R of acute X irradiation at 16 1/2 days of gestation. The decrease in reproductive capacity was less severe at 15 1/2 and 17 1/2 days of fetal life. Reproductive capacity was at least as high as in controls following irradiation at 18 1/2 days of embryonic life with the same dose, 200 R.

Russell et al. (61) reported that female mice receiving 300 R of acute X irradiation on the day of birth commonly produced nine or ten litters. This contrasted greatly with adult females which, after receiving the same dose, never produced more than two litters (mean: 1.4).

There is, however, a great increase in sensitivity shortly after birth. The population of oocytes in a female mouse in her second week after birth is even more drastically radiosensitive to killing than that in the adult. During this time, Russell et al. (61) reported an extreme sensitivity, even to low-intensity irradiation. A total dose of 85 R of continuous Cs^{137} gamma irradiation was administered during the second

week after birth (dose rate only 0.0084 R/minute). These females became sterile after either a single litter or a second litter of reduced size. In marked contrast, adult females receiving an almost identical dose, 86 R, at the same dose rate produced an average of 13.3 litters and 86 offspring per female.

Oakberg (32) found that female mice irradiated with 25 R of acute X irradiation ten days after birth produced only 35 percent as many offspring during their lifetime as did unirradiated controls. His studies have shown that a major cause of the difference in fertility between females irradiated at ten days of age or as adults is that in the young animals there are very low numbers of oocytes in the later, more resistant, stages of follicular development (31). A measure of the extreme radiosensitivity to killing of early dictyate oocytes is given by Oakberg's finding (31) that the LD_{50} (as determined 72 hours after exposure) of stage-1 dictyate oocytes was 9.4 R in the ten-day-old mouse.

Russell and Oakberg (56) have emphasized that the major effect irradiation has upon the fertility of female mice is the shortening of the reproductive life span, and the main cause of the shortened reproductive life span is the partial destruction of the pool of oocytes which occurs soon after the irradiation.

Just as Oakberg's histological studies have provided an explanation for the results in the young and adult female, Beaumont's (5, 6) histological studies in the rat have helped explain the fertility results found for fetal and newborn stages of development. As in the male, gametogenesis in the female is very similar for the mouse and rat.

Beaumont determined the survival of oocytes following doses of 50 R and 100 R of X irradiation at many intervals between eight days of gestation and five days after birth. Two periods of high radiosensitivity to killing were found: one occurring at 15 days of fetal life, when mitoses in the primordial germ cells were prevalent, and the second beginning shortly after birth, when the oocytes attain the dictyate stage of nuclear development (6). On the day before birth, 100 R was found to cause very little death among oocytes; however, at all other times this dose resulted in considerable killing of oocytes (6). Beaumont (5, 6) interprets these results as showing that the earliest stages of meiotic prophase (preleptotene and leptotene) are more susceptible to radiation damage than those that follow. Radiosensitivity is greatly decreased in the pachytene and diplotene stages of meiosis, and this is followed by a sharp increase in radiosensitivity which coincides with the entrance of the oocytes into the dictyate stage. Her findings on the high radiosensitivity to killing of early dictyate oocytes agree with those of Oakberg (31).

Beaumont's interpretation fits the mouse fertility data if it is assumed that the CF1 mice irradiated by Rugh and Jackson (40) were about two days slower in development than the Street strain mice documented cytologically by Borum (8). If this is not the case, then mouse oocytes must retain considerable radiosensitivity to killing for a short while after entering the pachytene stage.

Earlier Specific-locus Mutation-rate Studies in Mice

Russell (54) exposed adult F_1 hybrid females from the cross of

the 101 and C3H inbred strains to 400 R of 90 R/minute X irradiation. He found 23 specific-locus mutations in 14,842 offspring. This is a mutation frequency of 55.3 X 10^{-8} mutations/locus/R. The 95 percent confidence limits are 35.9 X 10^{-8} and 81.9 X 10^{-8}. The mean number of litters produced by females receiving this dose was 1.14 $\pm$ 0.04 (46). (All error values in this dissertation are standard errors of the mean.)

In another specific-locus experiment on adult females in which a single acute exposure of X irradiation was used, Russell (54) applied a dose of only 50 R, at 90 R/minute. Females receiving this dose produced 4.00 $\pm$ 0.21 litters (46). Two striking results came out of this 50 R experiment (54). Firstly, among the litters conceived within the first seven weeks after irradiation, the mutation frequency after 50 R was only 1/3 of that expected on the basis of a linear relation with the mutation rate at 400 R. This drop in mutation rate per R was statistically highly significant. Secondly, no mutations were found among the 78,191 offspring conceived more than seven weeks after irradiation (55). The mutation rate after seven weeks was statistically significantly lower than that before seven weeks.

Carter et al. (12) irradiated (C3H X 101)F_1 hybrid females at 17 1/2 days of fetal life with 200 R of X irradiation delivered at 72 R/minute. Three specific-locus mutations were found in 30,289 offspring. This is a specific-locus mutation frequency of 7.1 X 10^{-8} mutations/locus/R, with 95 percent confidence limits of 1.9 X 10^{-8} and 19.1 X 10^{-8}. This rate is highly significantly lower per R than the rate found by Russell after a dose of 400 R was given to adults, $P < 10^{-5}$ (for a one-tailed test).

Purpose of this Experiment

This experiment was designed to provide an estimate of the mutational response of oocytes present on the day of birth. The dose of 300 R of acute X irradiation was chosen so that these females could be irradiated simultaneously with the newborn males. In view of the extremely low spontaneous mutation frequency in female mice (55), no control was run in the main experiment. A small control was run for comparison of fertility effects. No specific-locus mutation experiments have been attempted on young females older than newborns because of the extreme radiosensitivity to killing of oocytes that prevails during most of the time from a few days after birth to adulthood (38, 61).

B. MATERIALS AND METHODS

Newborn (101 X C3Hf)F_1 females were irradiated with 300 R of acute X rays. The method of determining the time of birth and irradiation conditions were the same as those described in Chapter II. All females were irradiated within nine hours after birth. Most of the irradiated females were littermates of males irradiated as newborns and were irradiated simultaneously with them.

Almost all females were individually paired with test-stock males at some time between weaning and seven weeks of age. Only males that died or were obviously sick were replaced. From these matings, only the offspring that lived until weaning were counted. Litters were usually weaned sometime during the week following the time of full development of the external ears. Animals carrying specific-locus mutations were

genetically tested to determine if the mutation was actually an allele at the test locus predicted on the basis of phenotype. With the exception of some females that were held to determine longevity, females were discarded after they had been paired with males for four successive months without producing any offspring.

A fertility comparison was made between two groups of females treated identically in all ways except that one group received the 300 R X irradiation and the other group was sham-irradiated. The irradiated females tested for fertility were only a small part of the population of females in the main experiment.

All females in both groups of the fertility comparison were born within eight consecutive days. The females were distributed at random by litter between the two groups. The 50 irradiated females that lived to be mated came from 19 separate irradiated litters, and the 44 contemporary control females were from 22 sham-irradiated litters. No animals were weaned that weighed less than eight grams. All but one irradiated and three control females were sufficiently heavy to be weaned at 22 days of age. The remainder were weaned on day 24, the day on which all females in both of these groups were paired individually with mature and vigorous seven-week-old test-stock males. These males were distributed at random among the females. The pens of irradiated and control groups were intermingled on the shelves in the mouse room to insure a uniform environment.

Females in the fertility experiment were checked frequently for pregnancy, and any pregnant animals were observed nightly between 10:00

p.m. and midnight to determine the exact day of birth of the first litter and also the number of offspring born. All litters born during the lifetime of these females were kept until weaning age so that they, along with the litters born to the females in the main experiment, could be observed for the presence of specific-locus mutations.

C. RESULTS AND CONCLUSIONS

Mutation Rates

Three specific-locus mutations were found in 14,259 offspring born to irradiated females. This is a mutation frequency of 10.0×10^{-8} mutations/locus/R with 95 percent confidence limits of 2.7×10^{-8} and 27.1×10^{-8}.

In the past work on adult females, the acute dose closest to that used here was 400 R in the experiments by Russell (54) mentioned earlier. The mutation frequency per R in the newborn females is less than 1/5 that found by Russell following irradiation of the adults, a reduction which is highly significant, P = 0.0009 (for a one-tailed test). The interpretation of this result will be discussed later.

The mutation frequency per locus per R, found following the irradiation of newborn females with 300 R, is not significantly different from the rate of 7.1×10^{-8} mutations/locus/R found by Carter et al. (12) for females irradiated with 200 R at 17 1/2 days of fetal life.

The offspring of the few sham-irradiated control females were also observed for the occurrence of specific-locus mutations. None was found in 3107 total offspring. This is as expected considering the very low spontaneous mutation rate in adult females (55).

Allelism Testing

Two of the specific-locus mutations found were at the p locus, and the third was at the b locus. Each of these has been conclusively demonstrated to be an allele at the locus expected on the basis of phenotype.

Fertility

Since the first females that were irradiated as newborns conceived somewhat earlier than expected, the possibility that irradiation might advance the time of attainment of sexual maturity was explored. Forty-four sham-irradiated controls and fifty irradiated females were paired with males as described earlier. One of the control females and two of the irradiated females were omitted from the comparison because they died before they could have given birth. Another control female was omitted because the male with which she was initially paired proved to be sterile.

Assuming a constant gestation period of 19 days, the median age at the first conception that resulted in a litter being born was 34 days for the control, with a range from 30 to 59 days. The median for the irradiated females was 33 days, with a range from 28 to 71 days. Although no control females conceived before day 30, one irradiated female conceived on day 28 and three on day 29. Furthermore, seven irradiated females conceived on day 30 in contrast to only three control females. The data suggest that there may be a slight hastening in the onset of sexual maturity following irradiation, although there is no marked effect. It is quite clear that 300 R of X irradiation on the day of birth in no

way delays the time at which a female mouse can conceive and successfully maintain a pregnancy until term.

Although the irradiation of the newborn females did not have much effect, if any, on their onset of sexual maturity, it had a marked effect on their fertility. Comparisons of the total number of litters produced and the total number of offspring raised to weaning have been made between the irradiated females and their contemporary controls. In these comparisons, all females that died too young to have a litter were omitted. This left 48 irradiated females and 43 control females, all of which produced at least one litter. Of these, additional females were omitted from the comparisons for the following reasons:

1. One control female produced no young until she was mated with a new male, and the original male was found to be sterile.
2. One control female was killed accidentally during the course of the experiment.
3. Three control females were separated from males for longer than two weeks during their reproductive period.
4. Five control females and six irradiated females died within two months of the birth of their last litter, indicating that death may have intervened before the end of their potential reproductive period.

A total of 33 control females and 42 irradiated females are left for these comparisons.

The mean number of litters produced by control females was 14.85 $\pm$ 0.43. In comparison, the mean number of litters produced by irradiated

females was 6.05 ± 0.29. The range for controls was 6 to 19 litters and, for irradiated females, 1 to 10. The number of litters is highly significantly reduced for the irradiated females, $P \ll 0.001$ (for a two-tailed test; $t = 17.5$).

The mean number of offspring raised to weaning by the control females was 78.79 ± 2.65, whereas for irradiated females it was only 24.81 ± 1.45. The ranges for the two groups were 27 to 103 and 3 to 44, respectively. The irradiated females raised highly significantly fewer offspring to weaning, $P \ll 0.001$ (for a two-tailed test; $t = 18.9$). The overall average litter size for control females was 5.31 and for irradiated females only 4.10.

A decrease in litter size was already evident in the first litter born. To insure that only first pregnancies were considered, this comparison was restricted to those females which produced a litter during their first 50 days of life. There were 37 litters from control females and 42 litters from irradiated females involved in this comparison.

The number of offspring born in the first litter of control females was 7.30 ± 0.20; however, for irradiated females it was 5.50 ± 0.15. The range for controls was 3 to 9 and for irradiated 3 to 8. The size of the first litter born is statistically significantly lower in the irradiated females than in controls, $P \ll 0.001$ (for a two-tailed test; $t = 7.2$).

Although fertility is decreased considerably in the irradiated newborn females, it must be remembered that, in comparison to irradiated adult females, these females are exceptionally fertile. Whereas females

irradiated with 300 R on day 0 produced an average of 6.05 ± 0.29 litters, adult females exposed to only 50 R of acute X irradiation were able to produce only 4.00 ± 0.21 litters on the average (46). In the experiment on adults, litters were killed on the day of birth. Since this tends to increase the number of litters born, the contrast between newborns and adults is probably even greater than shown. Whereas females irradiated with 300 R as newborns reared an average of 24.81 ± 1.45 offspring to weaning, the adult females irradiated with 50 R of X irradiation gave birth to 23.17 ± 1.48 progeny on the average (46). Adult females irradiated with 100 R and 300 R of acute X irradiation gave birth to only 14.08 ± 0.58 and 7.58 ± 0.94 offspring, respectively (46).

Longevity

One hundred irradiated females were allowed to complete their natural life span. These included the females used in the fertility comparison and another fifty born about two months earlier. For comparison, the sham-irradiated control females were also allowed to complete their natural life span. One of the control females was accidentally killed and therefore omitted from this comparison. Most females were found after death, in which case their date of death was known within seven days. A few that disappeared during the course of the experiment, probably accidentally discarded by the animal caretakers, have been omitted from comparisons.

The distribution of deaths for both the irradiated and control groups showed a higher incidence of deaths up to 99 days and between 100 and 199 days than in the next few equal-sized intervals. Those dying

during the first two intervals have been considered as early deaths and omitted from the comparison of longevity. Thus, the comparison is restricted to those animals in both groups that survived at least 200 days. After these adjustments, there are 36 control females and 81 irradiated females involved in this comparison.

The mean life span in days of the control females was 738 $\pm$ 31 and, for the irradiated females, 670 $\pm$ 17. The mean life span of the females irradiated with 300 R on day 0 is significantly shortened, $P < 0.05$ (for a two-tailed test; $t = 2.12$).

This finding gains added interest in view of Gowen's (17) report that irradiation of adult females with 320 R acute X rays prolongs their life span. He attributed their longer life span to their greatly decreased fertility after irradiation. They were spared the hazards and strains of a lifetime of bearing and rearing young. If any such effect is acting in the females that were irradiated at birth, it is clearly more than offset by the deleterious effects of irradiation.

Sex Ratio

The percentage of males was 54.00 percent in the 3109 progeny of the sham-irradiated control females and 53.44 percent in the 14,259 offspring of irradiated females. The difference does not approach statistical significance.

Time of Occurrence of Mutations

Surprisingly, all three specific-locus mutations found in this experiment were born after more than 23 weeks had elapsed since the irradiation. Furthermore, assuming a gestation period of 19 days, all three

mutations were conceived in the narrow interval of time from 148 to 161 days after the time of irradiation. All occurred in the fifth litter of different females.

This restriction in time of appearance of the mutants could have been due to chance alone. However, should future experimentation confirm that there is a narrow interval of time following irradiation of newborn females during which all, or virtually all, mutants are conceived, this would suggest that some particular germ-cell stage (probably some subdivision of pachytene or diplotene) has a relatively higher mutation frequency; whereas germ cells on both sides of it in development have lower mutation frequencies. If oocytes were strictly regimented throughout development so that they were ovulated in the same order in which they passed through this possible mutationally responsive stage, then more mutant offspring would be born within a narrow interval of time.

As was mentioned earlier, Russell (54) found that following irradiation of adult females with 50 R of X irradiation, there is a drastically lower mutation frequency among offspring conceived more than seven weeks after the irradiation. He discussed three possible explanations for this. The decrease might be due to a lower mutational sensitivity in the less mature oocytes which give rise to the later litters. Secondly, the decrease might be due to a very efficient repair system in these oocytes. Thirdly, the decrease might be the result of selection. The fact that all three mutations in the present experiment were conceived more than 21 weeks after the time of irradiation shows that whatever the causes are for the sharp drop in the mutation rate at seven weeks in the adult

female, they are either not operating at all, or to a lesser extent, following irradiation of the newborn female.

Assuming a gestation period of 19 days, the three mutations found by Carter et al. (12) were conceived 103, 131, and 191 days following irradiation (personal communication, R. J. S. Phillips), thereby indicating that these 17 1/2-day fetal females, like those irradiated as newborns, differ from the adult in the time of occurrence of mutations. Not only were different germ-cell stages present at 17 1/2 days of fetal life than were present in the newborn, but also the dose of X rays administered was different. Thus, all mutations would not have been expected in, or near, the interval from 148 to 161 days after irradiation, even if a restricted mutationally responsive stage and regimentation of oocytes actually occur.

D. DISCUSSION

Several major qualitative differences have been demonstrated between the results of irradiating newborn and adult females. Fertility is affected much less adversely by acute X irradiation in the newborns. If the dose to adult females is lowered to permit more extended fertility following irradiation, all mutations are conceived in the first seven weeks. No similar restriction is found for those irradiated with 300 R as newborns.

In view of these differences, the interpretation of the seemingly lower mutation frequency in newborns is complicated. Perhaps the comparison of the 300-R exposure to the newborn female with the 400-R

irradiation of the adult is not really meaningful in view of the very different cell populations from which offspring are derived. Thus, in the adult, only oocytes in very mature follicles survive a dose of several hundred R, and such oocytes make up only a small proportion of the pool present before irradiation (3, 36). In the irradiated newborn, on the other hand, no mature or even intermediate follicles are yet present, and mutations derive from developmental stages that are not sampled at all in the irradiated adult.

Two other mutation-frequency comparisons can be made between newborn and adult females. Each of these involves still different portions of the original pool of oocytes. One of these comparisons is with the offspring conceived in the first seven weeks following a 50-R dose to adult females. All of the oocytes that would survive a 400-R dose, and, in addition, some that were slightly less mature when irradiated, presumably constitute the population of oocytes that are ovulated in the first seven weeks following a 50-R irradiation. The second comparison is with the offspring conceived more than seven weeks after 50-R irradiation. Presumably most of the oocytes involved in this comparison would have been in early or intermediate stages of follicular development when they were irradiated.

Among the 180,472 offspring conceived in the first seven weeks after a dose of 50 R, 13 specific-locus mutations were found (55). This is a mutation frequency of 20.6×10^{-8} mutations/locus/R, with 95 percent confidence limits of 10.6×10^{-8} and 33.8×10^{-8}. For the newborn females irradiated with 300 R, the mutation frequency was 10.0×10^{-8}

mutations/locus/R with 95 percent confidence limits of 2.7×10^{-8} and 27.1×10^{-8}. During the seven-week interval, the mutation frequency is not statistically significantly higher in the adult female than in the newborn female, regardless of which of the two estimates of the spontaneous frequency in the adult (55) is used in the correction for spontaneous mutations.

No specific-locus mutations were found after the seven-week interval. This zero mutation frequency, which has an upper 95 percent confidence limit of 12.0×10^{-8} mutations/locus/R, is not statistically significantly lower than the mutation frequency per R found in the newborn females.

Of the three comparisons between the mutation frequency in newborn females and those in different populations of oocytes in the adult female, the point estimate in the newborns is lower for the first two, being only 1/5 and 1/2 that of the adult, and higher for the last comparison. As was mentioned before, neither one of the last two comparisons differs statistically significantly from the adult.

In a discussion of mutagenesis in fetal and newborn female mice, Carter's (11) chronic exposure experiment should be mentioned. (C3H X 101)F_1 females received 300 roentgens of chronic and fractionated gamma irradiation in the interval from 12 1/2 to 17 1/2 days of fetal life. For six consecutive nights, starting at 12 1/2 days of fetal life, they were exposed to 50 R of Co^{60} gamma irradiation delivered continuously over a period of 16 hours each night.

Four specific-locus mutations were found in 18,753 offspring.

This is a mutation frequency of 10.2 X 10^{-8} mutations/locus/R. Its 95 percent confidence limits are 3.5 X 10^{-8} and 24.4 X 10^{-8}. The point estimate of this rate is slightly, but not significantly, higher than the rate Carter et al. (12) found for 17 1/2-day fetal females exposed to 200 R, and it is almost identical to the rate found in the present experiment. In view of the fact that Russell has clearly demonstrated a striking dose-rate effect in adult females (50), it is somewhat surprising that the rate found by Carter for chronically-exposed fetal germ-cell stages is not lower than that found following acute irradiation; however, his sampling error could easily have obscured a dose-rate effect.

No quantitative histological study has been made of the (101 X C3Hf)F_1 female mice to determine the exact proportions of the different types of oocytes present on the day of birth, and the survival of these various types following 300 R of X irradiation. Such a study will be requisite to a full understanding of the present genetic and fertility experiments. On the basis of known radiosensitivities of the different germ-cell stages that are thought to be present on the day of birth, it can be concluded that the present experiment has probably provided the mutation frequencies for pachytene and perhaps diplotene oocytes. It seems very likely that no irradiated dictyate oocytes survived.

Extensive killing of oocytes is probably the reason for the decreased number of litters. Since the female mouse cannot replenish losses from the oocyte pool established before her birth, sterility ensues when the supply of oocytes surviving irradiation is exhausted. Oakberg (32) has shown that severe depletion of the number of oocytes

can cause both a decrease in the number of litters and in the average litter size. Dominant lethals cannot, however, be ruled out as a possible cause of the decreased litter size (43).

It is a puzzling coincidence that, in both the male and the female, the first one or two days after birth are times of marked changes in radiosensitivity to killing of the germ cells by X irradiation. In the male the change results in decreased sensitivity, whereas in the female it results in increased sensitivity.

As was discussed earlier, the primordial germ cells in both sexes are cytologically similar until those in the female enter meiosis. Searle and Phillips (63) have exposed embryos of both sexes to low-dose-rate fast neutrons for one week before day 12 of embryonic life. (Times of irradiation ranged from 0.5 to 7.5 days post-conception to 4 to 11 days post-conception.) Specific-locus mutation rates were determined for the two sexes. They were quite similar, therefore suggesting that the mutation frequencies of the two sexes are not intrinsically different. When they diverge cytologically, their mutational responses change. Because both the radiation treatment and the germ-cell stages present were different, no attempt will be made to relate these neutron-induced mutation frequencies to the mutation rates discussed in this dissertation.

CHAPTER V

IMPLICATIONS OF THESE STUDIES UPON AN ASSESSMENT OF THE GENETIC HAZARDS OF ATOMIC RADIATION TO MAN

The development and maturation of the testis in man, in sharp contrast to that of the mouse, requires more than a decade. In man, the primordial germ cells enter the developing gonad during the fifth week of gestation. During the seventh week after conception it becomes evident histologically whether the gonad will develop into a testis or ovary (18). By the sixth month of fetal life, the cords of epithelial cells in the fetal testis develop into seminiferous tubules (18). These tubules do not form lumens, though, for many years. The early tubules contain gonocytes and small epithelial (future Sertoli) cells (26).

Histological study by Charny et al. (13) has shown that at birth the tubules are still small (average diameter about 60 microns) and filled with several layers of undifferentiated cells. As early as 3 1/2 weeks after birth, scattered cells are seen that have the appearance of spermatogonia (14). Histologically, the testis looks the same for the first four years (13). Between ages five and seven there is slight lumen formation; cells that appear to be spermatogonia are still found only occasionally. By eight and nine years of age there is a slight increase in the number of those cells which appear to be spermatogonia.

At ten years, the seminiferous tubules are somewhat larger (average diameter, 72 microns), and there are many spermatogonia. There is also scattered evidence of mitotic activity. By age eleven the testis

looks essentially the same as at age ten except that mitotic activity is now very pronounced, and primary and secondary spermatocytes and a few spermatids are seen. At twelve years of age, the tubules average 85 microns in diameter and more spermatids are present.

The age at puberty varied from eleven to fifteen in the boys studied by Charny et al. (13). At puberty, the seminiferous tubules are large (100 to 150 microns in diameter; the average diameter in the adult is 150 to 180 microns) and there is production of a few spermatozoa. Charny (13) sums up his studies of the testis with the conclusion that testis maturation begins at about age ten and progresses over a period of two to five years until it is complete.

This dissertation has presented the results of specific-locus mutation-rate experiments designed to estimate the mutational response of the mouse testis at ten different times during postnatal development. These ten different ages presumably are comparable to the state of development of the human testis at times ranging from about five months' gestation (12) to puberty. It seems quite possible that the mouse testis on the day of birth may be comparable to the relatively undifferentiated human testis which persists for almost ten years. Most, or all, of the other nine ages in the mouse which were screened for the presence of a high mutation frequency seem similar to the human testis during the two to five years of maturation that precede puberty.

Previous estimates of the genetic hazard of atomic radiation to man have been strongly influenced by the estimates of specific-locus mutation rates in the mouse (39, 55). In the past, such estimates had

to assume that the germ cells in the immature testis were similar in mutational response to those in the adult testis. One of the main goals of the present research has been to check the validity of that assumption. Because the human testis is immature for a sizeable proportion of the time before most procreation occurs, the finding of a markedly higher mutational response would have made it necessary to reassess the genetic hazard to man.

No evidence has been found of any marked increase in mutational response during any stage of development in the immature mouse testis. On the contrary, the testis of the newborn mouse has a statistically significantly lower mutation frequency than that of the adult, and the average mutation rate of the other nine ages combined is almost identical to that of the adult.

When the standards for maximum permissible radiation exposure of the population were set, the estimate of genetic damage in man was based largely on results in adult mice at high dose rates and large doses (55). As Russell has discussed (55), a much more reliable and important use of specific-locus mutation-rate data in the mouse has been the estimation of relative risks under different conditions such as dose rate, dose, sex, cell-stage, age at irradiation, and interval between irradiation and fertilization. Russell has discussed many of these results and their bearing on the estimate of hazard to man in a recent publication (55).

The present experiments have provided considerable information about relative risks in the immature mouse. Although some important

unanswered questions remain about X-radiation mutagenesis in the immature testis, it is reassuring that all evidence that has been obtained in this project supports the validity of the assumption that irradiation of the immature testis presents no greater hazard to man that irradiation of the adult testis.

The results from the experiment on newborn females raise some important questions. The full significance of these is not known at the moment because of the uncertainty about what germ cells in the human ovary, if any, are comparable to those in the newborn female mouse.

In the human female, variable numbers of primordial germ cells in the ovary enter prophase of the first meiotic division during the second month of intrauterine life. They pass through the leptotene, zygotene, and pachytene stages of meiosis and then enter the diplotene stage which, in contrast to the dictyate stage found in the mouse and rat, is their resting stage. Shortly after birth, all oocytes in the human female are in the diplotene stage (3).

Baker (2) has shown that diplotene oocytes in primordial follicles of the human contain lampbrush chromosomes which are similar in form to those occurring in lower vertebrates. The lampbrush loops are condensed and thought to be surrounded by a dense sheath of ribonucleoprotein. On the other hand, the dictyate oocytes in primordial follicles of the mouse and rat contain only irregular masses of heterochromatin, which are resolved with the electron microscope as a reticulum of fine threads. These are thought to have only a thin sheath of ribonucleoprotein.

Whereas mouse oocytes in primordial follicles are very radiosensitive to killing, such oocytes in the human are extremely radioresistant.

With this and other supporting evidence, Baker (2) has presented the hypothesis that oocytes in which lampbrush chromosomes are condensed and surrounded by a dense sheath of ribonucleoprotein are likely to be resistant to radiation-induced cell death, and conversely, the possession of dictyate chromosomes surrounded by a thin sheath of ribonucleoprotein causes such cells to be radiosensitive. His finding that mouse oocytes in advanced stages of follicular growth exhibit both a reversal to diplotene chromosome structure and high radioresistance to killing supports his hypothesis.

By means of isotope incorporation experiments utilizing tritiated uridine, Baker (2) found that chromosomes in pachytene and diplotene oocytes of the newborn rat also have a dense sheath of ribonucleoprotein similar to that found in the diplotene oocytes of monkey primordial follicles. (The monkey and human are thought to be similar in this regard.)

The vast majority of oocytes in adult mammals are in primordial follicles (2), but Baker's work (2) suggests that oocytes in such follicles of mouse and man may not be comparable. Whereas oocytes in primordial follicles in the adult mouse are in diffuse diplotene (the dictyate stage), such oocytes in the adult human are in compact diplotene. The possibility therefore arises that of all the oocytes of the mouse, some of those present in the newborn (namely those which have a condensed chromosome structure and survive 300 R) are most comparable to the majority of oocytes in the adult human. However, it could also be argued that oocytes in the mature follicles of adult female mice have returned to a chromosome structure similar to that found in human oocytes in primordial

follicles. This illustrates the difficulty of assessing the significance that should be given to the mutation-rate data from the newborn female mouse. In addition, it must be emphasized that a similarity in chromosome structure may not mean that there are similarities in mutational response.

Because of the possibility that the results obtained for the newborn female mouse may represent the mouse data most relevant to an assessment of genetic damage in the adult human female, it is important to consider the implications of the present results to such an assessment. Unfortunately, this was a rather small experiment and, along with that of Carter et al. (12), can only be considered as a beginning in the acquisition of a meaningful body of knowledge.

Three comparisons were made between the X-ray-induced specific-locus mutation frequency in newborn females and mutation rates in three different populations of oocytes in the adult female. The point estimate was lower in the newborn females for two of the comparisons, being only 1/5 and 1/2 that of the adult, and it was higher in the third comparison. Of these comparisons, only that showing a five-fold lower rate differed statistically significantly from the adult. Although the mutation frequency in the newborn female is not statistically significantly different from that found for two populations of oocytes in the adult, this frequency, 10.0×10^{-8} mutations/locus/R, is low in comparison to the mutation rate for spermatogonia of the adult male, which is 29.1×10^{-8} mutations/locus/R.

Russell has demonstrated the extreme efficiency of repair in the adult mouse (55). Should the oocytes in the newborn female have a repair

system comparable to that of adult oocytes, their mutation frequency at low dose rates or after a low total dose would be exceedingly low. The effect of a lower dose rate or lower total dose remains to be tested in the newborn female. However, it should be emphasized that even an acute dose rate and a moderate total dose give a low mutation frequency relative to the adult male.

There is only one feature of the present data that could indicate a higher mutation frequency than expected from the overall results, namely the finding that all three mutations occurred within a narrow interval of time after irradiation. This suggests that some precise developmental subdivision of pachytene or diplotene might have a high mutation frequency while the mutation rate of the great majority of the oocytes surviving irradiation at birth might be very low. If this suggestion should be confirmed, it would imply that there is virtually no mutational response in the oocytes of the adult human female, unless, by an outside chance, the responsive stage in the mouse would be exactly comparable to the resting stage of the primordial oocytes of the human female. If so, the present results would be misleading. It should be emphasized, though, that these results certainly give no basis for alarm because only by a series of hypothetical coincidences could this imply a greater genetic hazard for humans, and probably even this effect would be negligible, should repair occur as efficiently as expected. However, it is important to be aware of the possibility that there might be a germ-cell stage with a high mutation frequency.

The finding of these new complexities reemphasizes the fact that

more must be known about mutagenesis in immature mammals before it will be possible to extrapolate to the human with confidence.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Austin, C. R., and E. C. Amoroso, The mammalian egg, Endeavor, 18 (1959) 130-144.

2. Baker, T. G., Comparative aspects of the effects of radiation during oogenesis, Mutation Res., 11 (1971) 9-22.

3. Baker, T. G., Radiosensitivity of mammalian oocytes with particular reference to the human female, American Journal of Obstetrics and Gynecology, 110 (1971) 746-761.

4. Beaumont, H. M., Changes in the radiosensitivity of the testis during foetal development, Int. J. Rad. Biol., 2 (1960) 247-256.

5. Beaumont, H. M., Radiosensitivity of oogonia and oocytes in the foetal rat, Int. J. Rad. Biol., 3 (1961) 59-72.

6. Beaumont, H. M., The radiosensitivity of germ-cells at various stages of ovarian development, Int. J. Rad. Biol., 4 (1962) 581-590.

7. Beaumont, H. M., and A. M. Mandl, A quantitative study of primordial germ cells in the male rat, J. Embryol. Exp. Morph., 11 (1963) 715-740.

8. Borum, K., Oogenesis in the mouse, A study of the meiotic prophase, Exp. Cell Res., 24 (1961) 495-507.

9. Brambell, F. W. R., The development and morphology of the gonads of the mouse. Part I. The morphogenesis of the indifferent gonads and of the ovary, Proc. R. Soc. Ser. B., 101 (1927) 391-409.

10. Carter, T. C., Radiation-induced gene mutation in adult female and foetal male mice, Brit. J. Radiol., 31 (1958) 407-411.

11. Carter, T. C., Mutation induced in germ cells of the foetal female mouse, Genet. Res., Camb., 1 (1960) 59-61.

12. Carter, T. C., M. F. Lyon and R. J. S. Phillips, The genetic sensitivity to X-rays of mouse foetal gonads, Genet. Res., Camb., 1 (1960) 351-355.

13. Charny, C. W., A. S. Conston and D. R. Meranze, Development of the testis, Fertility and Sterility, 3 (1952) 461-479.

14. Charny, C. W., A. S. Conston and D. R. Meranze, Testicular developmental histology, Ann. N.Y. Acad. Sci., 55 (1952) 597-608.

15. Chiquoine, A. D., The identification, origin, and migration of the primordial germ cells in the mouse embryo, Anat. Rec., 118 (1954) 135-146.

16. Fazylov, U. T., and Pomerantseva, M. D., The mutagenic effect of various types of irradiation on the germ cells of male mice, VI. Genetic radiosensitivity of gonocytes of embryos and newly born mice, Genetika, Mosk., 7 (1971) 68-75 [Russ. with Eng. summ.].

17. Gowen, J. W., Lengthening of life span in mice in relation to two deleterious agents, in The Biology of Aging, AIBS symposium, No. 6, 1960, pp. 188-191.

18. Ham, A. W., Histology, edition 5, J. B. Lippincott Company, Philadelphia, 1965.

19. Huckins, C., The spermatogonial stem cell population in adult rats, I. Their morphology, proliferation and maturation, Anat. Rec., 169 (1971) 533-558.

20. Huckins, C., and Y. Clermont, Evolution of gonocytes in the rat testis during late embryonic and early post-natal life, Extrait des Archives d'Anatomie, d'Histologie et d'Embryologie normales et expérimentales, 51 (1968) 343-354.

21. Hughes, G., Radiosensitivity of male germ-cells in neonatal rats, Int. J. Rad. Biol., 4 (1962) 511-519.

22. Kimball, R. F., The relation of repair to differential radiosensitivity in the production of mutations in Paramecium, in F. H. Sobels (Ed.), Repair from Genetic Radiation Damage, Pergamon Press, Oxford, 1963, pp. 167-178.

23. Kimball, R. F., The relation between repair of radiation damage and mutation induction, Photochemistry and Photobiology, 8 (1968) 515-520.

24. Léonard, A., Radiation-induced translocations in spermatogonia of mice, Mutation Res., 11 (1971) 71-88.

25. Léonard, A., F. Imbaud and J. R. Maisin, Testicular injury in rats irradiated during infancy, Brit. J. Radiol., 37 (1964) 764-768.

26. Maximow, A. A., and W. Bloom, A Textbook of Histology, edition 7, W. B. Saunders Company, Philadelphia, 1957.

27. Mintz, B., Embryological phases of mammalian gametogenesis, J. Cell. Comp. Physiol., 56, Suppl. 1, (1960) 31-44.

28. Oakberg, E. F., A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal, Am. J. Anat., 99 (1956) 391-414.

29. Oakberg, E. F., Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium, Am. J. Anat., 99 (1956) 507-516.

30. Oakberg, E. F., Duration of spermatogenesis in the mouse, Nature, 180 (1957) 1137-1139.

31. Oakberg, E. F., Gamma-ray sensitivity of oocytes of young mice, Anat. Rec., 137 (1960) 385-386.

32. Oakberg, E. F., Effect of 25 R of X-rays at 10 days of age on oocyte numbers and fertility of female mice, in P. J. Lindop and G. A. Sacher (Eds.), Radiation and Ageing, Taylor and Francis, London, 1966, pp. 293-306.

33. Oakberg, E. F., Radiation response of the testis, in Excerpta Medica International Congress Series No. 184, Progress in Endocrinology, (Proc. of the 3rd Intern. Congr. of Endocrinology), 1968, pp. 1070-1076.

34. Oakberg, E. F., A new concept of spermatogonial stem-cell renewal in the mouse and its relationship to genetic effects, Mutation Res., 11 (1971) 1-7.

35. Oakberg, E. F., Spermatogonial stem-cell renewal in the mouse, Anat. Rec., 169 (1971) 515-531.

36. Oakberg, E. F., and E. Clark, Species comparisons of radiation response of the gonads, in W. D. Carlson and F. X. Gassner (Eds.), Effects of Ionizing Radiation on the Reproductive System, Pergamon Press, Oxford, 1963, pp. 11-24.

37. Oakberg, E. F., and R. L. DiMinno, X-ray sensitivity of primary spermatocytes of the mouse, Int. J. of Rad. Biol., 2 (1960) 196-209.

38. Peters, H., Radiation sensitivity of oöcytes at different stages of development in the immature mouse, Rad. Res., 15 (1961) 582-593.

39. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation, General Assembly, Official Records, Session 21, Suppl. 14 (A/6314), U.N., 1966.

40. Rugh, R., and S. Jackson, Effect of fetal X-irradiation upon the subsequent fertility of the offspring, J. Exp. Zool., 138 (1958) 209-221.

41. Russell, L. B., Genetic and functional mosaicism in the mouse, in M. Locke (Ed.), Role of Chromosomes in Development, Academic Press, New York, 1964, pp. 153-181.

42. Russell, L. B., Definition of functional units in a small chromosomal segment of the mouse and its use in interpreting the nature of radiation-induced mutations, Mutation Res., 11 (1971) 107-123.

43. Russell, L. B., and W. L. Russell, The sensitivity of different stages in oogenesis in the radiation induction of dominant lethals and other changes in the mouse, in J. S. Mitchell, B. E. Holmes and C. L. Smith (Eds.), Progress in Radiobiology, Oliver Boyd Ltd., Edinburgh, 1956, pp. 187-192.

44. Russell, L. B., and W. L. Russell, Genetic analysis of induced deletions and of spontaneous nondisjunction involving chromosome 2 of the mouse, J. Cell. Comp. Physiol., 56, Suppl. 1 (1960) 169-188.

45. Russell, L. B., S. K. Badgett and C. L. Saylors, Comparison of the effects of acute, continuous, and fractionated irradiation during embryonic development, Int. J. Rad. Biol., Suppl. entitled: Immediate and Low Level Effects of Ionizing Radiations (1960) 343-359.

46. Russell, L. B., K. F. Stelzner and W. L. Russell, Influence of dose rate on radiation effect on fertility of female mice, Proceedings of the Society for Experimental Biology and Medicine, 102 (1959) 471-479.

47. Russell, W. L., X-ray-induced mutations in mice, Cold Spring Harbor Symposia Quant. Biol., 16 (1951) 327-336.

48. Russell, W. L., Genetic effects of radiation in mammals, in A. Hollaender (Ed.), Radiation Biology, McGraw-Hill, New York, 1954, pp. 825-859.

49. Russell, W. L., Lack of linearity between mutation rate and dose for X-ray-induced mutations in mice, Genetics, 41 (1956) 658-659.

50. Russell, W. L., The effect of radiation dose rate and fractionation on mutation in mice, in F. H. Sobels (Ed.), Repair from Genetic Radiation Damage, Pergamon Press, Oxford, 1963, pp. 205-217.

51. Russell, W. L., Evidence from mice concerning the nature of the mutation process, in S. J. Geerts (Ed.), Genetics Today (Proc. 11th Intern. Congr. Genet., 1963), Vol. 2, Pergamon Press, Oxford, 1964, pp. 257-264.

52. Russell, W. L., The nature of the dose-rate effect of radiation on mutation in mice, Japan. J. Genetics, 40, Suppl. (1965) 128-140.

53. Russell, W. L., Studies in mammalian radiation genetics, Nucleonics, 23 (1965) 53-56, 62.

54. Russell, W. L., Repair mechanisms in radiation mutation induction in the mouse, Brookhaven Symp. Biol., 20 (1967) 179-189.

55. Russell, W. L., The genetic effects of radiation, in Peaceful Uses of Atomic Energy, IAEC, 1972, pp. 487-500.

56. Russell, W. L., and E. F. Oakberg, The cellular basis and aetiology of the late effects of irradiation on fertility in female mice, in R. J. C. Harris (Ed.), Cellular Basis and Aetiology of Late Somatic Effects of Ionizing Radiation, Academic Press, 1963, pp. 229-232.

57. Russell, W. L., and L. B. Russell, The genetic and phenotypic characteristics of radiation-induced mutations in mice, Radiat. Res., Suppl. 1 (1959) 296-305.

58. Russell, W. L., J. W. Bangham and J. S. Gower, Comparison between mutations induced in spermatogonial and postspermatogonial stages in the mouse, Proc. 10th Intern. Congr. Genetics, 2 (1958) 245-246.

59. Russell, W. L., L. B. Russell and E. M. Kelly, Radiation dose rate and mutation frequency, Science, 128 (1958) 1546-1550.

60. Russell, W. L., L. B. Russell and E. F. Oakberg, Radiation genetics of mammals, in W. D. Claus (Ed.), Radiation Biology and Medicine, Addison-Wesley Publishing Co., Inc., Reading, Mass., 1958, pp. 189-205.

61. Russell, W. L., L. B. Russell, M. H. Steele and E. L. Phipps, Extreme sensitivity of an immature stage of the mouse ovary to sterilization, Science, 129 (1959) 1228.

62. Searle, A. G., Comparative Genetics of Coat Colour in Mammals, Logos Press, London, 1968.

63. Searle, A. G., and R. J. S. Phillips, The mutagenic effectiveness of fast neutrons in male and female mice, Mutation Res., 11 (1971) 97-105.

64. Shaver, S., X-irradiation injury and repair in the germinal epithelium of male rats, II. Injury and repair in immature rats, Am. J. Anat., 92 (1953) 433-449.

65. Steel, R. G. D., and J. H. Torie, Principles and Procedures of Statistics, McGraw-Hill, New York, 1960, p. 109.

66. Widmaier, R., Über die postnatale Hodenentwicklung und Keimzellreifung bei der Maus, Zeitschrift für mikroskopisch-anatomische Forschung, 70 (1963) 215-241.

VITA

Paul Bruce Selby was born in Owatonna, Minnesota, on December 5, 1945. His parents, Mr. and Mrs. F. M. Selby of Medford, Minnesota, were teachers in various Minnesota school systems for many years. His father served as both principal and superintendent during most of his 53 years of teaching. Paul attended Medford Elementary School, and was graduated valedictorian of his class from Medford High School in 1963.

The following September Paul entered Westmar College in Le Mars, Iowa, where he became quite interested in genetics through course work and association with Dr. James Divelbiss. During the summer before his senior year at Westmar, Paul's career plans were greatly influenced by his being a participant in Oak Ridge Associated Universities' Summer Student Trainee Program. His sponsor was Dr. L. B. Russell, of the Oak Ridge Biology Division. In June of 1967, he received his Bachelor of Arts degree, with a major in Biology and a minor in Chemistry, from Westmar, from which he was graduated summa cum laude.

In the fall of 1967 he enrolled as a graduate student in the first class of The University of Tennessee--Oak Ridge Graduate School of Biomedical Sciences. His doctoral research was done under the direction of Dr. W. L. Russell. He received the Doctor of Philosophy degree with a major in Biomedical Sciences from The University of Tennessee in August, 1972.

He is married to the former Patricia Jean Ross of Oak Ridge, Tennessee.