UCU is incorporated at a lower level, and tyr-UAU is sometimes incorporated at a still lower level. Further, von Ehrenstein, by using isolated sRNA's in an *in vitro* protein-synthesizing system has shown val, tyr, and cys sRNA's not to respond to poly U; whereas ilu, ser, and the *three* leu sRNA's which respond normally to poly UC, poly UA, and poly UG, respectively, *all* do respond to poly U under this suboptimal condition (von Ehrenstein, unpublished results). I have assumed also that CIII can be mistakenly read as UIII on the basis of the results of Nirenberg and coworkers[20] and Söll *et al.*,[14] showing that phe sRNA appears not to distinguish between the two phe codons UUU and UUC. Of course, this may not be an error at all, but as discussed above, may represent an actual recognition of two codons by one sRNA—which for the present purposes we can consider an "error," however.

[13] Davies, J., L. Gorini, and B. Davis, *J. Mol. Pharmacol.*, **1**, 93 (1965).

[14] Söll, D., E. Ohtsuka, D. S. Jones, R. Lohrmann, H. Hayatsu, S. Nishimura, and H. G. Khorana, these PROCEEDINGS, **54**, 1378 (1965); Nirenberg, M. W., P. Leder, M. Bernfield, R. Brimacombe, J. Trupin, F. Rottman, and C. O'Neal, these PROCEEDINGS, **53**, 1161 (1965).

[15] We shall confine our attention at present to the effect of *translation* errors, realizing, of course, that high rates of error could and probably did exist at the same time for the transcription and/or replication processes. The interrelationship between the latter type of error and translation errors will be discussed elsewhere.

[16] Fox, S., *Bioscience*, **14**, 13 (1964).

[17] Orgel, L. E., these PROCEEDINGS, **49**, 517 (1963).

[18] It must be appreciated what relatively profound effects (selective advantage) small increases in the translation efficiency could have. The relation of cause to effect here—cause being the decreased translation error frequency, effect being the "improved" cell function— is an "*n*th power" sort of phenomenon. The probability of making a perfect translation of a given mRNA is approximately $(1 - E)^n$, where E is the average probability of making an error in reading a codon, and n is the number of codons in the mRNA. Therefore, if E is initially rather large, small changes in it will have a drastic effect on the function $(1 - E)^n$. Thus, adjustments in codon assignments which produce only slight improvements in translation error frequency in these error-ridden cells could still have very profound effects in terms of improved cell function, in terms of selective advantage. The feedback between improved translation and fidelity of transcription and/or replication would also play a role here.

[19] The name "ur-enzymes" has been suggested by K. C. Atwood to describe enzymes such as duplicases, transcriptase, etc.—in general, the tape-reading systems—absolutely essential to any cell.

[20] Leder, P., and M. W. Nirenberg, these PROCEEDINGS, **52**, 1521 (1964); Bernfield, M. R., and M. W. Nirenberg, *Science*, **147**, 479 (1965).

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*EFFECT OF THE INTERVAL BETWEEN IRRADIATION AND CONCEPTION ON MUTATION FREQUENCY IN FEMALE MICE**

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Determinations of the effect of germ-cell stage on induced mutation frequency have been very limited in female mammals, owing to the fact that permanent sterility sets in quickly after exposure to the doses and dose rates of X and gamma radiation that are necessary for an adequate yield of mutations. Thus, accurate measurements of oöcyte mutation frequencies have been made only in mature and nearly mature follicle stages. The adult mouse contains no germ cells of developmental stages preceding the dictyate oöcyte, and, although this nuclear state persists

until just hours before ovulation, there are marked progressive changes in the surrounding follicle and some changes within the dictyate oöcyte itself.[1] It has now been possible to study mutation induction in some of the earlier follicle stages by the use of neutrons.

The work reported here showed that neutron irradiation has an advantage over X and gamma irradiation in that the breeding period over which significant mutation-rate data can be collected is much longer. There are two reasons for this. First, with the acute doses used, the relative biological effectiveness (RBE) for neutron compared to X-ray effects on fertility in females is only of the order of one, whereas the RBE for genetic effects has turned out to be much higher. Thus it has been possible to give an acute neutron dose which will allow fertility to persist for many weeks and which, at the same time, will produce enough mutations for a reliable measure of mutation frequency. Second, the dose-rate effect is smaller than that for gamma rays. With gamma irradiation, dose rates which are low enough to give extended fertility yield a mutation frequency which, even at high total doses, is so low that accurate measurement of mutation frequency would require tremendous numbers of mice. With fission neutrons, on the other hand, although there is some dose-rate effect on mutation in oöcytes, as the data presented here will show, this is not as great as that with gamma rays. Consequently, large numbers of mutations are still observed in the early interval after irradiation even when the dose-rate is lowered to the level at which it will give extended fertility compared with acute irradiation.

The use of neutrons for this purpose has now shown that the interval between irradiation and conception has a large effect on the mutation frequency observed in the offspring of irradiated female mice. This finding has been briefly mentioned before.[2, 3] The more extensive data now obtained are presented here.

Methods.—The animals were exposed at the Oak Ridge National Laboratory Health Physics Research Reactor[4] (HPRR), which, through the excellent cooperation of the Health Physics Division, we were able to use for mouse mutation experiments as soon as construction and testing were completed. The HPRR is a small fast reactor similar to the Godiva II reactor at Los Alamos[5] and the Sandia Pulsed Reactor Facility.[6] The reactor core, which is suspended beneath the reactor framework, can be positioned remotely in the center of a large circular rack of mouse cages. The facility is housed in a low scatter building. The fast neutron to gamma radiation tissue dose ratio is approximately 7 (based on more accurate measurements than those used for an earlier estimate[4]); the thermal neutron dose to mice is less than 1% of that due to fast neutrons for the irradiation geometry used, and the neutron spectrum approximates a fission spectrum.

Seven repetitions of the experiment were made at each of two dose rates. In the first of this series of experiments, 960 female mice were exposed at each dose rate. In the second, the number was 900, and in each subsequent repetition the number of animals exposed was 480 at each dose rate. Age of females at time of irradiation ranged from 7 to 26 weeks, with a median age of 13 weeks and with more than 70% of the females falling in the 9–16-week age group. During exposure they were kept in one side of our standard mouse cages constructed of $^1/_8$ inch polystyrene, and the distance from the center of this side of the cage to the reactor core was 2 meters. In the first six repetitions of the experiment the animals were caged, during exposure, in groups of six. The wide distribution of length of fertile period at the high dose rate indicated that some animals might be receiving lower doses, perhaps from shielding by other animals in the cage. In order to test this, in the seventh repetition one fifth of the animals exposed were caged singly.

The neutron dosimetry, for which we are indebted to D. R. Johnson of the Health Physics Division, was based on measurements taken with a polyethylene-lined, cyclopropane-filled proportional chamber[7] and normalized for each exposure to the activation of a sulfur pellet located on the reactor. The proportional chamber was calibrated with a 1-c Pu-Be source using a dose conver-

sion factor of 4.0×10^{-9} tissue rads per n/cm^2 (ref. 8). The output of this source was measured by the National Bureau of Standards and found to be 1.855×10^6 neutrons per second $\pm$ 2.3%. After calibration, the chamber was placed at the experimental position near the reactor and measurements were made of the first collision tissue dose rate with the reactor operating at a power of approximately 0.2 watt. The resulting dose rate was 3.0 millirads per unit activation of the sulfur pellet. This value was used to calculate first collision neutron doses for each of the animal exposures.

The doses given in Table 1 were those obtained with the above dosimetry. Dr. H. H. Rossi of Columbia University kindly offered to provide an independent method of measurement, and Mr. L. J. Goodman of his group measured the tissue Kerma rate in free air at various reactor power levels. The neutron dose estimates obtained by the Columbia University group run about 15% higher than those obtained by the Oak Ridge National Laboratory group.[9] At the same time Mr. Goodman was able, with the small dosimeters designed by Dr. Rossi's group, to provide information on the amount of neutron dose reduction that occurs as a result of shielding when mouse phantoms are placed in front of the dosimeter.[10] The effects were large enough to account for the variability in fertility response described earlier. Further biological confirmation of some shielding effect comes from the comparison between animals caged singly and animals caged in groups in the seventh repetition of the experiment. In the low dose-rate portion of the experiment 52% of the offspring from animals caged singly came from conceptions occurring more than seven weeks after irradiation, whereas the animals grouped six to a cage had 64% of their offspring in this period. In the high dose-rate part of the experiment, the corresponding percentages were 30 and 49%, respectively. The animals caged singly apparently received somewhat higher doses which shortened their fertile periods.

Owing to these complications, it is not at present possible to determine accurately what the average dose to the ovaries was in the present experiments, but, from the various dosimetries and the biological evidence for shielding effects, we conclude that the true mean doses may have been lower than those given in Table 1, but were probably not higher. Uncertainty about the doses does not affect the main conclusion of this paper.

Mutation frequency was determined by our standard specific locus method.[11]

Results and Discussion.—The results are shown in Table 1. Since there is no evidence of any variability between repetitions of the experiment, the data have been pooled. In the offspring born from conceptions occurring up to seven weeks after irradiation, there is strong evidence of some dose-rate effect on mutation in oöcytes, although not as great an effect as that with gamma rays.[12] There is also a dose-rate effect on fertility, as is shown by the larger total number of offspring obtained at the lower dose rate.

The most remarkable feature of the results, however, is the absence of any mutations in 120,483 offspring obtained in the later time interval after irradiation, and the contrast between this and the 59 mutations obtained in the smaller total of 89,301 offspring from the earlier time interval.

The possibility that litters in the later time interval after irradiation came predominantly from animals that received lower doses as a result of shielding by other

TABLE 1

FREQUENCY OF SPECIFIC LOCUS MUTATIONS AT TWO INTERVALS AFTER IRRADIATION OF FEMALE MICE WITH FISSION NEUTRONS

		Interval between Irradiation and Conception			
		Up to 7 Weeks		More Than 7 Weeks	
Dose (rads)*	Dose rate (rads/min)	No. of offspring	No. of mutations at 7 loci	No. of offspring	No. of mutations at 7 loci
63	0.17	46,301	22	80,391	0
63	79	43,000	37	40,092	0

* Dose to gonads estimated to be somewhat lower (see text). Includes a gamma component equal to approximately one seventh of the neutron component.

animals in the cage, and that this accounted for the absence of mutations, is clearly excluded in the low dose-rate experiments. Here, in the offspring from females that had mutant young, 64.0 per cent came from conceptions in the later period, and in offspring from females that did not have mutant young, 63.6 per cent came from the later period. Thus, as judged by their having almost exactly the same productivity in the later period, it would appear that the average doses received by mutant-producing and non-mutant-producing females were equal. In the $6^1/_4$ hr of exposure the movement of the animals presumably tended to equalize any shielding effects on one another. Thus there is no bias in taking the mutation rates in the two periods at face value. The same criterion does indicate some bias in the high dose-rate experiments where the exposure time was only 45 sec. The corresponding percentages of offspring that fell in the later period for mutant-producing and non-mutant-producing females were 37.3 and 48.6 per cent, respectively. However, the bias is not large and the only effect of allowing for it is to reduce slightly the effective number on which the standard error of the zero mutation frequency in the later period is based. In short, it is clear that the marked difference in mutation frequencies in the early and later periods is due to some other factor.

What clues are there as to the nature of this factor? Two litters are usually produced from conceptions in the earlier time interval. The low mutation frequency in the later period comes, therefore, from oöcytes that were in immature follicle stages. It is of great importance to find out whether the marked difference in mutation frequencies is due to a low mutational sensitivity of oöcytes in early follicle stages, to an efficient repair mechanism in these stages, or to cell selection. If it is due to selection, the mechanism could conceivably be the survival of only those oöcytes that escaped having a proton track pass through their genetic material. In this case, the effect would not be seen with X and gamma rays. X-ray data are now being obtained. These and gamma-ray data already reported[3] are not yet adequate to settle the above point. Both experiments show very low mutation frequencies in later matings. As they stand, these frequencies are lower than those in early matings, but, with the small total number of mutations obtained, the differences are not significant. Because of the low doses or dose rates that have to be used to avoid early sterility, it is an enormous task to obtain an adequate number of mutations.

The finding that the interval between irradiation of the female and conception has a marked effect on mutation frequency is in complete contrast with our results on spermatogonia in males. Of course, one would not necessarily expect results in oöcytes and spermatogonia to be similar. The reason for comparing them is that these are the two cell stages that are most important in genetic hazards. In the extensive data on spermatogonia there is no evidence of any significant change in mutation frequency with time. This is true even up to the end of the reproductive life of the irradiated males. In contrast, the mutation frequency in successive litters from the irradiated females in the current experiment rapidly plunges to an observed value of zero. Even the upper 99 per cent confidence limit of this zero frequency is below the spontaneous mutation rate in male mice which is 28 specific-locus mutations in 531,500 offspring (a reliable value for the spontaneous rate in females is not yet available, although it is lower than that in males).

It may be noted that this finding of a dramatic change in mutation rate with time

does not invalidate our earlier conclusion concerning the existence of a large dose-rate effect of radiation on mutation in females. As was emphasized from the beginning, that conclusion is valid even for comparisons restricted to matings made shortly after irradiation.[13, 14]

Whatever the nature of the factor is that causes the sudden drop in mutation frequency with increasing time between irradiation of the female and conception, it is potentially of great importance in the estimation of genetic hazards of radiation. We cannot yet conclude that the results will apply to the human female, because the oöcyte stages involved in the two species may not be comparable in their responses. However, there is certainly a possibility of a similar effect. In any case it would seem desirable to re-evaluate some of the data collected on human radiation exposures. The mouse results suggest that in studies such as those made at Hiroshima and Nagasaki the likelihood of detecting genetic damage might be increased when distinction can be made between pregnancies occurring within a few months after maternal exposure and those occurring later.

Summary.—Irradiation of female mice with fission neutrons has shown that the interval between irradiation and conception has a striking effect on the mutation frequency seen in the offspring. In the first seven weeks after irradiation with approximately 63 rads, the mutation frequency is high. A total of 59 specific locus mutations was observed in 89,301 offspring conceived in that period. After that, no mutations were found in a total of 120,483 offspring. There is some evidence for a similar effect with gamma irradiation. It is not yet known whether the result is due to a low mutational sensitivity of oöcytes in immature follicle stages, to an efficient repair mechanism in these stages, or to cell selection. It is not safe to assume that the results will apply to the human female, but this is certainly a possibility that should be considered in the evaluation of human data and in the estimation of genetic hazards of radiation.

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*TRANSDUCTION OF ESCHERICHIA COLI GENETIC MATERIAL BY PHAGE P22 IN SALMONELLA TYPHIMURIUM × E. COLI HYBRIDS**

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Phage P22 can transfer *Escherichia coli* genetic material from hybrid *Salmonella typhimurium* × *E. coli* donors to *S. typhimurium* recipients, but as a rule little integration takes place for the heterologous *typhimurium-coli* loci.[1, 2] Abortive transductants are observed, however, which demonstrates that the phage is capable of picking up *E. coli* genetic material and injecting it into *S. typhimurium* cells. From such observations, Demerec and Ohta[1] concluded that while gross homology between chromosomes of the two species is good,[4] fine-point homology of the loci under consideration is poor.

In their transductions, Demerec and Ohta used recipients that were entirely *S. typhimurium* in genome. While these were suitable for testing transduction from *E. coli* or *S. typhimurium* donor loci to *S. typhimurium* recipients, they could not be used to test whether *E. coli* loci in hybrid recipients could also be transduced. To determine that, it would be necessary to have an auxotrophic mutation within the *E. coli* segment of the hybrid genome. Fortunately, it now has been found that in some hybrids the *E. coli* segment covers *thy*$^+$ locus, making it possible to obtain readily the appropriate *thy* hybrid via mutation and selection with nitrosoguanidine and aminopterin.[3] This mutant with *thy* in the *E. coli* segment of the hybrid genome is a suitable recipient for testing transduction of *E. coli* loci by phage P22.

Materials and Methods.—Donors were: wild-type *S. typhimurium* LT2; HybA57, which is a hybrid prototroph in which the *E. coli* segment carries the linked loci *lys*$^+$*thy*$^+$*argB*$^+$*cysC*$^+$ (Fig. 1; see Sanderson and Demerec[4] for complete map); and strain #KSU 2368, which is *thy345 argB69* double mutant of *S. typhimurium*, but which now carries the *E. coli* episome F15 containing *lys*$^+$*thy*$^+$*argB*$^+$.[3, 5] Recipient strains were: *S. typhimurium thy283; thy341 argB69; lys6 thy331; lys6; argB69; try85;* and *hybrid TC thy363*, which was initially HybA57 but which now has a thymine mutation.

Plates used for transduction (called 2X) contained a medium composed of 10.5 gm K_2HPO_4, 4.5 gm KH_2PO_4, 0.05 gm $MgSO_4$, 1.0 gm $(NH_4)_2SO_4$, 5 gm glucose, 2 gm nutrient broth powder, 1000 ml distilled water, and 15 gm agar. Appropriate supplements of lysine, thymine, or arginine were added as needed.