eligible for reversions. If the latter cells are the primary source of reversions then, as observed, no difference is expected in rate between the consecutive egg-laying intervals.

The question of whether the phenotypic reversion of recessive mutants to wild type or near wild type is in fact back mutation has been discussed *in extenso* elsewhere in connection with the problem of spontaneous back mutation.[1] The arguments militating for spontaneous back mutation as a specific change or alteration of a particular mutant gene apply equally well for the radiation-induced reversions of f^{3n} reported here and need no further comment. In short, these results amplify results based primarily on partial reversions in *Drosophila*[6] and of reversions in *Neurospora*[7] that X-rays and gamma rays can induce back mutations which, insofar as current criteria permit, do not differ from spontaneous back mutations.

Summary.—*Drosophila melanogaster* ♀ ♀ homozygous for one or the other forked pseudoalleles, f^{3n} and f^{1}, both known to back-mutate spontaneously, were irradiated with 4,000 r X-rays or gamma rays.

Back mutations of f^{3n} were significantly increased; those of f^{1} apparently not.

The back mutations are not associated with detectable chromosome alterations.

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DEPENDENCE OF MUTATION FREQUENCY ON RADIATION DOSE RATE IN FEMALE MICE

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In studies of specific locus mutation rates in mouse spermatogonia, a much lower mutation rate was obtained from chronic gamma than from acute X-irradiation.[1–4] Differences in both intensity (dose rate) and quality of radiation were involved in the comparison, but it was pointed out[2] that, although gamma rays frequently show a biological effectiveness somewhat lower than that of X-rays, the observed difference between the mutation rates appeared to be greater than could be attributed to this factor. This point of view has since been confirmed by three separate experiments conducted to test it.[4] In these experiments, no appreciable differences were found in the effectiveness of *acute* gamma rays (from Co^{60}), on the one hand, and acute X-rays, on the other, in inducing dominant lethals in spermatozoa, specific

locus mutations in spermatozoa and other postspermatogonial stages, or specific locus mutations in spermatogonia. It can be concluded that most of the difference between the *chronic* gamma and acute X-ray mutation rate results must be due to dose rate.

However, the finding of a dose-rate effect in spermatogonia did not necessarily prove that this effect was on the mutation process itself. It might, instead, have been due to secondary causes, such as cell selection resulting from spermatogonial killing or from other interference with the dynamics of the cycle of the seminiferous epithelium.[1, 2] To determine whether the mutation process itself could be affected by dose rate, it became important to carry out a dose-rate comparison in females where there seems little possibility that mutation-rate measurements can be interfered with by such secondary processes as elimination of sensitive cells. The adult ovary contains no oögonia, and the population of germ cells consists solely of oöcytes, which are non-mitotic and which appear to be remarkably uniform.[5] Furthermore, the continued fertility of females in chronic radiation experiments[6, 7] provides no evidence of extensive killing, selective or otherwise, of the oöcytes.

The first part of the dose-rate comparison for mutation rates induced in oöcytes has already been reported. The specific locus mutation rate in adult females exposed to continuous chronic Cs^{137} gamma irradiation at a dose rate of 86 r per week proved to be considerably lower than that produced by acute X-rays in spermatogonia.[6] Carter[8] also observed a low mutation rate in females exposed to interrupted chronic Co^{60} gamma irradiation of somewhat higher intensity than that used by us. The mutation rates from chronic irradiation of females, as far as they go, are not significantly different from the rates obtained from chronic gamma irradiation of spermatogonia.[4]

The completion of the dose-rate comparison for mutation rates induced in oöcytes, namely, the determination of mutation rates in females exposed to acute irradiation, promised a clear-cut answer of broad significance. For, if this rate proved to be no higher than that from chronic irradiation, then it would show that oöcytes are much less sensitive than spermatogonia. On the other hand, if the rate from acute irradiation of females turned out to be similar to that from acute irradiation of spermatogonia, namely, much higher than from chronic irradiation of both sexes, then it would indicate that there was a dose-rate effect directly on the mutation process.

It was, therefore, most important to obtain a reliable mutation rate from acutely irradiated females, even though this task is beset by the practical difficulty that the number of offspring that can be obtained before sterility ensues is very small. The experiment reported here was accordingly started to extend the limited data available from an earlier pilot experiment.[6] A brief statement of the new findings has already been presented.[4] In the present account, the results to date are described in more detail. Although the collection of data is still proceeding, the results are already sufficient to give a clear answer to the questions posed.

Materials and Method.—Young adult F_1 hybrid females from a cross of 101 × C3H strains were exposed to 400 r of whole-body X-radiation (250 Kvp; 15 ma.; inherent filtration 3 mm. Al; H.V.L. 0.4 mm. Cu; dose rate 92 r/min; animals in lucite containers on turntable rotating above masonite scatter block). The females were mated for 2 weeks, beginning on the day following irradiation, to

males of our T stock, which is homozygous for seven recessive marker genes. Young subsequently born were examined for mutations at the seven loci. Details of the experimental procedure have been described earlier.[9]

The data obtained in the present, 1958, experiment are additive with those of the 1950–1951 series,[6] since the stocks used were the same and the methods were identical except for one detail: in 1950–1951, when fertility effects of radiation were still being explored, females were kept with their mates indefinitely instead of being separated after 2 weeks, as in the 1958 series. However, the bulk of the young (97 per cent) were conceived within 2 weeks after irradiation, since most females were sterile by the time they could have had a second mating, and all were sterile 5 weeks after irradiation.

In 1950–1951, 379 females were irradiated, and 319 of these gave 1,729 young surviving to the age at which mutations are scored (i.e. approximately 3 weeks). In 1958, 6,130 young have, so far, been scored for mutations. These were offspring of 1,088 irradiated females. The results of the two acute X-ray experiments are presented in Table 1, together with data from chronic irradiation of females and control data. The data from the chronic gamma experiment have been brought up-to-date since the last report.[6]

TABLE 1

MUTATIONS AT SPECIFIC LOCI IN MOUSE OÖCYTES

RADIATION	DOSE (r)	INTENSITY (r/min)	NO. OF OFFSPRING	NO. OF MUTATIONS AT LOCUS							
				a	*b*	*c*	*d*	*p*	*s*	*se*	*d se*
—*	0	—	5,845	—	—	—	—	—	—	—	—
X*	400	96	1,729	—	—	—	—	—	1	—	—
X	400	92	6,130	—	2	—	—	1	2	—	1
—	0	—	40,918	—	—	—	—	—	—	—	—
Gamma (Cs^{137})	258	0.0085	26,468	—	1	—	—	—	—	—	1

* 1950–1951 experiment (Russell, Russell, Gower, and Maddus, these PROCEEDINGS, 44, 901–905, 1958).

Conclusions.—On combining the 1950–1951 and the 1958 series on acute irradiation of oöcytes, the mutation rate is 31.8×10^{-8}/r/locus (95 per cent confidence interval: $12.8–65.5 \times 10^{-8}$). The mutation rate calculated from the up-to-date tabulation of the chronic gamma data for females is 4.2×10^{-8}/r/locus (95 per cent confidence interval: $0.51–15.1 \times 10^{-8}$). A test of the significance of the difference between the mutation rates per roentgen in the two experiments gave $P = 0.006$ for a one-tailed test. Thus a significantly greater frequency of specific locus mutations is induced in oöcytes by acute than by chronic irradiation.

It might be argued that the oöcyte stages irradiated were not strictly comparable in the chronic and acute radiation experiments. Thus, in the former, offspring were obtained for almost a year, representing, therefore, oöcytes irradiated in follicles of a variety of stages of development, from youngest to most mature. In the acute irradiation experiment, on the other hand, all offspring resulted from oöcytes irradiated in relatively mature follicles. However, as already noted, the state of the oöcyte nucleus in the adult ovary appears quite uniform (dictyate) throughout the development of the follicle, except for the last few hours before ovulation (a stage avoided in the present experiment by not mating females until 1 day after irradiation). Development of the follicle seems to involve only growth in oöcyte size and multiplication of follicle cells. Moreover, a difference between

chronic and acute irradiation can be demonstrated even if one uses only that part of the chronic radiation data which pertains to oöcyte stages more comparable to those involved in the acute radiation experiment, i.e., only litters conceived within 2 weeks following accumulation of the dose. In Carter's experiment,[8] in which all litters were of this classification (because of subsequent sterility from the dose distribution used), one mutation occurred among 10,117 offspring. Of the 26,468 offspring from chronically irradiated females in our experiments, 4,303 were conceived in the first 2 weeks, and one of the two mutations was among these. These two sets of results from chronically irradiated later oöcyte stages, taken together, give a significantly lower rate than that now found to result from acutely irradiated females ($P = 0.005$, for one-tailed test), indicating that, as suspected, the difference between the acute and the total chronic data is not due to oöcyte stage.

The finding of an effect of dose rate on the frequency of mutations recovered from irradiated females—where, as explained, secondary factors, such as cell selection, cannot be easily invoked—argues strongly that the mutation process itself must be affected by dose rate. Furthermore, although secondary mechanisms cannot yet be definitely ruled out as the cause of the intensity effect demonstrated earlier for spermatogonia, it is simpler to assume that the explanation for the results in oöcytes also applies to spermatogonia.

That the frequency of radiation-induced mutations is independent of dose rate has been one of the basic tenets of radiation genetics. The finding that dose rate affects the frequency of specific locus mutations observed following irradiation of mouse spermatogonia[1-3] did not by itself violate this tenet, since the result might have been due to secondary factors. However, the present demonstration of a dose-rate effect on mutation rate in oöcytes shows that the tenet does not have universal validity. The classic findings of intensity independence were made in *Drosophila* spermatozoa.[10] Recent experiments on mouse spermatozoa[4] are in complete agreement with the *Drosophila* results. Therefore, the explanation for the new phenomenon of intensity dependence resides in gametogenic stage. Hypotheses concerning the mechanism for this effect have been discussed elsewhere,[4] and it was shown that a threshold response for all mutations in spermatogonia and oöcytes is not a necessary consequence of the findings.

The specific locus mutation rates in oöcytes, 4.2×10^{-8}/r/locus and 31.8×10^{-8}/r/locus for chronic and acute irradiation, respectively, may be compared with the rates in spermatogonia, namely (as calculated from straight lines fitted in the range of 0–861 r[4]), 5.2×10^{-8}/r/locus and 21.3×10^{-8}/r/locus for chronic and acute irradiation, respectively. It will be noted that, at each dose rate tested, there is, at present, no evidence of marked difference between oöcytes and spermatogonia. Before the mutation rates from acute irradiation of females were known, it was argued,[6] on the basis of the results then available, that the low mutation rate obtained with chronic irradiation was due either (*a*) to the same factor in males and females, namely, the low dose rate, or (*b*) to different factors in the two sexes, namely, to secondary causes in males and to a low sensitivity of the sex or of the oöcyte stage in females. The new results, showing a high mutation rate from acute irradiation of females, obviously disprove the second hypothesis. The conclusion,[6] that "the mutation rate in women exposed to chronic gamma radiation may be less than that estimated from our earlier yardstick, namely, the mutation

rate obtained from acute X-irradiation of male mice," can now be extended to chronically exposed *men* also, since the new results have made secondary factors unlikely. Carter[8] thought it most likely that the low mutation rate found following chronic irradiation of oöcytes was attributable to sex. As has just been pointed out, this interpretation is not upheld by the results reported in this paper. Carter's emphasis on the consequence of his interpretation, namely, that only a small part of the genetic hazard from medical irradiation would come from exposure of females, now appears to have been misleading.

Of course, more information is needed on the relative mutational sensitivities of males and females under various conditions of irradiation—intensity, fractionation of dose, etc.—but there is no justification at present for assuming less genetic hazard from irradiation of females. In fact, the present point estimate from the mutation rate induced by acute radiation in oöcytes is actually slightly higher than the spermatogonia rate. Although the difference is not significant in the present data, the possibility that the mutation rate may be somewhat greater in oöcytes than in spermatogonia is not out of line with the higher mutation rates found from irradiation of postspermatogonial stages,[11] among which are spermatocytes. In *Drosophila*, specific locus mutation rates are about half to three-fourths as high in oöcytes as in spermatozoa,[12] but the spermatozoa rate is about four times the spermatogonia rate.[13] Thus the relative mutation rates of oöcytes and spermatogonia may be similar in the two species.

It may be noted from Table 1 that no mutations have, to date, occurred among 46,763 offspring of our control females. Control males have, so far, yielded 17 mutants among 288,616 young[4] at our laboratory and 4 mutants among 41,262 young at Harwell.[8] Thus there is some indication that the spontaneous mutation rate in females may be lower than in males in the mouse. A similar situation exists in *Drosophila*.[14]

Summary.—The specific locus mutation rate in females is significantly higher following acute than following chronic irradiation. Since conditions in the adult mouse ovary are such that complications from secondary factors are unlikely, it can be concluded that in oöcytes there is a dose-rate effect on the mutation process itself. The influence of dose rate on the frequency of mutations following irradiation of spermatogonia, which was demonstrated earlier and which could have been due to secondary factors, is now, in the light of the oöcyte results, also considered to be due to a dose-rate effect on the mutation process.

The most significant features of the results, from the point of view of human hazards, are, first, that there is, at present, no evidence that females are less sensitive than males and, second, that the finding of a dependence of mutation frequency on radiation dose rate, now extended from males to females, applies to those germ cell stages that are important in human hazards.

The authors are grateful to Miss E. M. Kelly and Mrs. K. F. Stelzner for their extensive assistance in this experiment.

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*MODIFICATION OF IRRADIATION EFFECTS IN THE PIGEON, COLUMBA LIVIA. I. EFFECTS OF HOMOLOGOUS AND HETEROLOGOUS BONE MARROW TRANSPLANTS**

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INTRODUCTION

Previous investigations have demonstrated that the lethal effects of total-body roentgen irradiation on mammals (mice, rats, rabbits, and guinea pigs) can be modified by isologous, homologous, or heterologous bone marrow transplantation.[1,10,11] These modifications appear to depend upon (1) the establishment and proliferation of the hematopoietic elements of the transplant and (2) either partial or complete repopulation of the host's depleted hematopoietic system by the cells proliferating from the graft.[3,4,7,8]

The present report includes preliminary findings of experiments in which homologous and heterologous bone marrow injections were administered to pigeons previously subjected to 2,500 rads (approximately the $LD_{100/30}$) total-body X-irradiation. The evidence indicates strongly that partial and eventually total repopulation of the erythrocytes results from the homoglous and heterologous transplants. Also, host-species-specific hemagglutinins (hemagglutinins specific for the erythrocytes of host species) appeared in the circulation of irradiated pigeons which received heterologous bone marrow.

MATERIALS AND METHODS

Dosage response studies have shown that the $LD_{50/30}$ for the pigeon is approximately 2,050 rads and that the effective $LD_{100/30}$ was 2,500 rads. These studies also