The International Symposium on the Behaviour of Tritium in the Environment, San Francisco, California, October 16-20, 1978

INDUCTION OF SPECIFIC-LOCUS MUTATIONS IN THE MOUSE BY TRITIATED WATER*

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Abstract

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The results reported are the first obtained on transmitted gene mutations induced by tritium in any form in any mammal. They are, therefore, of obvious practical importance in the estimation of the possible biological hazards of man-made tritium in the environment. Male mice were injected intraperitoneally with either 0.75 or 0.50 mCi per gram of body weight of tritiated water. They were then used in our standard specific-locus mutation test in which the treated wild-type stock of mice is mated to a stock homozygous for seven recessive marker genes. Mutations at any of the seven loci are scored in the offspring. The earlier matings provided information on the mutation frequency in germ cells irradiated in postspermatogonial stages, and the later matings gave the mutation frequency in treated spermatogonia. The spermatogonia are the important cells so far as human risks are concerned, and the mouse results for this germ-cell stage yielded a relative biological effectiveness (RBE) of approximately 2 for tritiated water compared with low-dose-rate gamma irradiation. There are various uncertainties involved in arriving at this figure, and the difference between it and 1 is probably not statistically significant. However, for risk estimation, it seems prudent to use the RBE value of 2, which is, after all, the best point estimate computed from the present data.

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INTRODUCTION

Increasing concern over the possible biological impact of tritium in the environment from the various man-made sources has stimulated research in this field, including the genetic study described here and that of Carsten and Commerford on dominant lethal mutations [1]. Prior to the experiments reported here, there were no mammalian specific-locus mutation data or other mammalian gene mutation information available for tritium in water, in organic molecules, or in any other form. In addition to the concern over the possible genetic hazards of tritium, there is also scientific interest in a comparison of the genetic effects of low-energy β particles with those of higher-energy external radiation sources.

Preliminary accounts of this work have appeared in the Annual Reports of the Biology Division of Oak Ridge National Laboratory [2,3,4].

MATERIALS AND METHODS

The genetic results reported here were obtained by our standard specific-locus test [5] and are limited to those occurring in the offspring of treated males. (101 X C3H)F_1 wild-type male mice were injected intraperitoneally with a single dose of tritiated water (HTO) and mated to our T stock females, which are homozygous for seven recessive marker genes. The offspring were scored for presumed mutations at the seven loci, and the presumed mutants were bred to establish allelism of the mutations and to determine the viability of the mutations in homozygous condition.

The treated mice were kept for two weeks after injection in an isolator system developed to prevent tritium release [6]. Two types of experiments were conducted. In the first, each male was mated with from three to five untreated females immediately after injection, and with the same number of new females at weekly intervals for four weeks thereafter. Thus, in this case, the first two weekly batches of females were placed with males in the isolators. This permitted the collection of data from germ cells exposed in postspermatogonial stages (Experiment 1a). With the doses of HTO used, a temporary sterile period ensued in the males two to four weeks after treatment. When fertility returned, each male was caged with one female. The conceptions occurring from then on came from germ cells that had received most of their dose in spermatogonial stages (Experiment 1c). In a replicate (Experiment 1b) each male was mated to five females a week for the first three weeks after injection, and then not used for later matings. In the second type of experiment (Experiment 2), the males were not mated until after their removal from the isolators and after the end of their temporary sterile period. In this experiment each male was kept with two females.

In the first series of experiments, a few males were injected with 0.75 mCi of HTO per gram of body weight. With this dose, fertility prior to the temporary sterile period usually lasted only two weeks, and litter sizes were greatly reduced, particularly in the second week. The length of the temporary sterile period was similar to that observed following an acute dose of 1000 R of X-rays. The injected dose was, therefore, reduced to 0.50 mCi per gram of body weight for the remainder of the males in this experimental series and for all males in the rest of the study.

There are many complications in estimating the dose of radiation actually received by the genetic material of the germ cells. Our attempts

to circumvent as many as possible of these, and our methods for arriving at a weighted mean dose for the germ cells in the experiments reported here, are described in a companion paper by Cumming et al. [6].

No contemporary controls were run in this investigation. The spontaneous mutation rate, which is low in comparison with the induced mutation rates obtained here, was estimated from the cumulative controls of extensive past experiments.

RESULTS

The mutation frequencies in the offspring from matings in which the germ cells had received their radiation dose in postspermatogonial or spermatogonial stages are shown in Table I. The distribution of the mutations among the seven loci and their viability in homozygous condition, when this was determined, are listed in Table II. Confirmation of allelism, i.e., that a mutation actually occurred at the locus to which it had been assigned on the basis of its phenotypic appearance was established by breeding tests for all except five of the mutations. These include the three sterile mutants and two which died before testing: the mutation at the p locus listed as "untested," and one of the s-locus mutations listed as "untested" in the group from irradiation of spermatogonial stages. The other two s locus mutations listed as "untested" in the viability test were, however, tested for allelism. Although the allelism of the five mutations designated above was not established by breeding tests, it is highly likely, on the basis of extensive past experience, that the phenotypes of these five were correct indications of the loci involved.

The weighted mean dose for the experiment with irradiated postspermatogonial stages was calculated by estimating the accumulated dose for each succeeding day after HTO injection, and weighting by the number of offspring conceived on that day. For the experiment with irradiated spermatogonial stages, the weighting was by weekly, rather than daily, intervals. In both cases, the limited number of offspring obtained following the 0.75 mCi/gm injected dose have been included, by appropriate weighting, into the estimated mean dose.

The induced mutation rate is obtained by subtracting the spontaneous mutation frequency for males obtained in past experiments, namely 28 mutations in 531,500 offspring. For the offspring from irradiated postspermatogonial stages this gives an induced mutation rate, per locus, per rad, of 44×10^{-8}.

For the offspring from irradiated spermatogonial stages the induced mutation rate is 15×10^{-8} per locus, per rad. In computing this, each of the two clusters of the mutants listed in Table I has been counted as two mutations with, however, a minor correction for the fact that the males who gave the clusters had a slightly larger number of offspring than the mean number of offspring for the rest of the males. Without this correction the mutation rate is 15.4×10^{-8}; with the correction it is 15.0×10^{-8}.

Each of the two clusters has, of course, been counted as a single mutational event in Table II, where we are concerned, not with mutation frequencies, but with the relative frequencies of different types of mutational event.

DISCUSSION

Both from the scientific point of view and for the practical application to risk estimation, it is of interest to compare the results obtained here from an internal emitter with those from external radiation sources such as x- and gamma radiation. Before attempting to make a quantitative comparison, we should first examine the data to see if the mutations induced by tritium differ in any important qualitative way from those obtained with x- and gamma-rays.

One way of checking for qualitative differences is to examine the frequency distribution of the mutations among the seven loci. The distribution of the mutations in offspring from spermatogonial irradiation is not significantly different from that occurring with x- and gamma irradiation, where a low frequency is always observed at the a and se loci. The frequency at the s locus in the present data is lower than expected on the basis of results from external radiation, but the difference is not statistically significant. Furthermore, in a replication of Experiment 2 not yet completed, and not otherwise reported here, five out of the total of eight mutations being tested are apparently at the s locus, bringing the total at this locus more in line with the relative frequency expected from external radiation.

The mutations in the offspring from irradiation of postspermatogonial stages appear to be more evenly distributed among the loci, a characteristic of those obtained from external irradiation of these germ cell stages [7]. With external irradiation, the frequency of detected chromosome aberrations is higher for treated postspermatogonial stages than for spermatogonia. For example, deficiencies involving the d and se loci simultaneously are rare for irradiated spermatogonia, but common for treated postspermatogonial stages [7,8]. No d se deficiencies have been found in the small total number of mutations from postspermatogonial stages in the present data, but there is other evidence of possible chromosomal aberrations. Thus, three of the mutants were sterile, and the s locus mutant listed as "untested" had a high frequency of sterility in her offspring.

The appreciable proportion of mutations, particularly those from irradiated spermatogonia, that turned out to be viable in homozygous condition, is also in line with results from external radiation. One viable mutation at the c locus in the offspring from irradiated spermatogonia is intermediate in phenotype between albinism and wild type, and one viable mutation at the b locus in the offspring from irradiated postspermatogonial stages is intermediate between b and wild type.

In summary, the present data show no evidence that the mutations induced by injection of tritiated water differ in any marked qualitative fashion from those induced by external x- or gamma radiation. In view of this, it seems reasonable to proceed with a quantitative comparison of the mutagenic efficiency of the two types of radiation.

The mutation rates given here are based on mutant animals that survive to weaning, by which time mutations at all of the seven loci have become clearly discernible. It is known that a few mutations which were detectable at an earlier age occurred in animals that died before weaning. Thus the true mutation rate must be somewhat higher than that recorded at weaning. However, since the scoring of mutations has been done in the same way for external irradiation, it is valid, for comparative purposes, to use the mutation rates reported here.

For postspermatogonial stages, the experiment with external radiation which used a dose closest to that in the experiment reported here was one in which 300R of x rays was given at approximately 90R per minute. The dose rate in the tritium experiments was considerably below this figure, but mutation rate in postspermatogonial stages appears to be independent of dose rate or, at least, not markedly affected by it [9]. Since more than 95% of the offspring born in the tritium experiment on postspermatogonial stages were conceived in the first two weeks after injection, the results from the first two weeks of mating in the x-ray experiment are selected for comparison. The mutation frequency for these weeks was 25 mutations in 18,693 young [10]. Subtracting the spontaneous mutation rate gives an induced rate of 61.2×10^{-8} per locus, per R. Dividing this into the tritium-induced mutation rate of $4^{\ell} \times 10^{-8}$ per locus, per rad, and making a conversion from R to rad, yields a relative biological effectiveness (RBE) of 0.7 for tritium, compared with x rays, for specific-locus mutations induced in postspermatogonial stages. With the limited number of mutations scored, this estimate is clearly not statistically significantly different from 1.

For comparison of results with spermatogonial stages, the dose rate is important, since there is a marked effect of dose rate on these stages [9,11]. In the tritium experiment the dose rate is relatively high in the first few days after injection as compared to what it is later, but even in the first half-day it probably does not average much more than about 0.1 rad per minute. In extensive exploration of the dose-rate effect with gamma rays on spermatogonial stages [11], it has been shown that, although there is a marked effect on mutation frequency as dose rate is lowered from 90 to 0.8 R/minute, there appears to be no further change in mutation frequency when the dose rate is dropped to lower levels. It is appropriate, therefore, to compare the tritium results with gamma irradiation results at dose rates below 0.8 R/minute. The induced mutation rate for all data from spermatogonia irradiated in this dose-rate range was calculated in the review paper by Searle [12] to be 6.59×10^{-8} per locus, per R. Dividing this into the tritium induced rate of 15×10^{-8} per locus, per rad, and converting from R to rad, gives an RBE of 2.2 for tritium, compared with gamma rays, for specific-locus mutations induced in spermatogonia. With, again, the limited number of mutations in the sample, with uncertainty as to the number of independent events involved when clustering occurs, and with some uncertainty as to the true doses, it is unlikely that this figure differs significantly from 1, but it is not possible to put a precise probability value on the null hypothesis.

We realize that a comparison of the effects of tritium with those of x and gamma radiation at the microdosimetric level may be more complicated than is implied in the making of the above simple calculations. However, the RBE's derived above are of the kind which, in the absence of additional information, are used in risk estimation. They are presented primarily for that purpose.

With regard to applying these data to the estimation of genetic hazards of radiation in man, the spermatogonial stage is of prime importance. It would seem prudent at this time to assume that the RBE for exposure of these stem cells in the testis to tritiated water might be approximately 2.

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TABLE I. MUTATION FREQUENCY AT 7 SPECIFIC LOCI IN OFFSPRING OF MALE MICE INJECTED WITH TRITIATED WATER

Cell stage exposed to radiation	Experiment	Injected dose (mCi/gm of body weight)	No. of offspring	No. of mutations	Estimated wtd. mean dose to germ cells (rad)
Post-spermatogonial	1a	0.75	515	1	430
		0.50	3,327	4	
	1b	0.50	4,101	6	
Spermatogonial[a]	1c	0.75	2,408	3	615
		0.50	18,218	13[b]	
	2	0.50	11,481	7[b]	

[a]A negligible portion (<1%) of the dose was received in postspermatogonial stages.

[b]The number includes both mutants of a cluster of two in the offspring of one male in this experiment.

TABLE II. DISTRIBUTION OF MUTATIONS ACCORDING TO LOCUS AND VIABILITY OF HOMOZYGOTE

Cell stage exposed to radiation	Result of viability test	No. of mutations at specified locus:							
		a	b	c	p	d	se	s	total
Post-spermatogonial	Viable		1	1			1		3
	Sublethal[a]				1				1
	Lethal[b]	1	1		1				3
	Sterile[c]		1			1		1	3
	Untested							1	1
	Total	1	3	1	2	1	1	2	11
Spermatogonial	Viable		3	4	2	1			10
	Sublethal					2		1	3
	Lethal		2		2			1	5
	Untested				1			2	3
	Total	-	5	4	5	3	-	4	21

[a]Defined here as: Lethal after perinatal period and before breeding age.

[b]Lethal before birth or perinatally.

[c]Mutants themselves were sterile and, therefore, could not be tested.