THE FREQUENCY OF X-RAY INDUCED RECESSIVE VISIBLE MUTATIONS IN THE RAT

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Received February 19, 1968

DELETERIOUS mutations which act postnatally are considered to be more serious in man than are prenatal lethals. The former class frequently represents a human tragedy, while lethals which act in the early embryonic stages may pass completely unnoticed. Recessive visible mutations in experimental mammals are roughly equivalent to recessive genetic diseases in man, and in some cases probably represent homologous mutations. Early work with mice (RUSSELL 1951) showed that most detectable radiation-induced visible mutations are recessive. Recessive visibles therefore constitute a class of mutations which deserve particular attention.

Unspecified visible mutations have not been considered favorable endpoints in mutation studies. One reason visibles have not been extensively studied is that the discovery of an unspecified mutation is somewhat subjective. Although many mutants would be noticed by any careful observer, those with small effects may not be detected. Some mutations may mimic common environmentally produced abnormalities and may, therefore, not be tested for genetic etiology.

In the present study an attempt is made to estimate the effect of cumulative spermatogonial irradiation on the frequency of recessive visibles in the rat.

MATERIALS AND METHODS

Male rats from a highly inbred line (MI₄) were given a total dose of 450r administered as 100, 150, and 200r fractions at 10, 12, and 14 weeks of age, respectively. The dose rate was approximately 22r per minute. Matings were delayed 12 weeks or more after the final treatment to insure that the fertilizing sperm originated from irradiated spermatogonia. The male progeny were in turn mated to non-irradiated daughters of irradiated males. In this way the "load" of recessive mutations was allowed to accumulate. A total of nine generations of irradiation was completed. In the third and subsequent generations a portion of the matings were between full-sibs. (Due to a shortage of breeding animals irradiation was suspended in generation 4, and no full-sib litters were produced in generation 5.) The progeny from full-sib matings were discarded at 14 weeks of age, and the lines were continued with the progeny of "unrelated" parents within the highly inbred line. A contemporaneous control line was maintained. Further details of the experimental procedures have been given by HAVENSTEIN, TAYLOR, HANSEN, MORTON, and CHAPMAN (1968). The only deviation from this pattern was in generations 13 and 14. Females from the treated and control lines of generations 11 and 12 were mated to males of an unrelated stock. The F₁'s were full-sib mated to produce the F₂'s in generations 13 and 14.

Published with the approval of the Director of the Agricultural Experiment Station. Paper No. 1191 from the Genetics Laboratory. This work was supported in part by contract AT(11-1)-697 of the Atomic Energy Commission.

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A consequence of this cumulative irradiation is that some induced recessives will appear more than once, while others will be lost without being expressed. However, in the absence of any heterozygote selection, the expected frequency of autosomal recessives is the simple function:

$$L = U_s G + U_i D$$

where L is the expected number of mutations per gamete, U_s is the spontaneous mutation rate per gamete, G is the number of generations during which spontaneous mutations have been allowed to accumulate, U_i is the induced mutation rate per gamete per r, and D is the cumulative radiation dose. Since the probability that two sibs will receive any particular allele carried by one of their parents is one-fourth and the two parents carry four alleles (any one of which may be mutant) it follows that the expected frequency of heterozygote $\times$ heterozygote matings (among the full-sib matings) in generation $n + 1$ is equal to L in generation n . Even if both parents are heterozygous for the same mutation, segregation may not be evident due to the small litter size. The probability of at least one mutant progeny being produced from such a mating is $(1 - 0.75^t)$, where t is the sibship size at the time of observation. This value when calculated for each sibship and summed over all litters is the effective number of completely tested matings, N_e .

$$N_e = \Sigma (1 - 0.75^t)$$

Then, $\hat{L} = \frac{k}{N_e}$, where $\hat{L}$ is the estimate of L , and k is the number of litters observed to be segregating for a recessive. In the unlikely event that a sibship is observed to be segregating for more than one mutation, it would be counted as many times as there are different mutations in computing k .

The data consist of a number of groups with varying values of N_e , G , D , and k , corresponding to the control and irradiated litters in the various generations. A convenient and efficient method of combining the information from these data into single estimates of U_s and U_i is needed. A weighted multiple regression technique is used. The value of $\hat{L}$ in each group is regressed on the corresponding values of G and D . The regression equation is

$$L_n = U_s G_n + U_i D_n$$

This is a case of regression through the origin.

Each statistic $\hat{L}_n$ is weighted by the inverse of its estimated variance (FISHER 1966). The variance of $\hat{L}$, $V(\hat{L}) = V(k)/N_e^2$. If k_n is assumed to have a Poisson distribution, $V(k)$ is equal to the expectation ($E[\]$) of k .

$$\begin{aligned} E[k] &= N_e E[\hat{L}] \\ &= N_e (U_s G + U_i D). \end{aligned}$$

Therefore:

$$V(\hat{L}) = \frac{U_s G + U_i D}{N_e}.$$

Estimates of the variance of $\hat{L}_n$ are obtained by taking trial values of U_s and U_i and then following a process of iteration, replacing the old values of U_s and U_i with the new estimates in the variance formula until the estimates no longer change appreciably.

The sum of squares of deviations about the regression line is distributed as chi-square with degrees of freedom equal to the number of observations (generation—group) less the number of parameters estimated from the data, if the assumptions are correct (MATHER 1965). This provides a test for linearity of accumulation of mutations. The test applies to the entire model.

This regression procedure therefore provides variances of the mutation rate estimates and a test for nonlinearity. It should be pointed out that the assumption of independence inherent in estimating these variances has been violated. Litters within the same line are related and are, therefore, putting some of the same mutational opportunities to test. (This lack of independence

will be reflected in the same mutation appearing in more than one litter.) This will result in somewhat underestimated variances. Consequently, an apparently significant departure from linearity could reflect the underestimated variances as well as true nonlinearity. True nonlinearity could result from either heterozygote selection or varying thoroughness on the part of the investigators in searching for mutations.

The values of D are based on the irradiation history of the group and reflect the number of generations of irradiation put to test in the group. The values of G are determined by assuming that the original frequency of mutations in the lines was equivalent to three generations of accumulated mutations and adding one for each successive generation. G was halved in the F_2 groups since any visibles introduced through the tester males would have been recognized by clusters of mutant litters descended from particular tester sires. The value of N_e , a function of t , is based on the number in the litter which survived to 21 days of age. This is approximately the median age of onset of the detected mutations.

The rats were routinely examined at 1, 21, 42, and 69 days of age.

RESULTS AND DISCUSSION

Number of mutations detected: During the course of the experiment six mutations appeared in the irradiated line compared to three in the control line. One of these appeared in both lines. None of these eight different mutations strongly resembled descriptions of previously reported mutations, so these are tentatively considered as previously unknown loci and have been given names and symbols. One of these mutations (crawler) was detected among special matings rather than in the main experiment, so was not used in estimating mutation rates.

Description of mutants: I. Control line. (a) Stubby (*sb*). These animals are identified by their very short limbs at birth. These individuals generally resemble "bulldog" mutants reported in other species. They usually die within the first few days after birth. (b) Crawler (*cr*). These individuals exhibit trembling at about two weeks of age. By three weeks the rear legs are paralyzed to the extent that movement is accomplished by pulling themselves by their forelimbs. Death occurs around four weeks of age. Matings between two heterozygotes produce less than the expected 25 percent mutant progeny, presumably due to excess mortality among the mutants. (c) Skinny (*sk*). Mutants are emaciated and small at birth. Death usually occurs in the perinatal period, but mutants have been known to survive to weaning age.

II. Irradiated line. (a) Stubby (*sb*). This mutation appeared in both lines. See (a) under Control line. These may represent independent mutations, but the possibility of contamination cannot be excluded. (b) Runt (*rt*). Affected individuals are strikingly smaller than their litter mates at birth and die in the perinatal period. (c) Masked (*mk*). This mutation affects the hair coat at about five weeks of age. It is characterized by a nearly complete loss of hair followed by regrowth, which is subsequently partially lost in a characteristic persistent pattern. The vibrissae are not affected. Viability and body weight are reduced somewhat, but both sexes have reproduced. (d) Spastic (*sp*). Affected individuals exhibit a neuromuscular disorder which makes them incapable of normal crawling or walking. They fall on either side and hold their limbs out in rigid position with their backs arched. The condition becomes noticeable prior to two weeks

and death occurs between three and four weeks of age. (e) Vibrissaeless (*vb*). Mutant individuals are almost completely devoid of hair, including the vibrissae. Mutants can be recognized at birth by their abnormal vibrissae which are short and reduced in number. Mutants may develop one or more large blisters anywhere on their bodies during the first weeks and, as adults, exhibit many scratches. Body weight and viability are reduced but females have survived and reproduced. (f) Chubby (*cb*). Mutants develop slowly from about two weeks of age. Opening of the eyes is delayed several days. At three weeks they are small, have short limbs, small ears, and move slowly. Their chubby appearance is due to their reduced skeletal size. Death occurs prior to 35 days.

Frequency of mutant litters: Table 1 gives the cumulative radiation dose (D), the number of generations of accumulation of spontaneous mutations (G), the

TABLE 1

Radiation dose, spontaneous accumulation, number of tested matings, number of mutant litters, and mutations represented in each generation-treatment group

Generation	Treatment group†	Radiation dose D (roentgens)	Spontaneous accumulation G (generations)	Number of tested matings N_e	Number of mutant litters k	Mutations represented‡
3	C	...	4	31.7	0	
	R	225	4	59.4	0	
4	C	...	5	19.6	0	
	R	450	5	12.9	0	
6	C	...	7	44.1	0	
	R	900	7	36.0	0	
7	C	...	8	30.4	0	
	R	900	8	22.2	0	
8	C	...	9	27.6	0	
	R	1125	9	24.8	0	
9	C	...	10	25.6	0	
	R	1350	10	12.2	0	
10	C	...	11	43.0	0	
	R	1575	11	35.9	1	1 <i>cb</i>
11	C	...	12	51.0	0	
	R	1800	12	50.4	2	1 <i>sb</i> , 1 <i>mk</i>
12	C	...	13	64.2	1	1 <i>sb</i>
	R	2025	13	68.5	4	3 <i>mk</i> , 1 <i>cb</i>
13	C	...	14	37.8	2	2 <i>sk</i>
	R	2025	14	36.8	2	2 <i>sp</i>
14	C-F ₂	...	7	55.4	0	
	R-F ₂	1012	7	40.4	1	1 <i>vb</i>
	C	...	15	38.5	0	
	R	2025	15	32.1	1	1 <i>rt</i>
	C-F ₂	...	8	46.7	1	1 <i>sb</i>
	R-F ₂	1012	8	32.9	2	2 <i>cb</i>

† C = Control; R = Irradiated; F₂ = Progeny of full-sib mating following outcross.

‡ See text.

number of tested matings (N_e), the number of mutant litters (k), and the mutations represented in each generation.

Mutation rate estimates: The spontaneous mutation rate estimate is $(0.75 \pm 0.38 \times 10^{-3}/\text{gamete/generation})$. The induced mutation rate estimate is $(1.59 \pm 0.65) \times 10^{-5}/\text{gamete/r}$. The value of chi-square for testing deviations from linearity, 16.6 (24 degrees of freedom), is non-significant ($P = 0.85$). Thus, there is no indication of either heterozygote selection or heterogeneity between the observers of the different generations.

It is of interest to compare these rates with studies in mice. Early attempts to measure the mutation rate to recessive visibles in mice revealed few or no mutations (CARTER and PHILLIPS 1952; CARTER and LYON 1961). These negative findings may have been a consequence of the small size of the experiment and/or small doses of radiation. LYON, PHILLIPS, and SEARLE (1964) reported results of an irradiation experiment in which male mice were exposed to two 600r doses of spermatogonial irradiation. Male progeny were tested for autosomal recessive visibles by mating F_1 males to several of their own daughters. This revealed six mutations in the irradiated series and one in the control series. The mutation rate to recessive visibles was estimated to be $1.76 \times 10^{-5}/\text{gamete/r}$. The spontaneous rate was $7.04 \times 10^{-3}/\text{gamete}$.

Similarly, SCHLAGER and DICKIE (1967) detected 28 visible mutations among a large number of inbred lines of mice maintained by full-sib mating. Their data indicate that the overall spontaneous mutation rate to recessive visibles is about $1.2 \times 10^{-4}/\text{gamete}$. They interpreted their data differently than the present author and estimated the rate per locus rather than per gamete. Their per locus rate was based on the assumption that the number of partially tested gametes was equal to the "number of mice examined", including a number of F_1 hybrids. The estimate presented here for the overall rate equates the number of partially tested gametes to twice the number of parents, excluding the parents of hybrid litters. The theoretical basis for this method of estimation is derived and discussed fully by WOOLF (1954).

RUSSELL, RUSSELL, and KELLY (1958) reported a specific locus mutation rate of $2.1 \times 10^{-7}/\text{locus/r}$ from acute X irradiation of spermatogonia. Approximately one-half of their observed mutations would be detectable as recessive visibles in the present experiment.

The present spontaneous mutation rate estimate is intermediate with respect to the estimates made in mice. The mouse estimates appear to be heterogeneous.

The present induced mutation rate estimate is in good agreement with the estimate of LYON *et al.* (1964). It is tempting to conclude that the mutation rate to recessive visibles from acute spermatogonial irradiation is approximately the same in the two related species and is not affected by either the total dose, or dose fractionation over the range of the treatments of these two experiments. On the other hand, the apparent agreement might be due to chance or to the cancellation of two or more differences that were confounded.

There are several factors that would tend to make the present estimate an

underestimate of the true mutation rate. In the first place minor abnormalities may have gone unnoticed. Two cases of abnormalities could not be tested because no close relatives were available. Most of the detected mutations occur with a frequency somewhat less than the expected 25 percent in matings between two heterozygotes. Reduced viability of the mutant classes is presumably the explanation. If this is the rule for most mutations, the expression for the number of tested matings will be inflated. It is possible that some mutations were undetected because the interval between the onset of the expression and death was so brief that the animals were not observed in this period. Mutations with dominant detrimental effects on fitness would likely be eliminated in heterozygotes and therefore be undetected. This should result in a non-linear accumulation of mutations with dose. However, there was no evidence of this. Finally, coat color mutations would not ordinarily be detected because most of the animals screened for mutations were albino.

Since the observers generally knew whether they were looking at experimental or control litters, the possibility that some unconscious bias may have been introduced into the experiment cannot be excluded.

The disadvantages of recessive visibles as an endpoint in mutation studies are well known. The method does possess the following merits that some alternative methods lack. (a) Estimates of mutation rates apply to whole genomes rather than specific loci or some other fraction of the genome. (b) Recessive visibles represent an important class of mutations from the standpoint of mutagenic hazards to humans. (c) Analysis and interpretation of the data are relatively straight forward and do not require sophisticated statistical methods. (d) The discovery of new mutations will contribute to the formal genetic knowledge of the species, e.g., new marker genes, and linkage groups.

The author is indebted to DR. A. B. CHAPMAN, who directed the experiment from which the data are taken, and to both DR. CHAPMAN and DR. J. F. CROW for suggesting many valuable changes in the manuscript. DR. JANET HANSEN collected the first four generations of data.

SUMMARY

During an experiment on the genetic effects of cumulative spermatogonial irradiation in rats, 6 recessive visible mutations were detected in the irradiated lines as compared to 3 in the control line. Frequencies were estimated to be 0.8×10^{-3} /generation/gamete and 1.6×10^{-5} /gamete/r for spontaneous and induced mutations, respectively. These results are compared with similar studies in mice.

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