THE INFLUENCE OF DOSE ON THE SPECTRUM OF RADIATION-INDUCED MUTANTS AFFECTING A QUANTITATIVE CHARACTER IN YEAST

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INDUCED mutations affecting quantitative characters are an important part of the genetic damage in irradiated populations. One criterion for predicting the long-term effects on a quantitative character, at least within a given external environment and genetic background, is the spectrum of heterozygous mutant phenotypes in the immediate progeny of an irradiated experimental population. However, a quantitative character may be shifted in different directions by mutations at many loci; hence at high mutation frequencies, phenotypes may result from interactions of two or more mutations. The mutant spectrum would thus be dose-dependent, and this may partly explain the conflicting results reported, for example, by MULLER and FALK (1961) and WALLACE (1958, 1963).

The present investigation concerns ultraviolet-induced mutations that affect growth rate in diploid yeast. Evidence will be presented suggesting that the true mutation frequencies to different growth rates are obscured because of interactions as the number of mutations per cell increases.

PROCEDURES

The yeast was a homothallic strain of *Saccharomyces cerevisiae* var. *ellipsoideus* (Bordeaux). It was obtained by diploidization following germination of a haploid spore isolate. All stock slants were prepared from a single colony.

Relative rate of cell multiplication, the character under test, was calculated for individual lines from the rate of change in diameter of colonies incubated on the surface of agar medium. This method of estimating growth rates has been described in detail (JAMES and SPENCER 1958; JAMES 1960). The method permits the detection of very small differences in growth rate, but its principal attraction lies in the fact that estimates can be obtained before selection pressures of spontaneous mutations have obliterated genetic differences between lines.

Precautions were made that the information on mutation effects was not distorted by three extraneous influences of irradiation: (1) mitotic inhibition; (2) mitotic stimulation, which can appear at the conclusion of the inhibitory period or even in the absence of inhibition (JAMES and MÜLLER 1961); and (3) delayed mutation, which can introduce genetic heterogeneity into the progeny of a cell in the early divisions after irradiation (JAMES 1962). It was possible that some induced hereditary changes were a consequence of permanent damage to the cytoplasm. However, the one known example, respiratory deficiency, was considered to occur too infrequently to have an appreciable effect on the results.

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FIGURE 1.—Diagram of experimental procedure, showing derivation of test clones.

The experimental procedure is diagrammed in Figure 1. For each experiment a stock slant was incubated overnight. A suspension of the cells was then divided into four lots, three of which were irradiated. Immediately after irradiation, nonbudding cells were isolated by a micro-manipulator to the surface of an agar medium. Twice as many cells were isolated from the non-irradiated lot as from each of the irradiated lots. Reisolation of a budding cell was made as each clone reached the eight-cell stage. This procedure was designed to isolate radiation-induced mutants before nonmutants had overgrown the slower mutants. It was expected that only a fraction of the induced mutants would be recovered where heterogeneity was actually present. A final reisolation was made approximately 12 hours later when both nongenetic stimulatory and inhibitory influences of irradiation had ceased. At this time, budding cells were isolated in sets of five, three irradiated and two nonirradiated. Of the latter, one was termed a control and the other a zero-dose clone. The five members of a set were placed at designated positions, which were

FIGURE 2.—The growth curves of a set of five clones, including one which was classed as a major negative mutant.

varied with each experiment, on a block of agar and incubated at 23.8°C. Several sets were placed on each agar block; this permitted a correction factor to be applied where any consistent effect of location on growth was noted. Diameters were measured by microscope at 2-hr. intervals, beginning when the majority of diameters were approximately 0.1 mm and concluding when they exceeded 0.8 mm. Typical growth curves of the five members of a set are shown in Figure 2.

Two different statistics were considered for estimating growth rate, (1) the time interval between specified diameters (.25 mm and .8 mm), and (2) the time interval between cell isolation No. 3 and a specified diameter (0.8 mm). The values obtained by these two methods were strongly correlated. However, the latter method was used in the analysis of results because the peak of the distribution of values was more clearly defined. The growth behavior of a test clone was expressed in terms of its rate of multiplication relative to that of the control clone in the same set. This was the time interval of the test clone divided by that of the control clone. The log relative growth value was used in statistical analyses to provide linearity on the relative growth scale.

Ultraviolet light (UV) was provided by a germicidal lamp (15 watts) which delivered 95% of its UV energy in the 2537Å line. Cultures were irradiated as 10 ml broth suspensions in open Petri dishes and were agitated during exposure. The culture medium was nutritionally fortified with peptone and yeast extract. It has been described previously (JAMES and SPENCER 1958).

RESULTS AND DISCUSSION

Radiation-induced mutations affecting rate of cell division were detected at each of the dose levels of 25, 75, and 200 ergs/mm². Frequency distributions of the growth rates of test clones relative to those of the controls are presented in Figure 3. It is evident from this figure that the frequency of extremely slow-growing cells (major "negative" mutants) increased progressively with higher doses. An indication that lesser disturbances to the growth rate (minor "positive" and minor "negative" mutations) were also induced is less clear from a cursory inspection of the figure, and became evident only with statistical analysis of the data.

A few specific aspects of the zero-dose distribution may be considered. The variation in relative growth rates was remarkably small; the majority of the paired clones, 94%, differed in growth rate by not more than 5%; 56% of the paired clones had growth rates which differed by not more than 1%. Despite this high degree of uniformity, this sample probably included some mutants of spontaneous origin, although they could not be identified simply by examination of the data. Since the designation of test and control cells was random, the population distribution was symmetrical. A low value for relative growth rate could be due to the pairing either of a "fast" test clone with a "normal" control or of a "normal" test clone with a "slow" control. The isolated value of 1.923 is a case in point; examination of the growth curves for the two clones providing this value showed a normal test clone paired with an excessively slow control clone. A similar erratic value is present in each of the other three distributions because the same control clone was used at all four dose levels.

Lethality: Mean survival at the highest dose level was 83%. Death usually occurred at the two-cell stage subsequent to irradiation although reproduction occasionally continued to the eight-cell stage or even beyond. The survival curve, typical of diploids, (Figure 4) is based on more data than appear in Figure 3.

FIGURE 3.—The frequency distributions of relative growth rates of test clones in four populations exposed to different UV doses. Relative growth rate is the growth interval of a test clone divided by the growth interval of its control clone. The growth interval is the time required for an isolated budding cell to produce a colony of specified diameter on solid medium.

FIGURE 4.—The dose-survival curve for diploid cells exposed to UV. Each point is based on more than 360 cell-isolates.

Segregation for lethality sometimes occurred in later generations. When a non-reproducing “segregant” cell was selected by chance during reisolation, a lethal was recorded despite the fact that cell division continued in the remainder of the

FIGURE 5.—Estimated frequencies of induced mutant phenotypes following exposure to various UV doses. Major detrimental phenotypes are expressed as proportions of all viable clones. Positive and minor negative mutants are expressed as proportions of all viable clones excepting major detrimentals.

clone. For this reason, the estimated values were too low in terms of the usual criterion of survival, the ability of an irradiated cell to produce a visible colony.

Major detrimentals: The effect of dose on the frequency of major detrimentals is shown in Figure 5 where relative frequency is expressed as a proportion of all viable clones. The individuals classed as major detrimentals (log relative growth rate > 0.040) can have included few, if any, nonmutants; not only was the zero-dose population free of representatives of this class, but the clones themselves were individually identifiable as "slow growers" by visual examination. In fact, they grew so slowly that calculations of the relative growth values necessitated extrapolation of growth curves. This procedure tended to overestimate growth rates. The linearity of the curve has been strengthened by further data and indicates that major detrimentals were fully expressed at all doses and were epistatic to any other mutations which occurred in the same cells.

Minor mutants: A statistical analysis indicated that both positive and minor negative mutant phenotypes were induced by radiation. In this analysis, major detrimental mutations were excluded by truncating the distributions at the lower limit observed for the zero-dose group. Means and variances were then estimated for the remaining values in each of the four dose-groups (Table 1). The mean relative growth rates of the treated groups did not differ appreciably from that of the zero-dose group. Each of the variance estimates, on the other hand, was larger than that of the zero-dose group. A variance ratio test could not be used to compare variance estimates because of the nonnormality of the distributions. Instead, the KRUSKAL-WALLIS test was used (KRUSKAL and WALLIS 1952). Highly significant differences from the zero-dose group were found for all irradiated groups ($P < 0.01$). Excepting the induction of grossly detrimental mutations, these

TABLE 1

Means and variance estimates of truncated distributions of relative growth values following irradiation

Dose (ergs/mm ²)	Number in group	Mean	Variance estimate	Significance of difference from zero-dose group*
0	201	1.0002	.000146	
25	192	1.0008	.000239	P < 0.01
75	182	.9999	.000241	P < 0.01
200	143	.9991	.000314	P < 0.01

* KRUSKAL-WALLIS test.

results are very similar to those of BURDICK and MUKAI (1958) who used several different doses in an investigation of the heterozygous effects of X-ray induced mutations on viability of *Drosophila melanogaster*. That study also demonstrated the induction of both positive and negative mutants in high frequencies.

Estimates of the frequencies of radiation-induced positive and minor negative mutant clones in the three irradiated groups are presented in Table 2 and Figure 5. They support previous findings in yeast (JAMES 1959, 1960) that the yield of radiation-induced mutants of small effect is several times greater than that of mutants with severe effects. In calculating these frequencies, it was assumed that relative growth values which fell in the narrow range between 1.9975 and 0.0025 were mutant-free in all treatment groups. The proportion of such values was calculated for each group, after excluding the apparent major detrimentals and lethals. For any irradiated group, the ratio of this proportion to the corresponding proportion in the zero-dose group gave an estimate of the proportion of non-mutants in the group. Using this factor, the expected number of values in each population was subsequently calculated. The expected frequency of nonmutants in any interval on the growth-value scale was calculated from the observed proportion of values in the same interval of the zero-dose group. The difference between observed frequencies and expected frequencies of nonmutants provided estimates of the frequencies of radiation-induced mutant clones. These frequencies would be underestimated to the extent that the central class was not mutant-

TABLE 2

The estimated frequencies of radiation-induced mutants for growth rate in populations exposed to four doses of ultraviolet light

Dose (ergs/mm ²)	Positive Mean growth rate*		Minor negative Mean growth rate*		Major detrimental Mean growth rate*		Lethal	Total
No.	No.		No.		No.			
0	0		0		0		1	202
25	37	.970	53	1.029	9	1.449	1	202
75	43	.969	45	1.031	12	1.328	8	202
200	32	.961	21	1.051	39	1.440	20	202

* Mean growth rate relative to the unirradiated control.

free and to the extent also that the estimate of major negative mutants included any minor negative mutants.

In contrast to major detrimentals, the estimated frequencies of minor mutants did not increase linearly with dose over the range studied. Instead, the curves for the positive mutants flattened off, while the estimates of minor negative mutants declined at the higher doses.

Various explanations have been given for convex dose-mutation curves. One of these is a real reduction in the rate at which mutations are induced per exposed cell per unit dose of radiation at higher doses. In the present instance, this explanation appears implausible in view of the linearity of the dose-effect curve for major detrimentals (Figure 5). On the other hand, the curve for major detrimentals may be a two-component curve, one component of which is asymptotic while the other follows a 2-hit pattern typical of some chromosome aberrations. Another explanation involves the selective death of mutants at higher doses. This explanation is also unsatisfactory because frequency of death was only 17% at the highest dose level.

If the nonlinearity cannot easily be attributed either to reduction in induced mutation rate or to selective death of mutants, then it is worthwhile considering whether the shape of the dose-mutant curve can be satisfactorily explained in terms of gene interaction. Assuming that the frequency of induced mutations increased linearly with dose of radiation, it is evident from the high estimated frequency of minor mutants induced at 25 ergs per mm² that mutations must have been induced in nearly all if not all cells at higher doses. Further, the number of cells which contained two or more mutations must have been large. In some of these, all mutations would act in the same direction whether positive or negative. In others, both positive and negative mutations would occur. Whatever the phenotype of such cells, their increasing numbers would be expressed in a convexity of the dose-mutant frequency curve.

It might be expected that the estimated frequencies of mutant cells would eventually reach 100%. In fact, the combined frequency of positive and minor negative mutants did not exceed 50%, reaching its highest level at a dose of 75 ergs per mm². At least three factors may have contributed to this low ceiling. (1) Some fraction of the cells may have been resistant to radiation-induced mutation despite the fact that nonbudding cells were used. If so, the induced-mutation rate among sensitive cells must have been even higher than the data of Figure 5 imply. (2) Because minor mutant phenotypes did not differ greatly from normal, sampling errors would insure that a fraction of mutant clones was classified within the "nonmutant" category. This explanation is not, in itself, entirely satisfactory because the data of Table 2 indicate that there was a consistent drift toward the extremes in the mean relative growth values of both positive and minor negative mutants. This seems to imply either (a) a continuing accumulation of mutations within each cell whose effects were additive or more than additive, or (b) an increasing frequency of individual mutations of more extreme effect. In either case it would be expected that greater ease of mutant detection at higher doses would be reflected in a continued increase in estimated mutant

frequencies at higher doses. (3) Additive gene action in cells containing both positive and negative mutations might tend to produce cells of normal phenotype. If this tendency were strong an actual decline in mutant frequency would be expected at higher doses. Such a decline is suggested in the curve for minor negative mutations. Some of this decline may have been due to additivity of minor negative mutations which removed clones from the class of minor negative to that of major detrimental. On the other hand, past investigations (JAMES 1959) have indicated that segregations of severely affected clones usually yield a single highly detrimental mutant gene.

There was no indication that minor negative mutations are, as a class, epistatic to positive mutations. Since positive and minor negative mutations appeared with approximately equal frequencies at the lowest dose, one would expect such epistasis to be reflected in a negative shift in the mean relative growth values of the truncated groups with increasing dose. The data of Table 1 suggest that no pronounced shift occurred and it seems that negative mutations were as likely to be masked by positive mutations as the converse.

Various models incorporating these features were fitted by maximum likelihood methods to the observed frequencies. The superiority of one model over another cannot, however, be demonstrated on statistical grounds from these data. A fairly simple model for superimposed effects gave estimates of positive mutation rates that increased steadily with dose, but the estimated minor negative mutation rate showed a decline at the highest dose. A more complex model for superimposed effects is probably required, but with no basis for discrimination between models, further work on these lines was considered unwarranted.

A more thorough understanding of the interactions would, in principle, be provided by a genetic analysis of the segregants of the irradiated diploids. It is unfortunate that mutations of minor effect are not amendable to that type of analysis. As it is, these data provide no information about whether the positive mutants were expressions of simple dominance or of heterosis. Further, many of the minor effects may have been due to mutations which would have been severe, if not lethal, in the homozygous state.

Whatever the explanation for the nonlinearity of the dose-effect curve for minor mutants, it is evident that mutant frequencies at higher doses provide a poor estimate of induced mutant frequencies at lower doses. The ratio of minor to major mutants was more than 9:1 with exposure of 25 ergs per mm² and less than 2:1 with exposure to a dose of 200 ergs per mm².

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SUMMARY

The influence of radiation dose on the spectrum of heterozygous mutant effects on a quantitative character of diploid yeast was studied. The character was rate of cell division. Populations of homozygous cells were exposed to doses of 0, 25, 75, and 200 ergs/mm² of ultraviolet light. Vegetative progeny of individual cells

were tested for induced changes in rate of cell multiplication. The dose-effect curve was approximately linear for mutations causing a major reduction in rate of cell division. The dose-effect curves for positive mutants (increased rate of mitosis) and minor negative (decreased rate) were asymptotic or declined at higher doses. It was concluded that the frequency of detectable minor mutant phenotypes induced at higher doses gives a gross underestimate of mutation frequency.

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