RESPONSE OF POTASSIUM RETENTIVITY AND SURVIVAL OF YEAST TO FAR-ULTRAVIOLET, NEAR-ULTRAVIOLET AND VISIBLE, AND X-RADIATION*

By A. K. BRUCE‡

(From the Biology Division, Oak Ridge National Laboratory,§ Oak Ridge)

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ABSTRACT

Potassium retentivity and survival of yeast were studied after exposure to various kinds and conditions of irradiation. The radiations used were: 2537 A ultraviolet, 3500 to 4900 A long-ultraviolet and short visible, and 250 kvp[1] x-rays. Both potassium retentivity and survival are decreased by these radiations. The dose-response of survival is about 16 times as sensitive as is potassium retentivity after 2537 A irradiation. Potassium retentivity is about twice as sensitive as survival after irradiation of 3500 to 4900 A. Survival after x-irradiation under aerobic conditions is five times as sensitive as potassium retentivity. Survival of cells irradiated with x-rays under anaerobic conditions was about half as sensitive as under aerobic conditions. The response of potassium retentivity to x-radiation at 25°C. under anaerobic conditions is only slightly affected below 160 kr, at which dose the slope abruptly increases to that obtained under aerobic conditions; lowering the temperature to 0°C. moves this point to about 300 kr. These differential effects are indicative of interaction of radiations with the yeast cell at sites that independently control survival and the retention of potassium.

INTRODUCTION

Previous studies (Bair *et al.*, 1956) demonstrated that x-irradiation results in a decrease in the ability of the yeast cell to retain potassium. The response of this alteration was an exponential function of dose with a 50 per cent decrease in retentivity at about 60,000 r. A similar dose-response curve is found for the survival of yeast (Beam *et al.*, 1954), although the 50 per cent level is reached at a considerably lower dose. The site of interaction of radiation that results in cell death is considered to be nuclear (Lea, 1955). Substrate and

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‡ Present address: Biology Department, University of Buffalo, Buffalo 14, New York.

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[1] kvp, kilovolt peak.

temperature studies (Bruce and Stannard, 1957 *a*, 1957 *b*) suggest that radiation brings about an uncoupling of metabolic processes involved in active potassium transport. Such an alteration could either result from the interaction of radiation directly on some component of the cell surface involved in potassium exchange, or be mediated through an effect on the cell nucleus, that is in turn manifested by changes of the cell surface. This study was undertaken to determine whether the changes in potassium retentivity result from initial nuclear events, which would also be reflected in cell viability, or involve independent processes. If a direct relation between viability and retentivity of cellular potassium exists, the response of the cell to various radiations and conditions of irradiation would be expected to produce a similar effect on both criteria of radiation damage.

Materials and Methods

Suspensions of fresh, starved, bakers' yeast (Standard Brands) were exposed aerobically at 25°C. either to ultraviolet radiation of wave length 2537 A, to near-ultraviolet and short-visible radiation of wave length 3500 to 4900 A, or to x-radiation under oxygen or nitrogen. Control samples were manipulated similarly without being irradiated. For each type of radiation, dose-response curves of potassium retentivity and survival were determined. In addition, retentivity was studied after x-irradiation at 0°C.

Potassium loss was determined by the column elution technique (Rothstein and Bruce, 1957; Bruce and Stannard, 1957 *b*), with 500 mg. of yeast per column. This method permits the measurement of potassium efflux into a constant external environment. Samples were collected from the columns at 15 minute intervals with distilled water adjusted to pH 3.5 as the eluant, concentrated, and analyzed by flame photometry. The percentage of normal potassium retentivity is determined from the ratio of slopes of efflux from control to irradiated yeast over the period of measurement, usually 2 hours. Measurements of leakage were initiated within a half-hour after the termination of irradiation. Viability of the yeast was determined by surface-plating on potato–dextrose–agar (Difco) at pH 3.5 immediately after irradiation and scoring after 6 days' incubation at 25°C.

Since the phenomenon of photoreactivation is demonstrable in this organism after 2537 A irradiation (Warshaw, 1952; Dulbecco, 1955), its possible influence on these measurements was determined. After exposure to 2537 A radiation, a subsequent exposure to radiation of 3500 to 4900 A was given to a cell suspension and compared with the effect of either radiation alone. For this determination, 17 minutes of 2537 A radiation was used when potassium retentivity was to be measured, and 4 minutes for survival. The 3500 to 4900 A was given for 15 minutes for measurements of potassium retentivity, and for 5, 15, and 30 minutes for survival. Such exposures were chosen for placement of determinations within a measurable range of values. Manipulations were carried out under a yellow light of low intensity in this experiment and in all determinations of survival after 2537 A irradiations to prevent uncontrolled photoreactivation.

Radiations

2537 A.—The 2537 A irradiations were carried out in a 17 cm. crystallizing dish 18 cm. from a General Electric 15 watt germicidal lamp. The yeast suspension was 4 mg./ml. (wet weight). It was bubbled vigorously with air and stirred with two magnetic stirrers during irradiation.

3500 to 4900 A.—Four General Electric H-5 lamps were utilized for a source of near-ultraviolet and short-visible radiation. The yeast suspensions of 20 mg./ml. (wet weight) (4 mg./ml. for photoreactivation studies) were placed in pyrex tubes of 33 mm. diameter and bubbled with air for prevention of settling during irradiation. The sample tube was placed at the center of a constant temperature bath, 10.3 cm. in diameter, through which 10 per cent (*w/v*) copper sulfate solution was circulated to act as a filter and to maintain the sample at 25°C. The lamps provided with two Corning glass filters (Nos. 5850 and 7380) attached to the housings were equally spaced about the sample on a circle of 25 cm. diameter. The physical characteristics of this radiation are assumed to be similar to those described by Hollaender (1943).

X-rays.—The source of x-rays was a General Electric maxitron x-ray unit operated at 250 kvp and 30 ma., filtered with 1 mm. of added aluminum. Yeast suspensions of 20 mg./ml. (wet weight) were exposed in a Petroff type pyrex culture flask, bubbled vigorously with either oxygen or nitrogen, and placed in a controlled temperature water bath constructed of 7 mm. lucite and attached to the x-ray machine. The distance from the target to the center of the sample was 11.3 cm.

Dosimetry.—The incident intensity of the 2537 A lamp was measured with a meter designed and calibrated by Latarjet *et al.* (1953) and was 100 ergs/mm.[2] sec. The incident intensity from the source of 3500 to 4900 A was determined by actinometry with uranyl sulfate (Bowen, 1946) to be about fifty times that of the 2537 A source.[2] Dosimetry of the x-radiation was by the ferrous sulfate system (Hochanadel and Ghormley, 1953) with the oxidation of ferrous ion assayed by titration with potassium dichromate to the end point of diphenylamine. The dose rate was determined to be 4100 r.e.p./min.

RESULTS

The response of yeast to all radiations investigated involves a decrease in the ability of the cell to retain potassium and a decrease in survival. All curves were plotted semilogarithmically. Fig. 1 shows the responses to 2537 A ultraviolet light. Potassium efflux rates in the control samples averaged 6.0 mM/kg. of yeast per hour. Survival is far more sensitive to this wave length than is potassium retentivity.[3] The shapes of the curves are not exponential, survival curving upward at higher doses and potassium retentivity downward. Measurement of potassium retentivity could not be carried beyond an exposure

[2] The calibration of the 2537 A and the 3500 to 4900 A sources was kindly carried out by Dr. J. Jagger of this laboratory.

[3] Although the ultimate survival of the cells drops off rapidly with dose, at the time of measurement of potassium retentivity the cells are metabolically active.

of 20 minutes since flow rates through the columns became much lower at this point and the resulting pH across the column changed (see Bruce and Stannard, 1957 *b*). The packed cell volume was determined after such a dose by a hemotocrit type of technique and showed a larger cell volume than for the controls.

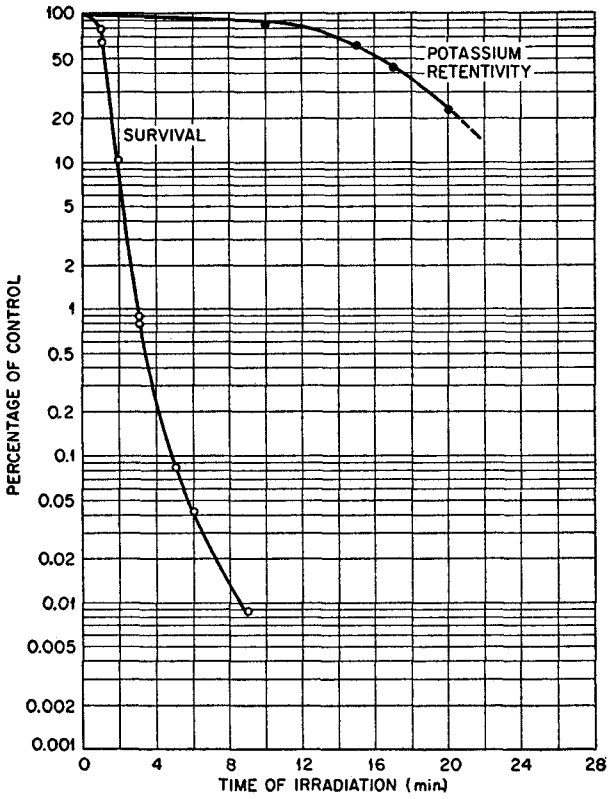

FIG. 1. Dose-response curves for 2537 A irradiation.

After exposure to radiation of 3500 to 4900 A, potassium retentivity is about twice as sensitive as is survival, as shown in Fig. 2. The response of survival is about exponential, although that for potassium retentivity curves downward at higher doses. In terms of absolute sensitivity, the 3500–4900 A radiation is far less efficient in producing effects on either measured process, being only $\frac{1}{300}$ and $\frac{1}{1000}$ as effective as 2537 A radiation for potassium retentivity and survival respectively.

The phenomenon of photoreactivation was present to a slight extent when the criterion was survival, but not when it was potassium retentivity. In fact,

the additional "reactivating" exposure brought about a further decrease in potassium retentivity. A dose of 2537 A radiation (17 minutes' exposure) gave 62 per cent of normal potassium retentivity. When followed by a reactivating exposure to radiation of 3500 to 4900 A (15 minutes), the retentivity was further reduced to 51 per cent of normal. The radiation of 3500 to 4900 A

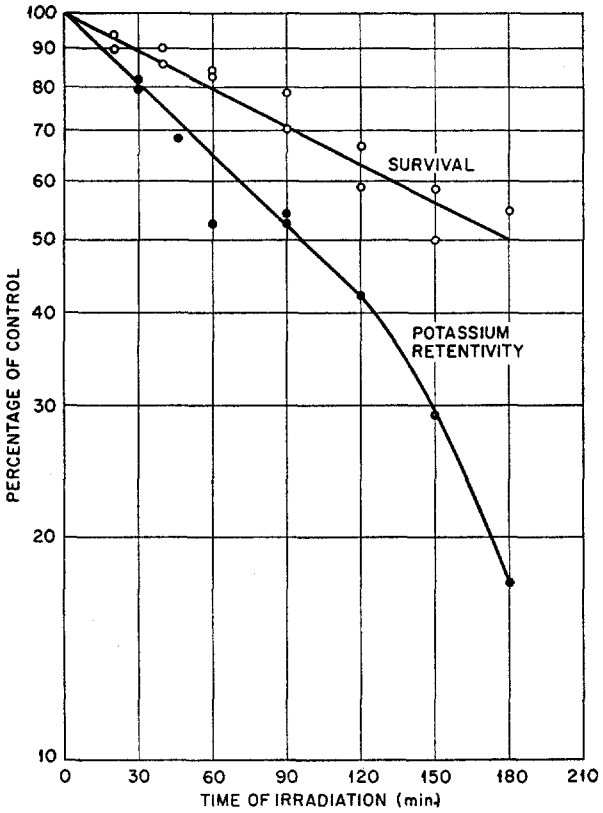

FIG. 2. Dose-response curves for 3500 to 4900 A irradiation.

alone gave 82 per cent of normal potassium retentivity. An exposure to 4 minutes of 2537 A radiation reduced survival to 0.95 per cent. When this was followed by reactivating exposures of 5, 15, and 30 minutes, progressively increasing survivals of 0.98, 1.2, and 1.6 per cent, respectively, were obtained. Exposure to 3500 to 4900 A (15 minutes) alone gave 85 per cent survival.

Survival is an exponential function of x-ray dose for this strain of yeast, regardless of whether oxygen or nitrogen is present during irradiation at 25°C. The 50 per cent survival level is reached at 12 kr in oxygen and at 30 kr in

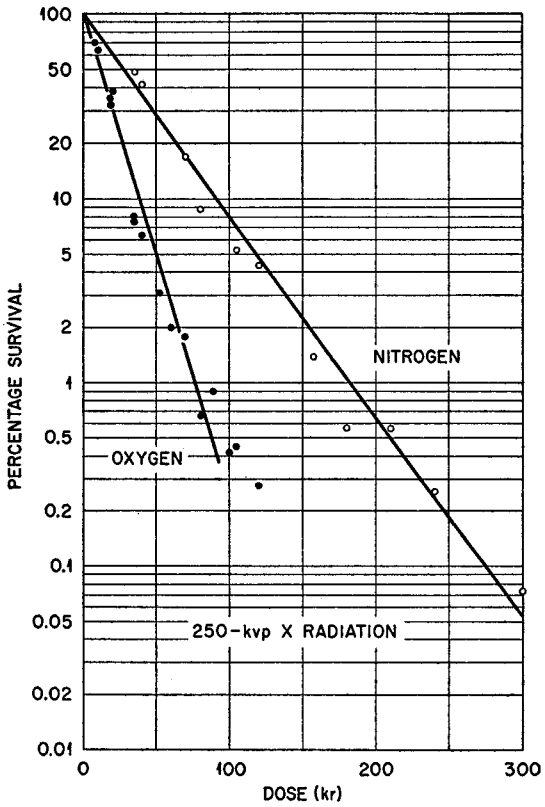

FIG. 3. Dose-response of survival for 250 kvp x-rays in nitrogen and in oxygen at 25°C.

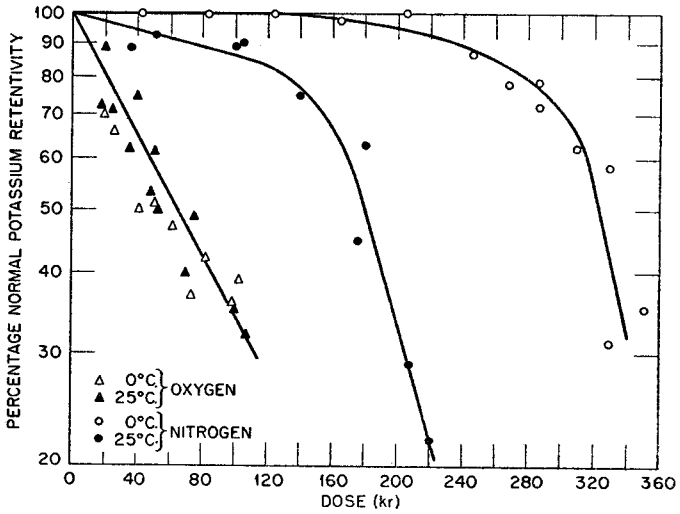

FIG. 4. Dose-response of potassium retentivity for 250 kvp x-rays in nitrogen and in oxygen at 0° and at 25°C.

nitrogen as shown in Fig. 3. This differential response was found in a number of organisms (Hollaender *et al.*, 1951; Burnett *et al.*, 1951). This simple relation was not obtained for potassium retentivity, as is shown in Fig. 4. Irradiation in oxygen yields an exponential response with the 50 per cent inactivation at about 60 kr. Irradiation in a nitrogen atmosphere not only changes the sensitivity of the response but also changes it to a sigmoidal relation. Lowering the temperature to 0°C. during irradiation shifts the response under nitrogen to considerably higher doses than when carried out at 25°C. Little, if any difference between response at 0° or 25°C. is observable if irradiation is carried out in the presence of oxygen.

The absolute rate of potassium efflux from the control yeast was altered as a result of treatments of temperature and atmosphere for time periods equal to exposure times. In oxygen at 25°C. the rate was 6.1 ± 0.8 (standard deviation) mM/kg./hr., while at 0°C. it was 6.8 ± 0.5 mM/kg./hr. After nitrogen treatment at 25°C. the rate was 8.0 ± 0.4 mM/kg./hr., while at 0°C. it was raised to 12.1 ± 0.8 mM/kg./hr. It should be noted that all measurements were carried out under aerobic conditions at 25°C. following the various treatments. Irradiated samples gave efflux rates ranging from that of the controls to approximately 40 mM/kg./hr. depending on the dose of radiation and the conditions under which it was carried out.

DISCUSSION

The comparative effect on potassium retentivity and survival is different for each change in the conditions of irradiation. The dose required to reduce each criterion of radiation damage to 50 per cent of normal is shown in Table I. The sensitivity of survival is seen to vary from 0.5 to 16 times that of potassium retentivity, depending on conditions. These differential effects are indicative of interaction of radiations with the yeast cell at sites that independently control survival and the retention of potassium.

Although the study of photoreactivation was undertaken mainly to determine whether measurements of potassium efflux had to be carried out under altered lighting conditions, the results further support the hypothesis that independent processes are involved in the manifestation of damage to the two systems studied. The conditions used were obviously not optimal for photoreactivation since the increase in survival was so small in comparison with the results of Warshaw (1952). This is caused by the limitations imposed by the high concentration of yeast required for potassium measurements. In spite of suboptimal reactivating conditions, the finding of effects in opposite directions for the two phenomena is a strong argument in favor of separate processes.

The loss of potassium by irradiated cells may have an effect on their behavior when studied on plates as in the assay of viability (see Rothstein and Demis, 1953). Studies by Bair and Stannard (1955 *a*, 1955 *b*) on the influence

of removal of potassium from normal and irradiated yeast by treatment with the cation exchange resin Dowex 50 revealed a lower metabolic rate, as measured by gas exchange, in the irradiated cells than in the controls. If potassium was restored to the cells, the metabolic rate of the irradiated cells approached that of the controls. This would be expected to result in a lengthened lag time for the formation of visible colonies from irradiated cells, as was found for growth in liquid culture. In the present experiments, as in those involving ultraviolet irradiation, irradiated yeast exhibited delayed colony formation (see also Hollaender and Duggar, 1937, 1938) requiring 5 to 6 days for a maximum of visible colonies rather than 2 days as in the controls. The loss of potassium may not be the only factor contributing to this behavior since, in preliminary experiments, the addition of potassium to the medium (to 0.2 M) resulted in a lower rather than higher colony count after 3500 to 4900 A irradiation.

TABLE I

Effect of Various Radiations on Potassium Retentivity and Survival

Radiation (at 25°C.)	Dose for 50 per cent of control value		*R:S*
	Survival (*S*)	Retentivity (*R*)	
2537 A	1 min.	16 min.	16
3500 to 4900 A	180 min.	96 min.	0.5
X-radiation (O_2)	12 kr	60 kr	5
X-radiation (N_2)	28 kr	180 kr	6.5

The high protection afforded the system responsible for potassium retentivity by x-irradiation in nitrogen as compared with oxygen, in contrast to survival, requires an explanation. Several possibilities exist in this respect: (1) The curves as shown could represent a multiple hit type of phenomenon in nitrogen. An extrapolation according to the Atwood and Norman (1949) analysis yields hit numbers from about 10 to 100, depending on the fitting of the experimental points and the temperature at which irradiation was carried out. Such values do not correspond to any known biological entity involved in potassium exchange, and the variation with temperature during irradiation renders this type of analysis invalid. (2) During anaerobic metabolism, compounds possessing considerable reducing potential (Conway and Kernan, 1955) are known to accumulate. Such compounds may act as "protective" substances. Burnett *et al.* (1951) and Alexander *et al.* (1955) demonstrated protection by ethanol and cysteamine. The shapes of the curves are indeed what would be expected for a limiting quantity of such a compound; *i.e.*, protection until the quantity of material present is exhausted by reaction with oxidizing products of the irradiation followed by an inactivation

at the rate of the unprotected system. The temperature data, however, do not support such a conclusion since increased temperature would be expected to yield a greater quantity of this protective material and result in a displacement of the sharply dropping portion of the curve to higher doses. Instead, the higher temperature displaces the curve to lower doses. (3) A change in slope could result if the curve for irradiation in nitrogen is not caused by an effect on a single system, but rather by two processes contributing to the retention of potassium. At the lower dosage levels, the potassium loss could be through the system primarily responsible for monovalent cation retention, although at the higher doses a more general damage to the membrane could result, releasing a number of intracellular constituents, including potassium. Microscopic examination of the cells after high doses delivered in nitrogen and preliminary studies on the dose-response of the loss of 260 mμ absorbing material and amino acids by methods similar to those used by Billen (1957) do not substantiate this explanation. The appearance of the cells is normal. Both 260 mμ absorbing material and amino acids are lost, but the dose-response to x-rays under nitrogen does not correspond to that for potassium retentivity. Further studies are in progress and projected that, it is hoped, will test the previously mentioned hypotheses and elucidate the behavior of the system responsible for potassium retention in the yeast cell in response to irradiation under anaerobic conditions.

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