Effect of High Oxygen Tension on Potassium Retentivity and Colony Formation of Bakers' Yeast

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ABSTRACT The ability of yeast cells to retain potassium and to form colonies was studied after exposure to pressures ranging from 2 to 143 atmospheres of oxygen. The investigations allow comparison of these responses with those found after x-ray exposure. Exposure to 2 to 8 atmospheres of oxygen for 2, 20, and 40 hours showed decreased potassium leakage as measured by an elution technique. Further experiments using 0.5 to 22 hour exposures to 10 to 143 atmospheres of oxygen showed decreased potassium leakage when glucose was present in the test media, but increased leakage (as did x-ray effects) in the absence of substrate. There was increased potassium leakage into the suspending media (distilled water) during oxygen exposure but this usually did not affect the leakage rates measured subsequently. Marked inability to form colonies was observed after 20 hour exposures to 100 atmospheres of oxygen, with a much smaller response at lower pressures. Increased oxygen concentrations, not pressure, evidently caused these effects, since comparable pressures of nitrogen produced almost no change. The ratio of potassium leakage to survival sensitivity was found to be approximately unity when comparing exposures causing 50 per cent damage. This is quite different from that seen with x-ray or ultraviolet irradiation.

INTRODUCTION

Investigations in the field of oxygen toxicity (1, 2) have shown many effects in both unicellular and highly developed organisms which are similar to those observed after exposure to ionizing radiation. Possibly similar basic mechanisms of action have been postulated on the basis of formation of free radicals (1). The radicals $HO^{\cdot}$ and $HO_2^{\cdot}$ are formed in water by ionizing radiation, and oxidizing radicals can also conceivably be formed (often denoted as $RO_2^{\cdot}$) by the combination of oxygen with normally produced free radical intermediates of metabolism.

Earlier work from this laboratory has shown that in addition to its well known effects on growth and colony formation, irradiation has pronounced effects on the mechanisms of electrolyte exchange in yeast as measured by the transfer of potassium or sodium ions. This is reflected, for example, in increased leakage of K^+ (3). It may be due, at least partly, to the inactivation at the cell surface of certain enzymes which are necessary for the active transport of the potassium ion (4).

The studies presented here explore the action of increased oxygen tensions upon the potassium leakage and colony formation of yeast, in order to allow further comparison between the effects of ionizing radiation and those of increased oxygen tension. A report indicating alteration of potassium exchange in yeast by high oxygen tensions had already been published by Mullins (5).

MATERIALS AND METHODS

The bakers' yeast used in these experiments was obtained weekly from Standard Brands Inc., and was washed and starved for 3 hours before use by bubbling washed air through a suspension of the cells in distilled water.

Exposure to 2 to 8 atmospheres of oxygen was performed in windowed chambers allowing a flow through of gas under pressure; exposures to 10 to 143 atmospheres of oxygen utilized heavy steel-walled, sealed chambers without flow through. Pressures were read from Hoke high pressure gauges, and the oxygen was obtained from standard oxygen tanks (peak pressure about 2100 psi). Exposures were always done with the cells suspended in distilled water. The 2 to 8 atmosphere exposures were performed with the suspension in open Petri dishes. The resulting suspension depth of 0.8 cm allowed near saturation of the suspension with the gas (by Henry's law, 1.26 mM O_2/atmosphere). The heavy steel chambers of the 10 to 143 atmosphere experiments admitted small cylindrical glass vials containing 15 ml of suspension; these gave a suspension depth of 5.6 cm. Gilbert *et al.* (6) using this apparatus and water volumes of 12 ml, have found that the time needed for 50 per cent saturation under 143 atmospheres was approximately 1.5 hours. It is thus possible that equilibrium was not reached in some of our shorter experiments. The pressures recorded must therefore be considered as exposure pressures and not a dosimetric unit. In the longer experiments, saturation was more nearly complete (75 per cent in 3 hours).[1]

After each exposure potassium leakage was measured by the column elution technique developed by Rothstein and Bruce (7), using 300 ml yeast suspension per column. Since concentrations used in the experiments were 12 or 15 mg/ml the amount of yeast on the sintered glass plate was 3.6 or 4.5 gm. This produced a thin layer which permitted easy passage of fluid.

The test medium was distilled water brought up to pH 3.5 with HCl (H^+ exchanges for K^+) with or without 0.005 M glucose as substrate. These test media were passed through the thin layer of yeast in each column under 3 psi of air pressure, so that any potassium leaked by the yeast was immediately eluted into the fraction being

[1] Shaking or stirring, while desirable, was not technically feasible.

collected below. Thus absolute amounts (rather than net) of leaked cation were measured. The effluents containing leaked cations were collected in 15 minute fractions over a 3 hour period, concentrated, and then analyzed for potassium using a Weichselbaum Varney universal spectrophotometer (Fearless Camera Corporation). Control samples were prepared and analyzed in exactly the same manner but were not exposed to increased oxygen tensions.

In spite of the greatest care as to time and conditions of storage, date of shipment, etc., there appeared an inherent (and unpredictable) variation from week to week in both the amounts of potassium leaked and the number of colonies formed by this commercial yeast (see Tables I through VI). Therefore, control samples were run with each group of experimental points, and all experimental values are considered with respect to their particular control values.

In addition, the leakage rates during the 1st hour, *i.e.* the first two or three fractions collected after exposure, showed wide variability and no consistent pattern from experiment to experiment. This was perhaps due to an undefined initial reaction when the yeast was placed in the acidic test medium. Effluent fractions taken during the subsequent periods of the test showed more uniform and consistent rates of K^+ leakage (average deviation about 10 per cent of the mean, range from 3.2 to 29 per cent of the mean in experiments at yeast concentrations of 10, 12, 20, and 60 mg/ml).

Yeast concentration was determined by yeast volume to suspension volume measurements taken from samples centrifuged for 5 minutes at 525 × gravity in Wintrobe hematocrit tubes. A "specific gravity" of unity was arbitrarily assumed but the determination was checked periodically by dry weight determinations.

Immediately after exposure 0.1 ml aliquots of the suspensions were diluted to yeast concentrations of 8×10^{-5} mg/ml in order to test for ability to form colonies. Plating 0.1 ml of these diluted suspensions per Petri dish (Sabouraud dextrose agar medium) gave between 50 and 100 visible and distinct colonies for unexposed yeast after 3 days' incubation at 25°C. Other details of technique are described by Stuart (8).

RESULTS

1. Potassium Leakage Rates

The first measurements presented are comparable to those described earlier (3) using x-irradiation. The rates of K^+ efflux, using the elution technique, after exposure to high pressures of oxygen or nitrogen are shown in Tables I and II. The figures represent the mean rate in the 2nd and 3rd hour after exposure, measured from the eight fractions collected during this period. The 1st hour is omitted for the reasons given in the section on methods.

The first series (Table I) utilized pressures from 2 to 8 atmospheres. No substrate was present in the eluting fluid. The rates of leakage showed a relative decrease (last column of table) in the cells exposed to oxygen at 2 to 6 atmospheres but almost no change at 8 atmospheres of oxygen or after exposure to 6 atmospheres of nitrogen. The rates showed no consistent tendency to decrease with time during the test.

The leakage rates after exposure to pressures from 10 to 143 atmospheres are detailed in Table II. The rates with glucose present in the test media (part A) are seen to be much higher than those in the absence of glucose (part B), and they tended to decrease during the test period.

The effect of high oxygen pressures is quite different in these two cases. In the absence of substrate the predominant effect is increase in K^+ leakage. In glucose it appears that one effect may be a reduction in the ability of glucose to increase the leakage rate normally seen in its presence.

TABLE I

POTASSIUM LEAKAGE AT 2 TO 8 ATMOSPHERES

		K^+ leakage rates, *mM K^+/kg/hr.*				
		2nd hr.		3rd hr.		Ratio*
Exposure pressure	Exposure time	Experimental	Control	Experimental	Control	Experimental/ control
atmospheres	*hrs.*					
			Oxygen			
6‡	0.5	2.3	3.2	1.8	2.4	0.74
6‡	0.5	2.1	3.2	1.7	2.4	0.68
2‡	2	4.5	5.8	4.5	5.4	0.80
5‡	2	3.8	4.8	3.8	4.4	0.83
8‡	2	4.6	5.1	4.8	4.4	0.99
2§	20	2.5	5.5	4.7	9.1	0.49
5§	20	2.9	5.1	6.6	9.3	0.66
8§	20	3.8	4.3	6.9	7.2	0.93
2‡	40	0.7	1.4	0.7	1.6	0.47
5‡	40	1.1	1.3	1.1	1.3	0.85
8‡	40	1.5	1.4	1.3	1.4	1.00
			Nitrogen			
6‡	0.5	3.0	3.2	2.4	2.4	0.96
6‡	0.5	3.1	3.2	2.4	2.4	0.98

* Average over 2 hour period.
‡ Yeast concentration 12 mg/ml.
§ Yeast concentration 15 mg/ml.

The effects of 20 hour exposures to oxygen pressures between 10 and 143 atmospheres are shown graphically in Fig. 1. The upper curve shows the increase in K^+ leakage seen in the absence of glucose while the lower curve (with 0.005 M glucose present) shows the opposite trend. The response in the absence of glucose increases markedly above 80 atmospheres. The response in the presence of glucose is essentially a decrease in leakage rate relative to the high normal rate in glucose and may reflect an inability of the cell to respond to the metabolic stimulus of this substrate.

An effect of exposure time is evident also. Results of a few experiments utiliz-

ing exposure times shorter than 20 hours (Tables I and II, and reference 8) showed considerably less effect (see below) while exposures for as long as 40 hours resulted in considerable loss of K^+ during the exposure period and a critical decrease in available K^+ for the leakage tests. Hence the data for 20 hours' exposure were chosen for Fig. 1 as most illustrative of the effects seen.

TABLE II

K^+ LEAKAGE RATES AT 10 TO 143 ATMOSPHERES OF OXYGEN OR NITROGEN*

Exposure pressure	Exposure time	K^+ leakage rates, (*mM K^+/kg/hr.*) 2nd hr. Experimental	2nd hr. Control	3rd hr. Experimental	3rd hr. Control	Ratio Experimental/control
atmospheres	*hrs.*					
		A. Glucose in test media				
10.2	2	20.4	20.5	14.0	13.3	1.02
10.2	20	17.3	13.9	14.2	15.0	1.09
20.4	20	8.7	8.0	7.0	5.0	1.21
40.8	20	10.7	13.3	8.6	8.9	0.87
74.8	20	8.2	14.0	7.9	9.4	0.69
140.8	0.5	13.1	14.9	9.6	10.3	0.90
142.8	22	8.6	11.2	4.5	10.0	0.62
61.2‡	20	15.3	17.7	12.5	15.3	0.84
120.6‡	20	11.7	10.5	8.1	7.0	1.13
		B. Without glucose				
34	20	3.0	2.5	2.9	2.4	1.20
61	20	3.8	3.4	3.0	2.6	1.13
74.8	3	1.8	1.8	—	—	1.00
74.8	20	4.6	3.0	3.9	3.4	1.33
91.2	20	1.9	1.6	1.6	1.1	1.30
92	20	3.8	3.3	2.2	2.0	1.13
107.8	3	1.8	2.0	—	—	0.90
108.4	20	9.1	3.0	6.2	3.4	2.39
142.2	1	3.6	1.6	—	—	2.25
141.2	10	6.4	4.4	—	—	1.45
142.5	20	11.6	3.0	10.3	3.4	3.42

* Yeast concentration 10 mg/ml.
‡ Atmospheres of nitrogen.

2. *Potassium Leakage during Exposure*

It was obviously important to know whether or not significant loss of potassium occurred during the exposure period. Therefore, analyses of the exposure media were made in some experiments after removal of the cells for rate studies by the elution technique. The amounts of K^+ accumulated in the media during exposures at pressures from 10 to 143 atmospheres are shown in

Table III. It is apparent that the quantities of K^+ lost during exposure were appreciable and were related to the oxygen pressure. Also the effect of time of exposures can be noted as in the leakage rate measurements.

Despite the fact that these data represent *net* K^+ loss rather than a true rate they show a response pattern quite similar to that in Fig. 1 (upper curve). Since exposure was always carried out in distilled water no information comparable to that in Fig. 1 (lower curve) was obtained.

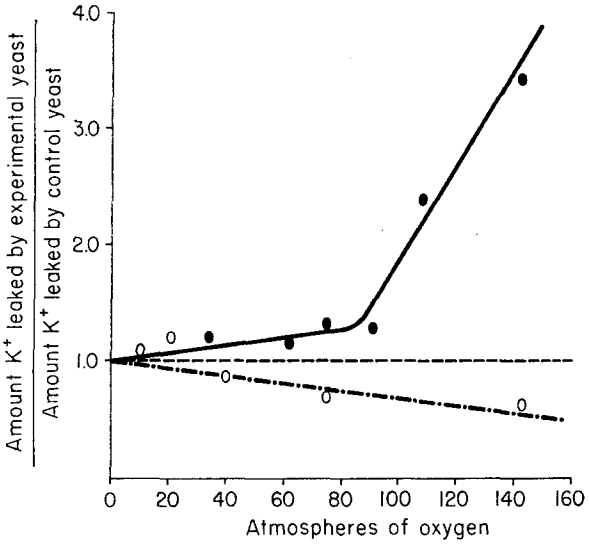

FIGURE 1. Effects of oxygen pressures on relative potassium leakage rates of yeast. All exposures, 20 hours; points represent averages over 2 hour measurement periods. Open circles represent leakage tests with 5×10^{-3} M glucose in test medium; solid circles represent leakage in distilled water. Individual points can be seen in Table II.

3. Total K^+ Loss

The sum of potassium losses during exposure and in the elution test is illustrated in Tables IV and V. These tables were constructed from the previous data by assuming the K^+ loss rate during the 1st hour of the elution test to be 1.5 times the rate during the 2nd hour. Selected experiments using 20 hour exposures are shown in these tables. Shorter times and additional pressures can be examined if desired in Tables II and III.

The amounts of potassium found in the exposure media were generally considerably less than those lost in the subsequent column elution test. The net potassium leakage during a 20 hour exposure approached that leaked during a 3 hour elution test only at the highest pressure studied. The highest loss during exposure was about 30 per cent of the total K^+ in the cell (100 mM/kg) and the highest total loss about 70 per cent of the total available potassium. While the

leakage rates in these extreme instances might have been still higher if the cell had possessed its initial K^+ content, these facts indicate that in general the cells had not been unduly depleted of exchangeable K^+ before the leakage rate was measured.

TABLE III

NET K^+ LOSS TO MEDIUM DURING EXPOSURE PERIOD

Exposure pressure	Exposure time	Potassium loss $mM\ K^+/kg$		Ratio
		Experimental	Control	Experimental/control
atmospheres	*hrs.*			
		Oxygen		
10.2	2	1.2	2.0	0.67
10.2	20	3.4	3.6	0.94
34.0	20	5.7	3.2	1.78
40.8	20	4.8	2.5	1.92
61.2	20	10.5	4.8	2.19
74.8	3	2.4	2.3	1.04
74.8	20	5.6	2.5	2.24
85.0	20	8.0	3.6	2.22
91.8	20	6.7	5.0	1.34
101.3	20	15.0	2.5	6.00
107.8	3	2.5	2.5	1.00
111.2	20	17.8	2.5	7.12
120.3	20	17.2	2.8	6.14
131.6	20	21.7	2.5	8.68
140.8	0.5	1.5	1.1	1.36
141.1	3	2.0	1.3	1.54
142.8	22	34.4	2.5	13.8
143.0	20	31.0	2.8	11.1
		Nitrogen		
61.2	20	5.7	3.6	1.58
61.2	20	3.4	2.9	1.17
119.0	20	6.0	3.6	1.67
120.3	20	4.5	2.8	1.61
120.6	20	4.4	2.8	1.57

4. Colony Formation

The relative numbers of colonies formed after exposure to high oxygen pressures were taken as an additional criterion of damage for comparison with x-ray effects. Fig. 2 shows the effect of exposure to high oxygen pressures for 20 hours upon the ability of this yeast to form colonies. The results are presented in terms of $\frac{\text{"No. of experiment colonies"}}{\text{No. of control colonies}}$, the growth plates being scored after 3 days' incubation. There is a significant decrease in the ability of the

TABLE IV

TOTAL POTASSIUM LOSS AT HIGH PRESSURES OF OXYGEN

Elution test in distilled water, pH 3.5. Exposure time, 20 hours

Pressure	Leakage during exposure	Elution test Leakage rate, mM K^+/kg/hr.			Total K^+
		1st hr.*	2nd hr.	3rd hr.	
atmospheres	*mM K^+/kg*				*mM K^+/kg*
34.0	5.7	4.5	3.0	2.9	16.1
Control	3.2	3.8	2.5	2.4	11.9
61.2	10.5	5.7	3.8	3.0	23.0
Control	4.8	5.1	3.4	2.6	15.9
74.8	5.6	6.9	4.6	3.9	21.9
Control	2.5	4.5	3.0	3.4	13.4
91.8	6.7	5.7	3.8	2.2	18.4
Control	5.0	5.0	3.3	2.0	15.3
108.4	17.8	14.0	9.1	6.2	57.1
Control	2.5	4.5	3.0	3.4	13.4
142.5	31.0	17.0	11.6	10.3	69.9
Control	2.8	4.5	3.0	3.4	13.7

* Calculated, see text.

TABLE V

POTASSIUM LOSS AT HIGH PRESSURES

Elution test in 0.005 M glucose, pH, 3.5, exposure time, 20 hours

Pressure	Leakage during exposure	Elution test Leakage rate, mM K^+/kg/hr.			Total K^+
		1st hr.*	2nd hr.	3rd hr.	
atmospheres	*mM K^+/kg*				*mM K^+/kg*
		Atmosphere: oxygen			
10.2	3.4	26.0	17.3	14.2	60.9
Control	3.6	21.0	13.9	15.0	53.5
20.4	4.0‡	13.0	8.7	7.0	33
Control	3.0	12.0	8.0	5.0	28
40.8	4.8	16.0	10.6	8.6	40.0
Control	2.5	20.0	13.3	8.9	44.7
74.8	5.6	12.0	8.2	7.8	33.6
Control	2.5	21.0	14.0	9.4	46.9
142.8	31.0	13.0	8.6	4.4	57.0
Control	2.8	17.0	11.2	10.0	38.2
		Atmosphere: nitrogen			
61.2	4.5	17.0	11.6	8.1	41.2
Control	3.2	16.0	10.5	7.0	36.7
120.6	5.0	23.0	15.3	12.5	55.8
Control	3.1	27.0	17.7	15.3	63.1

* Calculated, see text.

‡ Interpolated.

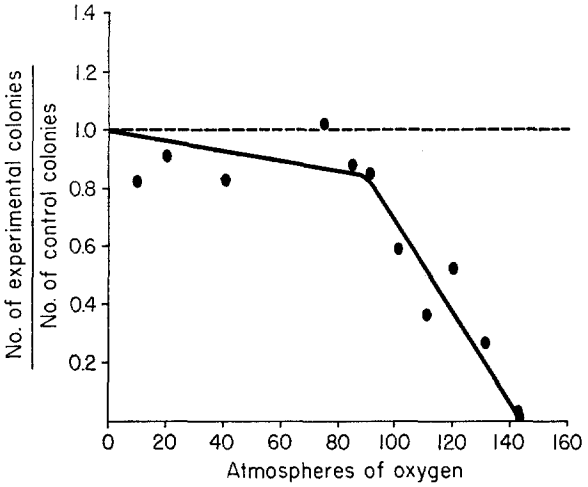

FIGURE 2. Effect of prior exposure to oxygen pressures above 8 atmospheres on colony formation in yeast. Exposure time, 20 hours, growth time, 3 days.

TABLE VI
EFFECT OF HIGH GAS PRESSURES ON COLONY FORMATION

Pressure	Exposure time	No. of colonies per plate*		Experimental/control
		Experimental	Control	
atmospheres	*hrs.*			
		Oxygen		
10.2	2	52	52	1.00
10.2	20	42	51	0.82
20.4	20	50	55	0.91
40.8	20	48	58	0.83
74.5	20	49	48	1.02
85.0	20	66	75	0.88
91.2	20	68	80	0.85
101.3	20	36	61	0.59
111.2	20	22	61	0.36
120.3	20	49	94	0.52
131.6	20	16	61	0.26
140.8	0.5	59	48	1.23
142.8	20	1	34	0.03
143.0	20	1	94	0.01
		Nitrogen		
61	20	81	75	1.08
119.0	20	78	75	1.04
120.6	20	52	52	1.00
120.6	20	85	94	0.90

* Mean of three or four plates.

exposed cells to form colonies. Also, as with the K^+ leakage effects, a possibly two component response was found, with an indication of progressively greater damage beginning between 80 and 100 atmospheres of oxygen. Complete data on colony formation are contained in Table VI. There was little effect of an exposure for 2 hours at 10.2 atmospheres, and no decrease in colonies after 30 minutes at 140.8 atmospheres.

5. Tests with Nitrogen

In order to determine the extent, if any, to which pressure alone contributed to the above effects, several tests were performed using the relatively inert gas nitrogen at comparable pressures. The results are contained in Tables I, II, III, V, and VI. This treatment did not appreciably alter the K^+ leakage rate at 6 atmospheres. There was a possible effect of N pressure on total potassium loss at 61.2 and 120.6 atmospheres (Table V). But since these effects are small and in opposite directions at the two pressures they are not considered significant. Analyses of the exposure media showed a slight increase in K^+ ion accumulation in the medium during exposure in nitrogen (Table III) but these are small in relation to the effects of oxygen at comparable pressures. The colony formation was essentially unaffected by nitrogen pressures of 61.2 or 120.6 atmospheres. Therefore, the effects observed in this study were at least principally and perhaps entirely due to the presence of high concentrations of oxygen and not to the pressures involved *per se*. In this connection, hydrostatic pressures of 10,000 psi have been found to have no effect upon the x-ray inactivation of *Saccharomyces cerevisiae* (9).

DISCUSSION

The present experiments indicate that exposure to high oxygen pressures can indeed bring about changes in the behavior of the yeast cell membrane as measured by potassium loss under several conditions. These are qualitatively similar to those observed after x-irradiation, but there may be quantitative differences. For example, in distilled water media there appears to be a decrease in K^+ leakage at the lower oxygen pressures, while at higher pressures a definite increase in potassium leakage is noted. No such dichotomous response was seen in the x-ray data gathered under similar conditions. (This may be only apparent because there were no x-ray doses below 10 kiloroentgens. However, the slope of the dose-response curve after irradiation gives no hint of an initial phase of decreased leakage for K^+ ions. Thus, the experiments of Bair *et al.* (10) indicate a single exponential dose-response curve from 10 to 90 kiloroentgens.)

Second, the responses to substrate appear to be different. Absolute leakage rates always increase markedly in the presence of glucose. In irradiation ex-

periments this increase is apparently identical in irradiated and control cells with no change in relative rates. After exposure to high oxygen pressure the leakage rate is increased *less* by glucose than in unexposed cells. Hence, the *relative* leakage rate is decreased by exposure to oxygen tensions above 20 atmospheres in contrast to the x-ray studies, indicating perhaps an interference with the ability of glucose to increase the leakage rate after high O_2 but not after irradiation.

Third, the potassium leakage response to high oxygen tensions appears to increase greatly beyond a certain "critical pressure" (about 80 to 100 atmospheres). This was not seen in the aerobic x-ray exposures since these could be described by a single exponential relationship (10). However, Bruce subsequently (11) found a two component response of potassium retentivity of yeast after exposure to ultraviolet irradiation and to x-rays under anaerobic conditions. These changes in slope were not as marked as in the present experiments. As was suggested by Bruce, the first part of this two component effect could reflect damage to the active transport system for potassium ion; the second part could indicate more general damage to the cellular membrane. The survival studies (Fig. 2) also show a possible two component effect with a change in shape of the response curve occurring also at about 80 to 100 atmospheres' exposure pressure.

A comparison of the effects of high oxygen pressure and several forms of irradiation is afforded by calculating the doses required to produce 50 per cent "damage" in each case. The ultraviolet light and anaerobic x-ray data are from Bruce (11); the aerobic x-ray points from Bruce and Stannard (3), plus a few direct comparisons done currently.[2]

	*K^+ leakage rate** (*R*)	*Colony formation* (*S*)	*R:S*
Ultraviolet radiation (3500 to 4900 A)‡	96 min.	180 min.	0.5
Ultraviolet radiation (2537 A)‡	16 min.	1 min.	16
X-irradiation (aerobic)	60 kr.	12 kr.	5
X-irradiation (anaerobic)	180 kr.	28 kr.	6.5
High oxygen pressure (20 hrs.)	109 atm.	113 atm.	0.96

* Without glucose in the test medium.

‡ The incident intensity for 2537 A was 100 erg/mm² sec.; that of the 3500 to 4900 A was about fifty times as great (11).

The K^+ retentivity and colony formation responses to high oxygen pressures show nearly equal sensitivities at the 50 per cent damage points giving an R:S ratio of about 1. This lies between those for near ultraviolet (3500 to

[2] It has been found that the radiosensitivity of the commercial yeast can vary with time. Thus, the yeast utilized here has been found to be 1.5 to 2.5 times more radioresistant in 1958 than was the case in 1955. However, simultaneous measurement of x-ray sensitivity and of oxygen effects showed the ratios quoted, and these agree well with those found by Bruce and Stannard (3).

4900 A) (below 1) and x-irradiation or 2537 A ultraviolet irradiation (above 1). The differences in these ratios make it seem likely that different sites of action are involved in the types of damage caused by these various agents and speak against nonspecific damage or cell death as an explanation of the effects. Because of these relations it would be unwise to attempt to draw specific damage equivalents for the different agents without much additional information. The possibility that the effects reflect only cell death or an all-or-none response in the case of high oxygen pressures is also negated by the effect of substrate, and measurement of cell staining with methylene blue. In all experiments, an aliquot of the suspension was stained with methylene blue as described by Bair (12). Failure of yeast to stain with this dye has been commonly accepted as a criterion of viability. In no case did more than 5 per cent of the cells take the stain after exposure to either oxygen or nitrogen.

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