ROLE OF ELECTROLYTES AND STARVATION IN ALTERING APPARENT RADIOSENSITIVITY OF BAKERS' YEAST*

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Ordinarily respiration and fermentation in yeast and other microorganisms are not markedly altered by radiation doses sufficient to prevent colony formation. However, the literature reveals various reports of both stimulation and inhibition of gas exchange processes after exposure to ionizing radiation. Although Wels and Osann (23) and Runnström (13) found no change in respiration or fermentation of yeast which had been exposed to doses as high as 120,000 r, other authors report varying degrees of both stimulation and inhibition. Von Euler (7) found a 40 per cent increase of respiration above normal following irradiation. In other work von Euler *et al.* (6) found a small decrease. Fardon *et al.* (8) produced a 20 per cent increase of oxygen consumption by exposing yeast to 45 kv. x-rays. Sherman and Chase (18, 19) found a 30 per cent inhibition of fermentation after exposure of yeast grown in a synthetic medium to 90,000 r x-rays. This dose was sufficient to inhibit colony formation 85 per cent. Schneider (14) and Barron *et al.* (4) report similar findings.

This situation indicated that several factors were probably operating and that experimental conditions were of considerable importance. The present study is part of a formal attempt to separate out the various factors involved (2, 3). It shows that the cationic content of the medium, particularly potassium, plays a major role in some of the observed effects of x-irradiation.

Materials and Methods

The bakers' yeast used in this investigation was obtained in pound blocks weekly from Standard Brands, Inc., and cultured 24 hours in a synthetic medium described

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by Spoerl *et al.* (20).[1] The yeast was cultured with aeration in an apparatus described by Brockman and Stier (5) and modified by Bair (2). Dry weight, cell counts, and turbidity measurements (employing the Rouy-Leitz photrometer) were used to estimate the quantity of yeast in a given suspension. All cultures were examined both microscopically and by plating procedures for possible contaminants. Despite the possibilities for contamination from block yeast no interferences occurred within the 24 hour culture period. Preliminary experiments revealed that suspensions of approximately 30 per cent transmission measured with a 520 mμ filter provided sufficient concentration of yeast (0.65 mg./ml., dry weight) to enable respiration and fermentation measurements to be made on 1 or 2 ml. of suspension. Yeast was harvested from the culture medium by washing twice with distilled water and centrifuging for 2 minutes at 4000 R.P.M. in a Servel, model SS1 angle centrifuge. This was sufficient to separate the large whole cells from the small cells and cell fragments. The yeast was irradiated in distilled water at a concentration of 0.65 mg./ml.

Irradiation Procedure.—The yeast suspensions were divided into two portions; one to be irradiated and one to serve as a control. The portion to be irradiated was placed in a container consisting of the bottom 1½ inches cut from a 2 liter beaker, and covered with an ⅛ inch sheet of lucite which was perforated to allow the escape of oxygen. Oxygen was bubbled through the suspensions throughout the irradiation period to provide stirring and the pO_2 necessary to yield maximum radiation effects (1, 9). The control was similarly treated except that it was shielded by ½ inch of lead while in the x-ray room.

The irradiation factors were standardized at the beginning of this work: 250 kv., 15 ma., approximately 1850 r/min. (varied slightly with each exposure), no filter, and 6½ inch target distance (to center of suspension). A 250 kv. Picker industrial x-ray unit was used. Dosage measurements were made in air with the center of the 1000 r Victoreen thimble[2] chamber 6½ inches from the target. After the dosage measurements had been made, the dish containing the yeast suspension was inserted into position without moving the machine. The dosage measurement was corrected for temperature and barometric pressure.[3] The error due to scatter was found not to exceed 10 per cent of the total dose.

All irradiation exposures were carried out under these same conditions and at room temperature of 26° ± 3°C.

Starving.—A distilled water suspension of yeast was placed in a 500 ml. aeration bottle fitted with a fritted glass disk of coarse porosity. Washed air passed through sterile glass wool was bubbled through the suspension for 24 hours. The yeast was then centrifuged and washed once with distilled water and resuspended in distilled water to the desired concentration.

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[1] The glucose content was reduced to 20 gm./liter and the thiamine hydrochloride was reduced to 400 mg./liter to conform more closely with the levels prescribed for other similar media (10).

[2] They were checked frequently with the 250 r chamber.

[3] The irradiation and dose rate measurements were performed by Mrs. Florence Van Slyke, Radiation Physiology Section, whose assistance is acknowledged with pleasure.

Treatment of Yeast with Ion Exchange Resin.—Approximately 50 ml. dowex 50 (50 to 100 mesh, obtained from Dow Chemical Corporation, Inc.) was placed in a 500 ml. graduated cylinder and washed several times with distilled water, decanting just as soon as the larger portion of the resin settled, thus eliminating the smaller fragments and dust.[4] The resin was then washed several times with a solution of 10 per cent triethylamine in distilled water until the pH of the supernatant was approximately 12. Then the resin was washed again with distilled water until the pH was 7. The dowex 50 would now exchange triethylamine for the cations of the yeast suspension.

Centrifuge tubes of 90 ml. capacity or, if larger volumes of yeast suspensions were to be treated, 250 ml. graduated cylinders, were fitted with glass capillary tubing extending to the bottom of the cylinder. The yeast suspension was added to the cylinder followed by moist triethylamine–dowex 50. The amount of dowex 50 added was 5 to 8 per cent of the volume of the yeast suspension, amounting to approximately 10 gm. wet weight of dowex 50 per 150 ml. of yeast suspension (0.65 mg. yeast (dry weight)/ml.). This amount was selected arbitrarily as providing more than adequate surface for the exchange of ions and was adopted as standard procedure. Air was bubbled through to insure adequate mixing. The treatments were of 1 hour duration, although it was later found that 5 minutes was just as effective. When ready, the capillary tubes were removed, the dowex 50 settled immediately, and the yeast suspension, completely free of resin, was poured off.

Manometric Techniques.—All gas exchange studies were by standard Warburg micromanometric techniques (22). Final concentration of glucose was 1 per cent, of phosphate buffer 0.067 M, and of triethylamine-succinate-tartrate buffer, 0.02 M. The preparation and use of this latter buffer are described by Rothstein (11). In those experiments in which potassium was added KCl was tipped in from a side arm to make a final concentration of 0.067 M potassium.

All measurements were made at 26°C. and pH 4.5 or 6.5, determined both before and after the run. Shaking rate was *ca.* 120, ¾ inch, strokes per minute. All determinations were run with duplicate and usually triplicate flasks. Therefore, all gas exchange data consist of the average of two or three determinations. In addition, most of the experiments were repeated and the results averaged. The manometer deflections in all experiments were maintained relatively in the same order of magnitude by adjusting the amount of yeast placed in the flask.

Data from a representative series of experiments were submitted to statistical analysis[5] in order to determine the experimental error which could be predicted for similar manometric experiments. The results indicated that the minimum experimental error to be expected was approximately 5 per cent. The analysis suggested that differences above 5 per cent *might be*, and those 10 per cent and above *were* very probably real. The data did not permit a prediction on the basis of the confidence interval.

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[4] The large dowex 50 particles which remained settled immediately thus allowing complete separation of the dowex 50 from the yeast in the dowex-yeast suspension.

[5] Statistical analysis of the data was performed by Dr. S. L. Crump, of the Statistics Section.

All measurements were begun approximately 3 hours after start of the x-radiation treatment. The results are expressed in terms of Q_{O_2}, $Q^{air}_{CO_2}$, and $Q^{N_2}_{CO_2}$. These are defined as the microliters of gas consumed or produced per hour per milligram of yeast (dry weight).

RESULTS

Role of Buffer Composition.—

The important role of the buffer composition in the metabolism of irradiated yeast at pH 4.5 is illustrated by data presented in Table I. The rates of respiration and fermentation of non-irradiated yeast were only slightly influenced by the composition of the buffer. The rates tended to decrease more rapidly in the absence of potassium (T-S-T buffer). The respiration and fermentation of irradiated yeast were much more sensitive to the presence of potassium. In potassium phosphate buffer the gas exchange rates of irradiated yeast displayed a stimulatory effect. Oxygen uptake was increased 10 per cent while CO_2 production was increased 30 per cent. When the measurements were made with the yeast in T-S-T buffer, the gas exchange rates suggested an inhibitory effect of irradiation. The greatest effect was the 30 per cent inhibition of aerobic and anaerobic CO_2 production. Presented in Table II are data obtained when the measurements were made at pH 6.5. These results indicated no marked ef-

TABLE I

Effect of Buffer Composition on Gas Exchange of Yeast at pH 4.5

	Time*	Potassium phosphate‡			T-S-T§		
		Control	Irradiated‖	Difference	Control	Irradiated‖	Difference
	hrs.			*per cent*			*per cent*
Q_{O_2}	1	82	79	−4	81	82	0
	2	95	105	+11	66	69	−5
	3	99	109	+10	59	55	−7
$Q^{air}_{CO_2}$	1	217	225	+4	199	181	−9
	2	221	256	+16	166	140	−16
	3	178	237	+33	148	102	−31
$Q^{N_2}_{CO_2}$	1	281	315	+12	264	229	−13
	2	265	366	+38	222	173	−22
	3	194	250	+29	216	147	−32

Substrate was 1 per cent glucose, temperature 26°C.

* Began 3 hours after start of x-radiation.

‡ 0.067 M potassium phosphate buffer.

§ 0.02 M tritheylamine-succinate-tartrate buffer.

‖ 90,000 r, 250 kv. x-rays.

fect of irradiation on gas exchange processes regardless of the buffer composition. (The low rates observed in T-S-T buffer at pH 6.5 are discussed by Rothstein (11).)

Thus, whether an inhibition, stimulation, or no effect of radiation was observed depended upon the composition of the buffer and the pH at which the gas exchange measurements were made.

In view of the known stimulatory effects of potassium on gas exchange processes in yeast, these results suggest that irradiation effects were somehow dependent upon the potassium concentration of the suspending medium. To explore these effects further, two procedures were adopted: (*a*) starving the

TABLE II

Effect of Buffer Composition on Anaerobic CO_2 Production of Yeast at pH 6.5

Time*	Potassium phosphate‡			T-S-T§		
	N_2 Q_{CO_2}		Difference	N_2 Q_{CO_2}		Difference
	Control	Irradiated‖		Control	Irradiated‖	
hrs.			*per cent*			*per cent*
1	274	250	−9	118	132	+12
2	295	274	−7	92	101	+10
3	284	250	−12	75	69	−12
4	261	232	−11	47	40	−13
5				48	46	−4
6				44	44	0

Substrate was 1 per cent glucose, temperature 26°C.

* Began 3 hours after start of x-radiation.

‡ 0.067 M potassium phosphate buffer.

§ 0.02 M triethylamine-succinate-tartrate buffer.

‖ 90,000 r, 250 kv. x-rays.

yeast by 24 hours' aeration in distilled water and (*b*) exposure of the yeast to the cation exchange resin, dowex 50, in order to deplete the intracellular cation concentration. Starving has been shown to lower the intracellular potassium level from 0.1 M to 0.07 M (12) and also to decrease substrate reserves (21), the presence of which may provide a protective action by competing for the ionization-produced radicals.

Effect of Starving.—

In Table III are shown the rates of respiration and fermentation of starved yeast comparing those of the irradiated with those of the non-irradiated in both potassium phosphate and T-S-T buffer. The yeast was starved for 24 hours prior to being exposed to 90,000 r x-radiation.

Irradiation of the starved yeast resulted in inhibition of both respiration and fermentation in potassium phosphate as well as in T-S-T buffer. With the yeast suspended in potassium phosphate buffer the oxygen uptake of the irradiated yeast averaged 28 per cent less than that of the non-irradiated. The anaerobic CO_2 production was 15 per cent less. When the same measurements were made with the yeast buffered by T-S-T, the oxygen uptake averaged 47 per cent and the anaerobic CO_2 production 55 per cent less as a result of irradiation. It was observed that in T-S-T buffer, the gas exchange of the non-irradiated starved yeast proceeded at a considerably lower rate than in potassium phosphate, the rate in potassium phosphate being equivalent to that of fresh yeast.

TABLE III

Gas Exchange of Starved Yeast at pH 4.5

	Time*	Potassium phosphate‡			T-S-T§		
		Control	Irradiated‖	Difference	Control	Irradiated‖	Difference
	hrs.			*per cent*			*per cent*
Q_{O_2}	1	75	50	−33	30	14	−54
	2	90	64	−30	31	16	−49
	3	84	65	−21	26	13	−50
	4				24	18	−25
	Average....	83	60	−28	28	15	−47
$Q_{CO_2}^{N_2}$	1	260	247	−5	143	83	−42
	2	267	237	−12	103	52	−50
	3	254	220	−14	89	38	−57
	4	287	206	−30	86	29	−67
	5				76	23	−70
	Average....	267	227	−15	99	45	−55

Substrate was 1 per cent glucose, temperature 26°C.

* Began 3 hours after start of x-radiation.

‡ 0.067 M potassium phosphate buffer.

§ 0.02 M triethylamine-succinate-tartrate buffer.

‖ 90,000 r, 250 kv. x-rays.

Effect of Dowex 50 Treatment.—

It was necessary first to determine when in respect to the irradiation exposure the yeast should be treated with dowex 50 in order to observe maximum radiation effects. Therefore, experiments were performed in which the yeast was treated with dowex 50 either before or after radiation. The respiration and fermentation measurements were begun 3 hours after the start of irradiation with the yeast suspended in T-S-T buffer, pH 4.5, and 1 per cent glucose.

In Table IV data are presented showing the effect of dowex 50 treatment on normal yeast. The rates of respiration and fermentation were markedly altered. This was true even for non-irradiated yeast (compare the rates in Table IV with the normal Q_{O_2} of 70, $Q_{CO_2}^{air}$ of 150, and $Q_{CO_2}^{N_2}$ of 250). Superimposed on this is a considerably greater depression of the irradiated cells. When dowex treatment preceded irradiation there was approximately 30 per cent inhibition of oxygen uptake and aerobic CO_2 production. Anaerobic CO_2

TABLE IV

Effect of Dowex 50 Treatment on Gas Exchange of Bakers' Yeast

Time*	Not irradiated‡	Irradiated§	
		Pre-x-ray treated‖	Post-x-ray treated¶
hrs.			
Q_{O_2} 1	58	41 (−29 per cent)	40 (−31 per cent)
2	64	42 (−34 per cent)	23 (−64 per cent)
3	59	44 (−25 per cent)	31 (−47 per cent)
4	63		33 (−48 per cent)
$Q_{CO_2}^{air}$ 1	66	44 (−33 per cent)	44 (−33 per cent)
2	79	54 (−32 per cent)	28 (−65 per cent)
3	78	59 (−24 per cent)	38 (−51 per cent)
4	84		44 (−48 per cent)
$Q_{CO_2}^{N_2}$ 1	91	57 (−37 per cent)	54 (−41 per cent)
2	139	111 (−20 per cent)	79 (−43 per cent)
3	140	97 (−31 per cent)	88 (−37 per cent)
4	131		88 (−33 per cent)

Yeast suspended in triethylamine-succinate-tartrate buffer, pH 4.5, and 1 per cent glucose. Temperature, 26°C.

* Began 4 hours after start of x-radiation.

‡ Average of two experiments; $Q_{CO_2}^{N_2}$, average of three experiments.

§ 90,000 r, 250 kv. x-rays.

‖ One experiment.

¶ One experiment; $Q_{CO_2}^{N_2}$, average of three experiments.

production was slightly less sensitive (on a percentage basis). When the yeast was treated with dowex 50 after irradiation, inhibitions of 50 per cent or more were observed except for anaerobic CO_2 production which was again affected less than the oxidative processes.

Data showing the effect of dowex 50 treatment on *starved* yeast are presented in Table V. It was shown in Table III that starving the yeast produced an increased sensitivity to irradiation resulting in inhibitions of 50 per cent and greater. Treating the starved yeast with dowex 50 caused no marked change in the rates of the non-irradiated yeast, but those of the irradiated yeast were dramatically decreased resulting in a 60 per cent inhibition of oxygen uptake,

70 per cent inhibition of aerobic CO_2 production, and more than 90 per cent inhibition of anaerobic CO_2 production.

Thus, it was very effectively demonstrated that the catabolism of irradiated yeast was much more sensitive to dowex 50 treatment than that of non-irradiated yeast and that previous starving tremendously increased the inhibition due to irradiation.

TABLE V

Effect of Dowex 50 Treatment on Gas Exchange of Starved Yeast at pH 4.5

	Time*	Untreated			Post-x-ray Dowex-treated		
		Control	Irradiated‡	Difference	Control	Irradiated‡	Difference
	hrs.			*per cent*			*per cent*
Q_{O_2}	1	30	14	−54	32	18	−44
	2	31	16	−47	30	12	−59
	3	26	13	−48	20	8.5	−58
	4	24	18	−25	24	8.1	−66
$Q_{CO_2}^{air}$	1	99	54	−46	77	46	−41
	2	63	35	−44	73	22	−70
	3	49	23	−54	51	15	−71
$Q_{CO_2}^{N_2}$	1	143	83	−42	137	50	−64
	2	103	52	−50	93	20	−79
	3	89	38	−57	78	8.8	−89
	4	86	29	−66	78	4.9	−94
	5	76	23.3	−69	72	2.9	−96

Yeast suspended in 0.02 M triethylamine-succinate-tartrate buffer, 1 per cent glucose. Temperature, 26°C.

* Began 3 hours after start of x-radiation.

‡ 90,000 r, 250 kv. x-rays.

Addition of Potassium.—

In an attempt to determine more clearly the role of potassium in the gas exchange of irradiated yeast potassium chloride was tipped into Warburg flasks containing non-irradiated and irradiated yeast in T-S-T buffer. In these experiments sufficient KCl was added to half the flasks so that the final concentration of potassium after being tipped into the yeast suspension was 0.067 M. Shown in Table VI is a summary of data obtained in these experiments. Only the results from the fermentation studies are presented but the results for the respiration studies were qualitatively similar. The rate of anaerobic CO_2 production is shown followed by the rate 2 hours after the addition of potassium chloride and then by the per cent increase. There was a 23 per cent increase in rate of CO_2 production when potassium was added to the fresh non-irradiated yeast and 45 per cent when added to the irradiated yeast. The

presence of potassium completely masked the inhibitory effect of irradiation in fresh yeast as indicated by the $Q_{CO_2}^{N_2}$ of 155 for non-irradiated yeast and 158 for irradiated yeast.

Addition of K^+ to starved yeast produced stimulation of both control and irradiated cells, about equal in terms of Q value, much larger percentagewise in the irradiated cells because of the low basal rate. With dowex-treated yeast both the smallest and the largest effects of adding K^+ were seen. In no case, however, did these restore the rate to that seen in fresh, non-irradiated yeast in KH_2PO_4 buffer, in contrast to the complete restoration seen with fresh irradiated yeast.

TABLE VI

Effect of Potassium on Anaerobic CO_2 Production of Yeast

Yeast	Not irradiated			Irradiated*		
	$Q_{CO_2}^{N_2}$ ‡		Increase	$Q_{CO_2}^{N_2}$ ‡		Increase
	T-S-T	+K+		T-S-T	+K+	
			per cent			*per cent*
Fresh	126	155	23	109	158	45
Starved	76	114	50	23	62	170
Postradiation Dowex 50 treated						
Fresh	116	139	11	81	96	19
Starved	72	138	92	3	19	533

Suspended in 0.02 M triethylamine-succinate-tartrate buffer 1 per cent glucose: temperature, 26°C.

* 90,000 r, 250 kv. x-rays.

‡ Values for the 2nd hour after the addition of K.

DISCUSSION

The increased sensitivity of irradiated yeast to the ionic content of the environment may partially explain the inconsistency of previous reports of radiation effects. It would be meaningless to tabulate from the literature the observed effects of radiation on respiration and fermentation with respect to the experimental conditions employed. Due to the many unevaluated variables such as yeast strains, culture history, radiation energy and dose rate, interval after irradiation at which measurements were made, substrate, buffer, etc., it would be impossible to obtain a coherent picture. However, using one set of experimental conditions it has been shown that the observed effects of radiation are dependent upon the buffer composition and that the presence of potassium greatly increases the otherwise lowered rate of gas exchange of irradiated yeast to the extent that the over-all picture may be one of stimulation rather

than inhibition. In short, the gas exchange of yeast becomes more potassium-dependent after exposure to large doses of x-irradiation.

The effect of starvation is undoubtedly not solely due to loss of cations. Depletion of enzyme and/or substrate reserves is known to occur (Stier and Stannard (21)) and this may account for the failure of added potassium to restore the fermentation completely. However, we have not been able to increase the radiosensitivity of our yeast by growing it in a nitrogen-deficient medium (2). Hence, simple lack of the full enzymatic complement will not provide a full explanation.[6]

It is interesting that some effect is produced by treating yeast with the cation exchange resin *prior* to as well as *after* irradiation. This suggests that the loss of cations (or secondary effects dependent thereon) made the yeast more susceptible to radiation effects. This may also account in part for the starvation effect. However, the mechanism is not obvious.

That the dowex 50 treatment actually resulted in loss of cations, especially sodium[7] and potassium, was confirmed by spectrographic analysis of treated and non-treated yeast (2, analyses done by Dr. L. T. Steadman).

Sheppard and Beyl (15) and Sheppard and Stewart (16) have shown that irradiated red cells leak potassium. This may not be true of other animal tissues (24). Structural damage to the cell membrane and/or functional interference with the ion transport mechanism may underly these events. It is interesting to note, however, that Zacek and Rosenberg (25) found electron microscope evidence for marked changes in the red cell membrane after irradiation. Large areas of membrane were destroyed leaving only a fibrillar network. Also pertinent is the recent work of Rothstein (11, 12) with yeast which indicates that the marked dependence of fermentation on K^+ in the medium may reside in enzymatic reactions at the cell surface.

It is clear that a dose as high as 90,000 r produces much damage in the yeast cell interior and further work is indicated to evaluate the interdependence of radiation damage, metabolism, and environmental factors in the medium. That some aspects of growth show phenomena similar to those described here while others do not will be reported subsequently (3).

SUMMARY

1. Respiration and fermentation of yeast receiving 90,000 r of 250 kv. x-rays were studied under a variety of conditions. This dose will nearly completely inhibit growth or colony formation.

[6] Our results do not correspond to those of Sherman (17) in that he could increase sensitivity of yeast by growth in a N-deficient medium. Some small difference in conditions must exist since we have been able to accomplish this by a medium deficient in nitrogen, copper, and zinc.

[7] Adding sodium had no effect in the situations in which potassium was active, however.

2. The apparent effects of irradiation are quite dependent on the K^+ and H^+ of the suspending medium. At pH 4.5 stimulatory effects were observed in KH_2PO_4 buffer and inhibition in potassium-free (T-S-T) buffer. At pH 6.5 the situation was reversed and the effects were very small (about 10 per cent). Addition of K^+ to irradiated yeast in T-S-T buffer at pH 4.5 can completely reverse the inhibition seen.

3. Starving increases the apparent radiosensitivity of respiration and fermentation, probably by depletion of metabolite and/or electrolyte reserves.

4. Treatment with a cation exchange resin (dowex 50) results in marked inhibition of these processes in irradiated yeast, either fresh or starved. This was most effective if given *after* irradiation. Almost complete inhibition of anaerobic CO_2 production occurs with starvation, irradiation, and dowex treatment combined.

5. The effects of starvation and cation exchange resin treatment can be reversed, though not completely, by adding K^+ to the medium.

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