

THE EFFECT OF ULTRAVIOLET LIGHT ON THE SODIUM AND POTASSIUM COMPOSITION OF RESTING YEAST CELLS

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(Received for publication, May 20, 1958)

ABSTRACT

The Na^+ and K^+ content of non-metabolizing yeast cells was determined before and after monochromatic ultraviolet (UV) irradiation. UV facilitated the uptake of Na^+ into and the loss of K^+ from the cells (net ion flux); the effect is greatest for the shortest wavelength employed (239 $m\mu$) and is partly dependent upon the presence of oxygen. The UV effect on net ion flux persists for at least 90 minutes during which tests were made and it occurs following dosages which are without measurable effect on colony formation. The UV effect on net ion flux is decreased by acidity and promoted by alkalinity. Addition of calcium ions in sufficient amount prevents the usual net ion flux changes observed in irradiated yeast. Increase in concentration gradient between the inside and the outside of the cell increases the net ion flux of irradiated yeast, Na^+ uptake leading K^+ loss in all cases. UV appears to act by disorganizing the constituents of the cell surface, permitting K^+ to leave the cell in exchange for Na^+ . At low intensities of UV this ionic exchange approaches equivalence, but at higher intensities more Na^+ is taken up than K^+ is lost. Some evidence suggests that the Na^+ in excess over that exchanged for K^+ is adsorbed to charged groups produced by the photochemical effect of UV on the cell surface.

The disruptive influence of ultraviolet (UV) radiation on the integrity of the cell surface has been studied in sea urchins (19, 36, 29, 40), in protozoa (6, 28), erythrocytes (11, 21-23, 35), and yeast (7, 37 a, 37 b) to mention only a few examples. The present investigation was planned to determine the wavelengths of UV effective in altering the surface of yeast and to follow the net flux of sodium and potassium ions immediately after irradiation. It was felt that such a study might give an indication of some of the events underlying the observations previously reported.

Materials and Methods

Yeast cells were chosen for the experimental material because of their resistance to plasmolysis and the consequent ease of manipulation. They have the further ad-

* The support of a National Institute of Health predoctoral fellowship administered by the National Cancer Institute, is gratefully acknowledged.

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vantage of being easily obtainable in a relatively uniform physiological state. All experiments reported here were performed with a single strain of *Saccharomyces cerevisiae*¹ maintained in the laboratory on agar slants, containing 1 per cent yeast extract, 3 per cent glucose, 0.05 M NaH₂PO₄, and 2 per cent agar. The liquid media containing the same nutrients but without agar were inoculated from the slant cultures and incubated at 30°C. under constant aeration (oscillatory shaker) for 24 hours before use.

Cells in two different states were employed in most of the experiments described: K⁺-rich and Na⁺-rich. Yeast cells just harvested from a nutrient culture are K⁺-rich. Those washed free of nutrient and fasted for 12 hours or more in M/20 NaH₂PO₄ (on the oscillating shaker at 30°C.) show a pronounced time-dependent exchange of K⁺ in the cells for Na⁺ in the medium (9, 33). They are therefore Na⁺-rich. Unfortunately, fasting also lowers the resistance of yeast to UV as judged by growth injury (17); therefore, the cells not only differ in their ion content when prepared in the manner designated but also in their UV susceptibility.

For an experiment, the yeast cells were centrifuged in a Goetz tube, rinsed twice in distilled water,² and suspended in a volume of distilled water 35 times the packed cell volume. Equal volumes of cell suspension and distilled water or 0.2 N NaCl were then mixed to obtain the desired dilution of cells for irradiation, approximately 2 × 10⁸ cells per ml. (1.4 per cent cell suspension). Suspensions were prepared 2 hours before an experiment to permit the yeast cells to reach a steady state with their environment. Unless otherwise specified, the cells were irradiated in 0.1 N NaCl. Comparisons were made with control cells handled in the same way except for irradiation.

The UV light source used in preliminary experiments (and in a few instances when larger volumes of yeast suspension were to be tested) was a 30 watt General Electric germicidal lamp which produces 85 per cent of the radiation at 253.7 mμ. For definitive experiments a quartz mercury arc operating at atmospheric pressure and at 450 volts D.C. and approximately 2.2 amps. was used (6). The intensity of the UV radiation from the natural quartz monochromator was measured by a thermopile. The relative effectiveness of different wavelengths was compared on the basis of dosages in terms of quanta of energy delivered per unit area.

Due to the limitations of band width and intensity when monochromatic UV radiation was used, a small radiation cell only 8.7 mm. in diameter and holding only 0.2 ml. had to be employed. The cell suspension in the reaction cell was continuously stirred during irradiation with a magnetic stirrer. After exposure the cells were washed three times in distilled water, using an air turbine microcentrifuge, a process taking less than 10 minutes. The cells were then killed by heating them for 30 minutes at 80–85°C., transferred volumetrically to 5 ml. beakers, and diluted for ion assay.

Determinations of K⁺ and Na⁺ in yeast cells were made with the flame photometer attachment of the Beckman DU spectrophotometer, both ions being ana-

¹ The yeast culture used in the experiments was the diploid strain 2382-Z-1 from a ploidy series kindly supplied by Dr. Herschel Roman.

² The designation "distilled water" in this paper means water from a second distillation in a pyrex glass still.

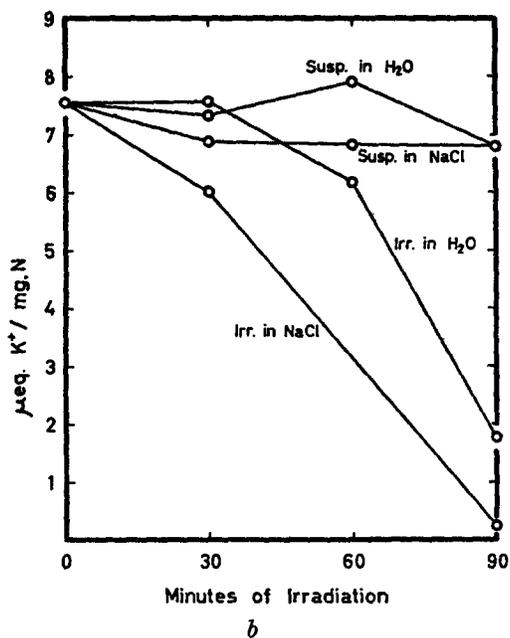
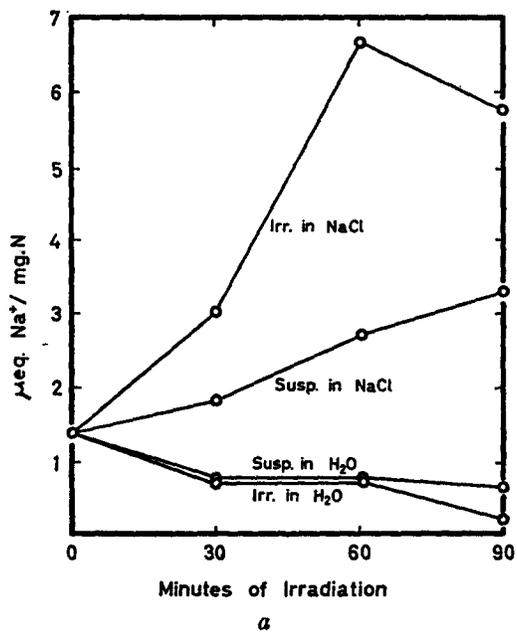


FIG. 1 a. The effect of continuous irradiation from a germicidal lamp on the Na⁺ content of yeast when irradiated in 0.1 N NaCl or distilled water. The respective controls are plotted for comparison. A similar plot of K⁺ content is found in Fig. 1 b.

lyzed in a single sample, each, of the control and irradiated suspensions. The photometer was adjusted to 100 per cent transmission with a solution containing 6.25 mg. of sodium ion and 12.5 mg. of potassium ion per liter. In most of the experiments reported, the cell fragments were not removed from the solution being tested (9). Concentrations are reported as milliequivalents of ion per milligram of nitrogen content of the yeast cells. Nitrogen determinations were made by routing Kjeldahl procedures. For comparison with other published values, 1 mg. of nitrogen is approximately equivalent to 12 mg. of dried cells.

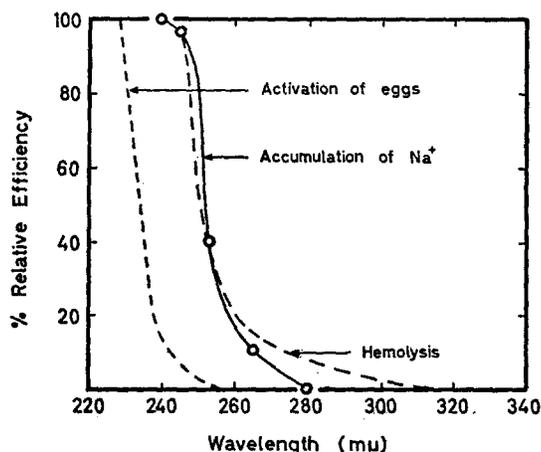


FIG. 2. An action spectrum for the accumulation of Na^+ in yeast cells receiving 5×10^{14} quanta at different wavelengths of UV. Wavelengths 360 and 430 $\text{m}\mu$ were also investigated and found to be ineffective.

Experimental Results

The feasibility of following the ionic exchanges in UV-treated yeast cells was ascertained in a preliminary study with a 10 ml. sample of yeast. For this purpose the germicidal lamp with an intensity of $40 \text{ ergs mm.}^{-2} \text{ sec.}^{-1}$ at the surface of a quartz-covered Petri dish containing the yeast was used. During irradiation the cells were continuously agitated with a magnetic stirrer, and samples were withdrawn for analysis after irradiation for 30, 60, and 90 minutes. The curves for net ion flux (Figs. 1 *a* and 1 *b*) indicate a progressive uptake of Na^+ and a loss of K^+ during the first 60 minutes of irradiation. At this time, as determined by counts with a hemocytometer, 97.5 per cent of the yeast cells were still able to decolorize methylene blue, indicating that the appropriate enzymatic reactions in the cell were still operating. The decrease in the total ion content in the subsequent 30 minute period (*i.e.*, after 90 minutes of irradiation) is consistent with deterioration of the cells, since by this time only 42.5 per cent of the cells were still capable of reducing methylene blue. The ion content of non-

irradiated cells, as well as those irradiated in distilled water, is also plotted in Fig. 1 for comparison.

Action Spectrum.—To ascertain which part of the UV spectrum is most effective in producing the ion exchange demonstrated above, aliquots of yeast suspensions were exposed at all available wavelengths between 239 and 440 $m\mu$, in each case to a dose of 5×10^{14} quanta. The results of these experiments are compared in Fig. 2, using the accumulation of Na^+ in yeast at the most effective wavelength (239 $m\mu$) as 100 per cent. Each point is the average value for three experiments. The magnitude of Na^+ uptake by yeast is greater than

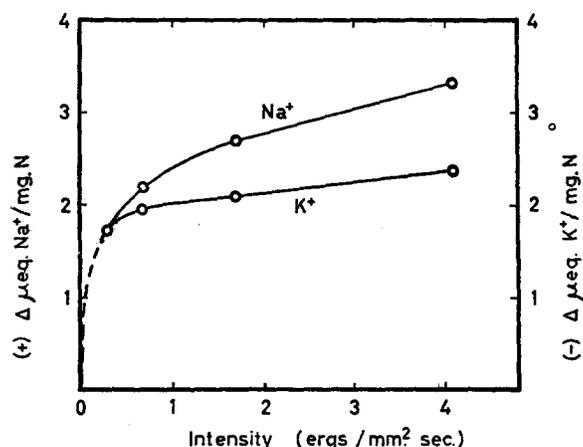
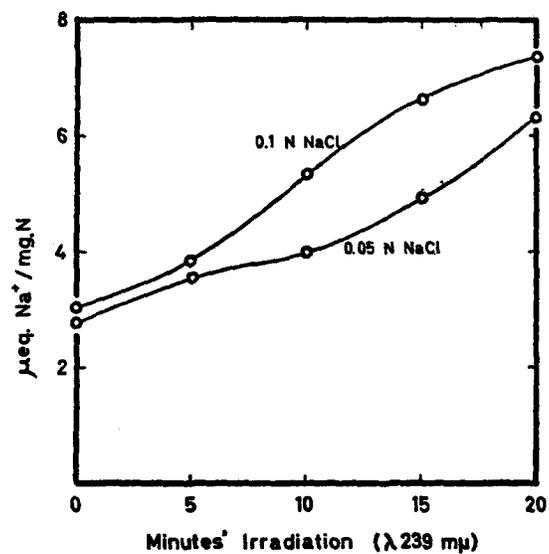
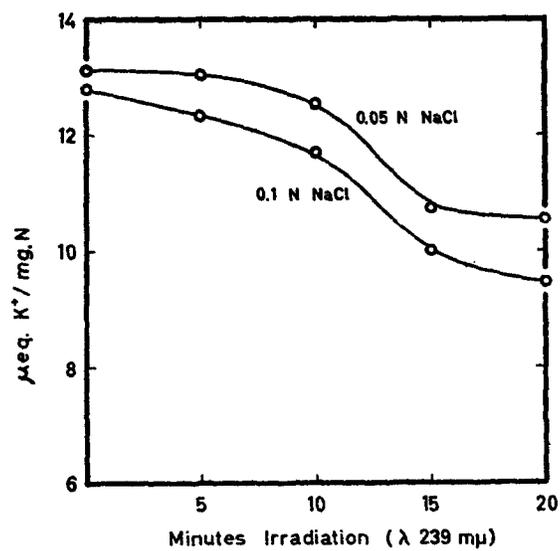


FIG. 3. The influence of intensity of UV (239 $m\mu$) on the uptake of Na^+ (left ordinate) and loss of K^+ (right ordinate).

K^+ loss, but the plots hold equally well for K^+ loss. In addition to the curve for Na^+ accumulation by yeast, curves for hemolysis (35) and activation of sea urchin eggs (19) are plotted in Fig. 2.

Interesting results were found by varying the administered dose of UV. If a comparison be made between the ion exchanges occurring at different intensities of radiation of a given wavelength acting for a constant period of time (20 minutes), a discrepancy is observed between the behavior of Na^+ and K^+ . At low intensities (*ca.* $0.3 \text{ erg mm.}^{-2} \text{ sec.}^{-1}$), approximately equivalent amounts of Na^+ and K^+ are exchanged. However, as intensity increases, sodium uptake increases more rapidly than K^+ loss (Fig. 3). It would be of interest to know whether at low intensities the Na^+ and K^+ curves are confluent to a point of zero effect (dotted line on graph), but such data were not gathered. When the reciprocal experiment is run (*i.e.*, time is varied at constant intensity), the K^+ loss also lags behind Na^+ gain and levels off sooner than the Na^+ curve as shown in Figs. 4 *a* and 4 *b*.

*a**b*

FIGS. 4 *a* and 4 *b*. The effect of (1) dosage of UV and (2) salt concentration of the suspending medium on the content of Na^+ and K^+ in yeast cells during irradiation. During each experiment the wavelength (239 $m\mu$) and intensity were maintained constant and exposure time and salt concentration of the medium varied.

UV Effect on Cell Viability.—It is important to know whether the net ion flux resulting from irradiation as reported above occurs before or after the yeast cell has lost its viability. To test this, an aliquot of a suspension of yeast was irradiated with a dosage of 5×10^{14} quanta of UV at $239 \text{ m}\mu$ and a dilution of the suspension was subsequently plated on nutrient medium for colony counts. After 48 hours' incubation at 28°C ., the colonies were counted and the results are recorded in Table I. It is evident that a dosage of UV, which produces a marked change in net ion flux, has little if any effect on the ability of the cells to produce colonies. Since cell division is a sensitive indicator of UV

TABLE I
Effects of UV on the Ability of Yeast to Produce Colonies

Dosage		Colonies per ml. of cell suspension	Viability
Quanta	Ergs		
(239 $\text{m}\mu$)			
0		1×10^8	<i>per cent</i> 100
2.5×10^{14}	2,195	9.5×10^7	95
5.0×10^{14}	4,390	9.3×10^7	93
1.5×10^{15}	13,170	2.9×10^7	29
(253.7 $\text{m}\mu$)			
0		1.7×10^8	<i>per cent</i> 100
3.2×10^{14}	2,500	1.1×10^8	0.064

damage, the results suggest that yeast cells are little affected by the "standard" dosage of UV employed in most of the experiments reported, except locally and superficially. However, larger dosages of this wavelength markedly reduce the viability of yeast as judged by their ability to develop colonies (Table I).

Irradiation of the yeast suspension with UV of wavelength $253.7 \text{ m}\mu$, on the other hand, reduces the capacity of the cells to form colonies much more readily than irradiation with UV of wavelength $239 \text{ m}\mu$, as seen in Table I. This might be expected since $253.7 \text{ m}\mu$ is near the maximum in the action spectrum for the bactericidal effect (24).

Postirradiation Ion Exchanges.—To determine whether the UV-induced change in net ion flux is temporary or whether it persists for a time after exposure to the radiation, a suspension of yeast was irradiated, kept in the dark, and sampled after 30, 60, and 90 minutes. For this purpose a quartz vessel holding 2 ml. of the yeast suspension was used instead of the 0.2 ml. vessel, necessitating a wider light beam (including wavelengths 245 and $239 \text{ m}\mu$) and,

TABLE II
Postirradiation exchanges of Na⁺ and K⁺*

Type of yeast	Time after irradiation												
	0 min.			30 min.			60 min.			90 min.			
	Na ⁺	K ⁺	Total	Na ⁺	K ⁺	Total	Na ⁺	K ⁺	Total	Na ⁺	K ⁺	Total	
	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	
1. Na ⁺ -rich cells	C†	12.20	6.13	18.33	11.92	6.22	18.14	11.63	6.31	17.94			
	I‡	13.64	5.82	19.26	13.82	5.44	19.26	13.92	5.06	18.98			
	D‡	+1.44	-0.31	+0.93	+1.90	-0.78	+1.12	+2.29	-1.25	+1.04			
2. Na ⁺ -rich cells	C	9.39	7.99	17.38	C‡								
	I	9.65	7.02	16.67	11.04	8.68	19.72	11.00	7.91	18.91	12.12	6.06	18.18
	D	+0.26	-0.87	-0.71	+1.65	-0.69	+2.34	+1.61	-0.08	+1.53	+2.73	-1.93	+0.80
3. K ⁺ -rich cells	C	3.52	12.20	15.72	3.44	11.48	14.92				4.42	11.60	16.02
	I	6.94	9.75	16.69	9.31	8.63	17.94				11.12	6.21	17.33
	D	+3.42	-2.45	+0.97	+5.87	-2.85	+3.02				+6.70	-5.39	+1.31
4. K ⁺ -rich cells	C	3.46	12.30	15.76	4.34	11.71	16.05				4.96	11.31	16.27
	I	8.16	8.95	17.65	10.32	7.83	18.15				12.30	5.86	18.16
	D	+4.70	-3.35	+1.89	+5.98	-3.08	+2.10				+7.34	-5.45	+1.89

* Irradiated with a wide beam including wave lengths 245 and 239 m μ .

† C = control cells suspended in 0.1 N NaCl, I = cells irradiated in 0.1 N NaCl, D = the difference between irradiated cells and controls (I - C).

‡ Comparisons after 30 and 60 minutes' irradiation made to zero time controls because of slight changes in controls with time (Experiment 1).

TABLE III
Influence of pH on the UV-Induced Exchanges of Na⁺ and K⁺*

pH	Na ⁺			K ⁺		
	Control	Irradiated	Difference	Control	Irradiated	Difference
Na ⁺ -rich cells						
	$\mu\text{eq./mg. N}$					
4.1	7.48	8.37	+0.89	6.95	6.53	-0.42
5.7	7.41	11.48	+4.07	7.15	6.31	-0.84
6.7	9.45	11.88	+2.43	7.36	6.03	-1.33
8.6	8.74	12.39	+3.65	6.82	4.82	-2.00
K ⁺ -rich cells						
	$\mu\text{eq./mg. N}$					
4.39	3.28	5.95	+2.67	12.76	12.00	-0.76
5.72	4.39	8.00	+3.61	12.23	10.55	-1.68
6.84	6.62	10.16	+3.54	13.08	9.40	-3.68
8.43	7.38	13.43	+6.05	11.72	8.24	-3.48

* Yeast irradiated 20 minutes with 239 m μ .

while an exposure of 20 minutes was used, the cells were less injured than in the experiments with the smaller cell since the intensity of the light was somewhat lower. The data for Na^+ and K^+ content in yeast cells so treated are recorded in Table II.

As seen in Table II, the data, although variable, show that the UV alteration of the cell surface persists in the dark and that it is not restricted only to the period of irradiation or immediately thereafter.

The possibility that the UV effect is being mediated by toxic substances in the suspending medium was eliminated by incubating normal cells in the supernatant solution from irradiated samples. Results from such experiments were uniformly negative.

pH Effects.—Due to their ability to withstand wide variations in pH, yeast

TABLE IV
Effects of Ca^{++} on UV-Induced Exchanges of Na^+ and K^+*

(Ca^{++})	Na^+ gain	K^+ loss	Na:Ca ratio
$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$
0.0 M	+5.7	-4.3	
0.005	+2.3	-2.0	20
0.01	-1.4	Variable (+ and -)	10

* Experiments with 239 $m\mu$.

cells permit an investigation of the relationship between UV and hydrogen ion concentration. The pH was altered by mixing 0.05 M stock solutions of mono- and dibasic sodium phosphate buffers in different proportions. The Na^+ concentration was maintained constant at 0.1 N by altering the concentration of the NaCl solution used as a diluent of the cell suspension. The yeast suspension was irradiated with UV of wavelength 239 $m\mu$ and sampled immediately after irradiation. Two of four experiments, similar except for nutritional state of the cells, are listed in Table III, from which the following conclusions may be drawn: (1) An increase in pH causes an increase in the Na^+ uptake of control cells, especially in K^+ rich cells were a change from pH 4 to 8, doubles the Na^+ concentration; (2) an increase in pH increases the Na^+ gain and the K^+ loss induced by irradiation, the K^+ loss more than the Na^+ gain.

Effects of Altering the Environment.—The dose dependency as a function of the chemical potential gradient was studied by suspending K^+ -rich cells in either 0.05 N NaCl or 0.1 N NaCl and irradiating for 20 minutes. Samples were taken at 5 minute intervals during irradiation to ascertain the dose dependency of the ion exchanges. These data are plotted in Figs. 4 a and 4 b. The displacement of the curve to the right for cells in 0.05 N NaCl suggests a reduced Na^+

flux rate consistent with the reduced chemical potential gradient as compared to the curve for cells in 0.1 N NaCl.

The similarity in form of the curves for the net flux of K^+ (Fig. 4 b) and for net flux of Na^+ suggests that K^+ outflux may occur in response to Na^+ influx. The curve for the less concentrated solution (0.05 N) lags behind that of the more concentrated, and within 20 minutes of observation does not reach the same level.

The alkaline earth ions at certain concentrations have been shown to have a stabilizing effect on films of proteins (12) and lipides (1) and on micell formation

TABLE V
Effect of NaCN on UV-Induced Exchanges of Na^+ and K^+*

Cells suspended in NaCl			Cells suspended in NaCl + 0.001 M NaCN		
Na ⁺					
Control (1)	Irradiated (2)	Difference (2) - (1)	Control (1)	Irradiated (2)	Difference (2) - (1)
$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$
6.00	8.67	+2.67	7.71	10.18	+2.47
2.84	12.66	+9.82	2.84	11.30	+8.46
3.34	11.08	+7.74	4.24	15.36	+11.12
K ⁺					
(1)	(2)	(2) - (1)	(1)	(2)	(2) - (1)
$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$	$\mu\text{eq./mg. N}$
10.50	9.05	-1.45	10.95	8.66	-2.29
11.56	4.86	-6.70	12.20	6.01	-6.19
13.42	7.30	-6.12	12.90	4.64	-8.26

* Experiments with 239 m μ .

(4). Similarly, irradiated yeast cells in 0.01 M $CaCl_2$ -0.1 M NaCl take up less Na^+ than do those in 0.05 M $CaCl_2$ -0.1 M NaCl or in 0.1 M NaCl alone. The Ca^{++} at 0.01 M appears to compete successfully with the Na^+ for available binding sites at the cell periphery. However, irradiated yeast cells suspended in 0.01 M $CaCl_2$ -0.1 M NaCl sometimes hold their K^+ , sometimes lose it, indicating less consistent stabilization of K^+ exchanges in the cell by calcium even at this concentration (Table IV).

To ascertain whether interference with the endogenous respiration could produce results similar to the irradiation effects, the respiration was decreased in two ways: by metabolic poisons and by lack of oxygen. NaCN, used to inhibit oxidative metabolism at a concentration of 0.001 M, had no appreciable effect, either on the controls or on the irradiated cells (Table V).

Yeast cells rendered anaerobic by gassing with nitrogen (washed with pyro-

gallol) were equilibrated for 15 minutes more in Warburg vessels with oxygen-free nitrogen, while controls were equilibrated with air. Both were then shaken in a Warburg bath (25°C.) for 1 hour before being used in the experiment. The anaerobic samples were taken while gassing the vessels with nitrogen and admitted to the irradiation chamber through the exhaust tube while it too was

TABLE VI
Effect of Oxygen Tension on UV-Induced* Exchanges of Na^+ and K^+

Experiment No.	Nitrogen			Air		
	Na^+					
	Control (1)	Irradiated (2)	Difference (2) - (1)	Control (1)	Irradiated (2)	Difference (2) - (1)
	$\mu\text{eq./mg. N}$					
1	3.76	4.87	+1.11	3.21	6.29	+3.08
2	2.88	3.64	+0.76	3.08	4.22	+1.14
3	3.18	5.23	+2.05	3.27	12.68	+9.41
4	6.00	6.82	+0.82	6.00	8.67	+2.67
5	2.88	6.49	+3.61	2.84	12.66	+9.82
6	3.42	7.25	+3.83	3.34	11.08	+7.74
Average . . .	3.68	5.72	+2.03	3.62	9.26	+5.64
K^+						
	(1)	(2)	(2) - (1)	(1)	(2)	(2) - (1)
$\mu\text{eq./mg. N}$						
1	13.00	10.50	-2.50	11.40	10.70	-0.70
2	12.92	14.10	+1.18	12.35	10.15	-2.20
3	13.05	11.70	-1.35	14.40	6.80	-7.60
4	10.50	9.80	-0.70	10.50	9.05	-1.45
5	11.20	8.15	-3.05	11.56	4.86	-6.70
6	13.25	8.61	-4.64	13.42	7.30	-6.12
Average . . .	12.32	10.48	-1.84	12.27	8.14	-4.12

* Irradiated with 239 $\mu\mu$.

being gassed. As seen in Table VI, in the absence of oxygen the irradiation-induced exchange of ions is considerably reduced (by more than 50 per cent). Short wavelength UV-induced protein denaturation is also reduced but not abolished in the absence of oxygen (15), an observation which has a bearing on the possible mechanism of action (see below).

DISCUSSION

As shown above, UV causes an effect on the yeast cell surface which permits an increase in the Na^+ and a decrease in the K^+ concentration in the cell. When

the wavelength of UV of the same dosage decreases from 265 to 239 $m\mu$ (4.7 to 5.2 electron volts per quantum) the radiation progressively increases ionic movements. An interpretation of these data centers around two main points: (1) Which cell constituents are affected by the UV? (2) How does this account for the observed ion shifts?

The cell constituents affected by the UV responsible for the effects described can be tentatively identified only by circumstantial evidence. The action spectrum for a biological effect of UV is sometimes taken as fairly direct evidence for identification of the cell constituent responsible for the effect, but only if the action spectrum is fairly detailed and corresponds closely with the absorption spectrum of a cell constituent. However, to make such deductions from an action spectrum the quantum yield should be independent of wavelength and the reciprocity law should hold. In the present instance (Fig. 2) not only is the action spectrum lacking in decisive detail but the reciprocity law does not hold. However, the short wavelengths used in the critical experiments reported are known to be absorbed in the peripheral region of the cell (5), probably very near the surface which is considered to be quite complex (32). The two main components in the peripheral region of the cell which absorb wavelengths shorter than 265 $m\mu$ are lipides and proteins. UV hemolysis, which results from injury to the surface of the cell, has been attributed to the effects of those radiations on both lipides (35) and proteins (11). Since both of these compounds absorb the short UV wavelengths, the action spectrum for net ionic flux change in yeast reported here does not permit selection between the two compounds as the locus of action of the radiations on the cell surface; therefore, other tests must be designed to do this.

The prevalence of unsaturated fatty acids in cellular phospholipides (25) makes them a likely locus for the UV action in the cell membrane, especially in view of the known hemolytic action of the altered lecithin molecule, lysolecithin. The UV oxidation of unsaturated fatty acids has recently been investigated (41) and the mechanism of the process reviewed (3, 20). The likely oxidation of a fatty acid by addition of oxygen at the carbon atom adjacent to the double bond, forms the unstable hydroperoxide. The hydroperoxide decomposes to the alpha or beta unsaturated ketone, which might conceivably have a disorganizing effect on the cell.

That a lipide locus of UV damage is unlikely as an explanation of the present experiments is indicated by the following facts: (1) Anaerobiosis merely reduces the radiation injury, by approximately half, instead of abolishing it as would be expected in the case of photooxidation of lipides (3); (2) such an interpretation is not consistent with the influence of pH on UV damage found here; (3) it was not possible to demonstrate fatty acid oxidation products by the thiobarbituric acid technique (41), even after prolonged (30 minute) irradiations with a germicidal lamp at 40 $\text{ergs mm.}^{-2} \text{sec.}^{-1}$ incident energy.

On the other hand, the peptide bonds in the proteins and the constituent amino acids (aliphatic and aromatic) absorb UV at wavelengths shorter than 253.7 $m\mu$, only the aromatic nucleus having an absorption peak at or near 280 $m\mu$ (2, 26, 34). Absorption of UV by proteins is attended by many changes, one being protein denaturation—an unfolding of the molecule, exposing charged groups (13, 30). The increase in surface potential of irradiated (235 to 253.7 $m\mu$) protein monolayers is a further indication of the photochemical release of charged groups (8, 27) including acid and basic groups (18). The exposure of charged groups by UV action might possibly explain some of the changes in cell ionic content observed. Furthermore, with decreasing hydrogen ion concentration, the charged groups of a native protein progressively dissociate (37). Since pH changes also strongly influence the action of UV upon ion exchanges, and, in the direction which would occur if it were affecting proteins, it is sug-

TABLE VII
Na⁺ and K⁺ Content of Yeast Cells in Equivalents per Liter

Experimental condition	Na ⁺	K ⁺	Na ⁺ gain	K ⁺ loss	Total Na ⁺ + K ⁺
As harvested	0.0266	0.1500			0.1766
Starved in 0.1 M NaCl.....	0.0643	0.1332	0.0377	0.0168	0.1975
Starved, irradiated.....	0.1084	0.1036	0.0818	0.0468	0.2120

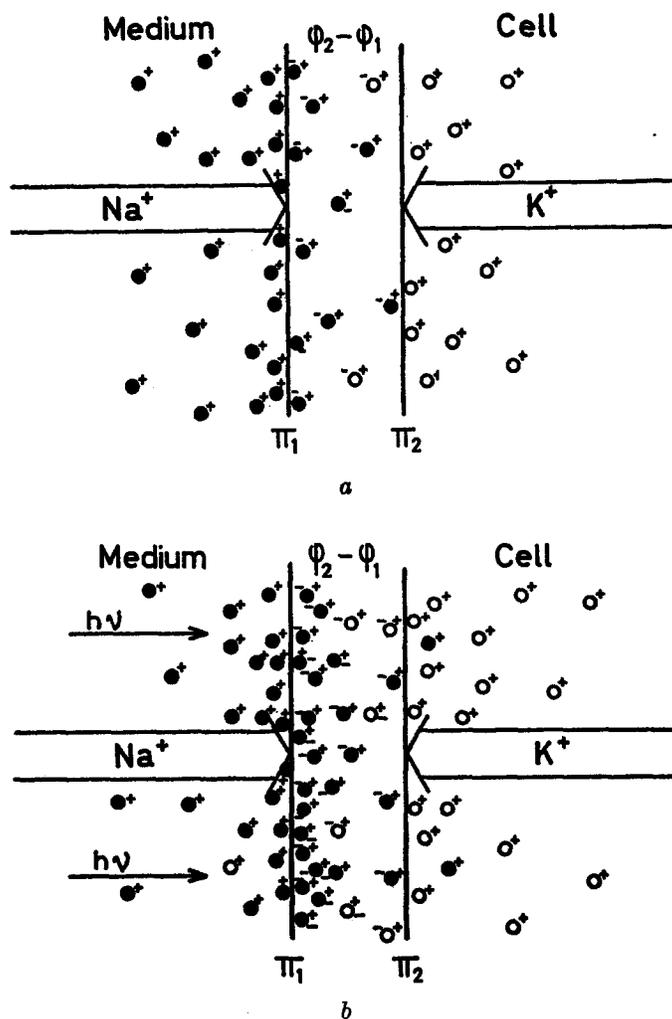
gested that proteins are the major locus of UV alteration of the cells observed in the experiments (14), although more data are desirable.

There are three possible ways in which damage to the proteins in the cell surface might explain the radiation-induced ion exchanges recorded in this paper.³ In the first place, inactivation by UV of an enzyme involved in active transport might be suspected, thus permitting equilibration of the two bulk phases, environment and cytoplasm, with the corresponding ion flux observed. However, the doses of UV required to inhibit reduction of methylene blue by yeast are much greater than those which induce the changes in net ion flux shown in Fig. 1, 97.5 per cent of the yeast still being capable of reducing the dye after 60 minutes' irradiation in 0.1 M NaCl, indicating intactness of the metabolic systems (see also reference 16). Furthermore, cells treated with 0.001 M NaCN, which virtually abolishes respiration, showed no appreciable changes in Na⁺ and K⁺ composition before irradiation, nor increased sensitivity to UV. All this evidence is contrary to what would be expected if a metabolic

³ Still another possibility is that the yeast cell has a resting potential, inside negative relative to the outside. This would account for an accumulation of Na⁺ but would not explain the nearly equivalent exchange at low intensities of UV, nor, assuming the potential metabolically maintained, the ineffectiveness of metabolic inhibitors on control cells and the lag in K⁺ loss (Figs. 4 a and 4 b).

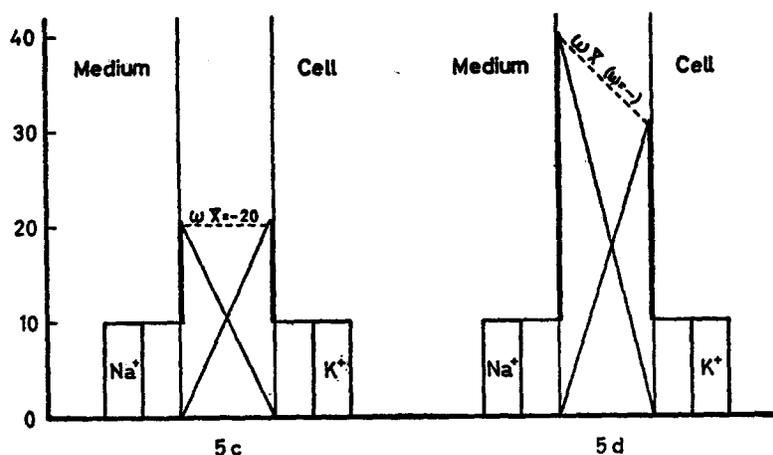
pump regulating ionic flux were operating in endogenous yeast cells as it does in actively fermenting yeast (10).

A second possibility is the UV alteration of the diffusion barrier in such a way as to produce holes and a "leaky" condition. If this were the case after irradi-



FIGS. 5 *a* and 5 *b*. Hypothetical distribution of ions in a normal and irradiated cell surface. π_1 and π_2 designate the Donnan potentials and ϕ_1 and ϕ_2 the membrane potentials at the respective surfaces of the membrane. The total membrane potential is the algebraic sum of the individual Donnan potentials and the potential difference within the membrane, $\pi_1 + (\phi_2 - \phi_1) + \pi_2$ (38).

ation an outward diffusion of K^+ and inward diffusion of Na^+ would ensue—driven by their respective electrochemical potential gradients until the cells had reached a steady state with their environment. In Table VII are listed the averaged values of Na^+ and K^+ from three experiments before and after UV treatment. The values are only approximate, as the cells were not in identical nutritional states; however, the K^+ value of the control cells compares favorably with published values (9, 31). It may be seen that almost twice as much Na^+ is taken up by the irradiated cells (5×10^{14} quanta) as K^+ is lost. This produces an increase in the total ion content of the cells. If the ions were



FIGS. 5 *c* and 5 *d*. Two possible profiles of membrane potential are plotted. The first indicates the condition in which $\pi_1 = \pi_2$ and, following their linear gradients, there will be an equivalent ion exchange. However, in Fig. 5 *d* in which membrane charge ($\omega \bar{x}$) is non-uniform and $\pi_1 \neq \pi_2$ the profiles are a matter of conjecture and are shown here only as an aid for orienting thinking on the problem.

dissolved in the cytoplasm of the cells, the osmotic pressure of the cells would increase correspondingly. It is difficult to say whether this is true since necessary information is lacking about the main driving forces of diffusion although evidence from the intensity-dependency curve (Fig. 3) makes this seem unlikely.

A third⁴ possible explanation of the observed radiation effects is the production by UV of charged (*i.e.*, ion-binding) groups on proteins present on the

⁴ If there is no electrical potential gradient between the bulk phases and only the activity gradient were effective, then on the basis of relative mobilities and concentrations, K^+ would exchange at a greater rate than Na^+ (Table VII). A reversal of the concentration gradient would permit Na^+ uptake to exceed K^+ loss as observed in the experiments reported here. This would follow if the interfacial Donnan potential were high, relative to the bulk concentration (38, 39).

surface of the cell. UV denaturation of proteins on the basic side of their isoelectric point does in fact increase the number of negatively charged groups, which under the conditions of the experiments, would bind Na^+ .

Considering all the experimental evidence it would appear that the observed UV effect is a composite, consisting of damage to the diffusion barrier (as indicated by the K^+ loss) as well as production of adsorption sites (equivalent to the Na^+ in excess of its exchange for K^+). Fig. 3 is of interest in this connection. At low intensities the UV injury is of such a nature as to permit equivalent exchanges of ions between the cells and the medium. At higher intensities, acting over the same period of time, there is a divergence between Na^+ uptake and K^+ loss, the former increasing at a greater rate. These data suggest UV damage to the barrier system at relatively low intensities. A subsequent increase of the rate of quantum flux increases the uptake of Na^+ , presumably as adsorbed ion, and only slightly increases the K^+ loss, as if a new mechanism had been evoked—the development of charged groups on the proteins. Thus, increasing the intensity of UV approximately four times increases the Na^+ content by a factor of 2 and decreases the K^+ content by a factor of 1.4.

An appreciable charge density at the surface of an irradiated yeast cell is indicated if, for purposes of calculation, we consider that the Na^+ taken up by the irradiated cell in excess of its exchange for K^+ is the consequence of the development of charged groups on the surface of the cells resulting from irradiation. The excess of Na^+ uptake over K^+ loss, approximately 1×10^{-4} m.eq./ 2×10^7 cells, when calculated to occupy only the surface of the cell, gives 3×10^9 groups/ 10^{10} A^2 of the surface of the cell. Since UV even of wavelength 239 $\text{m}\mu$ penetrates a short distance into the cell, the photochemically produced charge density would be three dimensional rather than two dimensional (Figs. 5 *a* to *d*). If, for purposes of argument, we assume that the surface charges are distributed through a depth of 100 A , the charge density is equivalent to a charge concentration of 5 N . Because of the complex structure of the peripheral region of the yeast cell (31), it is impossible to say with certainty what proportion of these charges is associated with the surface of the cell and what proportion the cytoplasmic surface. The concentration profiles in Figs. 5 *c* and *d* assume linear gradients, which are not likely to occur. There is probably a non-linear distribution of negative charges extending to the limits of penetration of the UV.

While the data included in this paper do not present a definitive explanation of the way in which the cell surface is affected by UV, they furnish a point of departure for obtaining quantitative data which might go far toward describing and explaining the nature of the ionic diffusion barrier in the yeast cell.

The authors are indebted to Professor Torsten Teorell and Dr. Karl Johann Öbrink of the University of Uppsala and to Mr. William Andrus and Mr. D. Stuart Nachtwey of Stanford University for a critical reading of the manuscript.

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