	Haploid	Diploid	Triploid	Tetraploid
Nuclear diameter	0·84 μ	1·35 μ	1·73 μ	2·01 μ
Standard deviation	0·0195	0·0663	0·055	0·0833
Mean volume	0·31 c μ	1·288 c μ	2·711 c μ	4·252 c μ

preparations. Figure 1 shows graphically the increments in mean nuclear diameters (indicated by dots) and their ranges of variation in preparations of pure cultures representing each degree of ploidy. The interphase nucleus is seen as a homogeneously stained, vesicular body with no detectable chromatin threads. One hundred such nuclei of each degree in the series were measured in whole, randomly selected cells. Where a nucleus was so closely pressed onto the vacuole as to have a slight dent, its broadest diameter was estimated.

Increments in mean nuclear diameters and volumes are tabulated above.

Since about 22 to 28% of the nuclei in each class were slightly dented by the vacuole, the calculated mean volumes are only approximate. The increments in ranges of variation of diameter, probably associated with growth of the nucleus during interphase[1], will be noted.

These observations are in obvious refutation of LINDEGREN's identifying as the centrosome, a structure here shown to conform to the theoretically expected nuclear area–ploidy relationship. It should be stressed that RANGANATHAN and SUBRAMANIAM's[2] view that even single cell or single spore isolations cannot yield "pure" cultures owing to "somatic doubling" or irregular segregations of split chromosomes, finds no support in the present work. Such mechanisms, if prevalent, would be expected to yield genetical heterogeneity, involving the occurrence of variously sized nuclei, in a culture of initially single-haploid-cell derivation. Meticulous search in haploid preparations has failed, however, to reveal nuclei larger in diameter than haploid nuclei. Neither were cytological differences detected between preparations of aerobically grown and fermenting cultures of haploid origin, the nuclei in both cases falling within the range indicated in Figure 1. This is contrary to SUBRAMANIAMS's thesis that fermenting cells differ cytologically from aerobically active cells and that endopolyploidy is a consequence of fermentative growth.

The size differences between the clones presently studied are as characteristic of the series as are the increases in total nuclear volumes; haploid cells are roundish and measure 2·81 μ in diameter while diploids measure 5·338 × 2·658 μ, triploids 7·041 × 3·591 μ, and tetraploids 7·964 × 4·23 μ. These figures represent the mean of one hundred estimations for each class. Only single, non-budding cells were measured. These observations contrast markedly with those of DURAISWAMI and SUBRAMANIAM[3] (whose "haploids", measuring 10·6 × 7·2 μ, are actually larger than their "tetraploids" measuring 6·9 × 6·6 μ) and indicate that their objection to using cell size as an aid to distinguishing genetical types is not universally valid. It is superfluous to add, in this connection, that yeast geneticists have subordinated morphological criteria to the precise genetical diagnoses possible with biochemical markers but can invariably associate undoubted haploid segregants, in Mendelian as well as non-Mendelian (other than polyploid) asci, with small roundish cells. Matings between compatible segregants reinitiate ascus-size vegetative cells.

Part of this work was carried out at the University of Chicago. I am indebted to Prof. I. GERSH for facilities and advice. A discussion of the nuclear cytology of yeast will appear elsewhere.

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BALAJI D. MUNDKUR[1]

Southern Illinois University, Carbondale, Ill., April 4, 1953.

Zusammenfassung

Durch Gefriertrocknung nach ALTMANN-GERSH wurden zytologische Präparate von einer genetisch geprüften polyploiden Serie von Hefe hergestellt. Die gemessenen Werte für Kern- und Zellgrössen stimmen in der theoretisch erwarteten Weise mit dem Grade der Polyploidie überein. Damit ist eine von höheren Organismen bekannte zytologische Erscheinung zum erstenmal für Mikroorganismen demonstriert.

Die Befunde stehen im Widerspruch zu LINDEGRENS Annahme einer «Kernvakuole» und zu SUBRAMANIAMS genetisch nicht fundierten Einwänden gegen die Brauchbarkeit von Einzelsporen-Kulturen, ebenso zur Ansicht des letztgenannten Autors, dass zytologische Unterschiede bestehen zwischen Zellen aus Gärungs- und solchen aus aeroben Kulturen.

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[1] H. B. FELL and A. F. HUGHES, Quart. Rev. micr. Sci. *90*, 355 (1949). – E. M. WERMEL and W. W. PORTUGALOW, Z. Zellforsch. *22*, 185 (1935).

[2] B. RANGANATHAN and M. K. SUBRAMANIAM, J. Indian Inst. Sci. *34*, 235 (1952).

[3] S. DURAISWAMI and M. K. SUBRAMANIAM, La Cellule *53*, 215 (1950).

Induction of Resistance to X-Ray Inactivation in Saccharomyces by Pre-Exposure to 2537Å Ultraviolet Radiation[1]

An exploratory study of the effects of combined ultraviolet and X-irradiation revealed a marked increase in the resistance to X-ray inactivation of a portion of the survivors of an ultraviolet irradiated population. In contrast, no change in sensitivity to ultraviolet radiation was observed in the survivors of an X-irradiated population.

A haploid stock, #13894, and a non-sporulating diploid stock, #11296 of *Saccharomyces cerevisiae* from the Carbondale pedigree were employed in this investigation. The ploidy for each stock has been established through genetical analyses and nucleic acid determination[2]. 24 h old clones of each organism were maintained

[1] This work was supported by research grants from the U.S. Atomic Energy Commission, Contract AT(11-1)-176, and the Office of Naval Research, Department of the Navy, Contract (NR 164-140).

[2] M. OGUR, S. MINCKLER, G. LINDEGREN, and C. LINDEGREN, Arch. Biochem. Biophys. *40*, 175 (1952).

at 4°C as stock inocula. Samples were prepared for irradiation by transferring a heavy loop of cells from a stock inoculum to a 250 ml flask containing 25 ml of clarified V-8 juice (pH 3·9), a commercial mixture of several vegetable juices. After 44 to 48 h growth on a rotary shaker at 30°C, the cells were harvested, washed twice with M/15 KH_2PO_4 and resuspended in M/15 KH_2PO_4 to a concentration of approximately 5×10^5 cells per milliliter. 30 ml of the suspension was then transferred to a sterile PETRI dish to be irradiated. Cells prepared in this manner possess no buds, exhibit no cytologically detectable glycogen or volutin reserves and contain numerous prominent mitochondria.

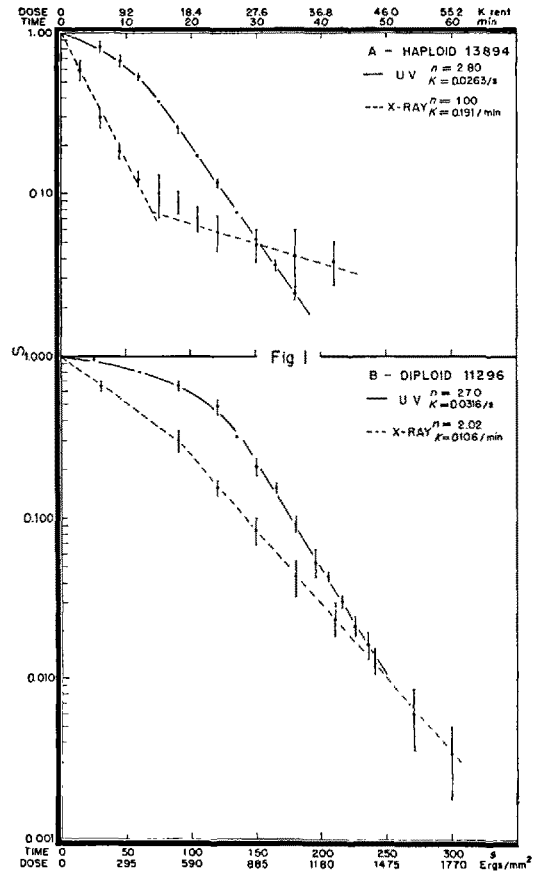

Fig. 1.—Standard survival curves for haploid and diploid yeasts were obtained by averaging the survival points for a number of independent determinations: 6 ultraviolet and 5 X-ray runs for the haploid, and 6 ultraviolet and 4 X-ray runs for the diploid. Standard deviations are shown for all points. The target value, n, and rate of logarithmic inactivation, K, for each curve is presented in the legend. The break in the haploid X-ray survival curve is similar to that reported by LATARJET and EPHRUSSI[1]. All haploids from the Carbondale yeast breeding stocks, which have been subjected to X-ray analysis, yield survival curves that characteristically break between 5% and 10% survival.

The X-ray dosage was given at a rate of 920 R/min (measured by a 2500 r Victoreen dosimeter) from a tungsten target tube operated at 180 KVP with a total filtration of 2 mm of Al. The HVL was 0·22 mm Cu. The ultraviolet radiation was delivered at a rate of 5·900 ERGS/mm²/s (at the surface of the suspension) by a Hanovia quartz envelope lamp yielding 95% of its ultraviolet energy at the wave length 2537 Å. During irradiations the suspensions were continuously agitated by a magnetic stirrer. 1 ml samples were removed at intervals (Fig. 1 and 2) diluted in M/15 phosphate and plated in duplicate (controls in quadruplicate) in a medium containing: 2·0% dextrose, 0·2% BUDWEISER yeast extract, 0·1% $(NH_4)_2SO_4$, 1·5% agar. Per cent survivals for given doses of radiation were determined from average macroscopic colony counts taken with the aid of a Quebec colony counter after three days incubation at 30°C.

Survival curves were plotted on semi-log paper with S, the fraction surviving, plotted along the axis of ordinates and the dose D, measured in ergs per square

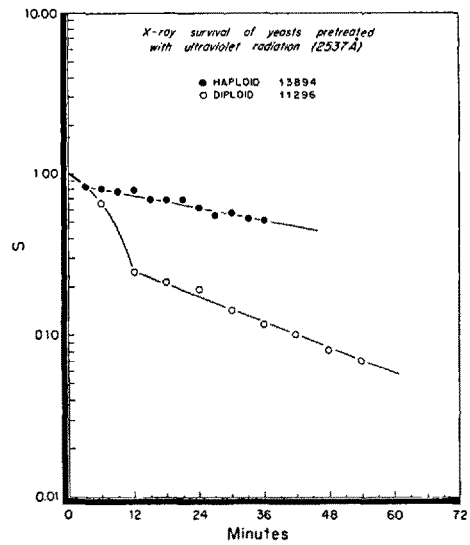

Fig. 2.—X-ray survival curves of the fraction of haploid and diploid yeast suspensions viable after pre-irradiation with ultraviolet to 5% survival. Breaks in diploid survival curves have not been observed previously.

millimeter per second for ultraviolet and kiloroentgens per minutes for X-rays on the axis of abscissae.

The straight line portions of the control curves were fitted by the method of least squares to the equation

$$\mathrm{Ln}\, S = \mathrm{Ln}\, n - KD$$

where K is the slope of the line, D is the dose and n its intercept on the axis of ordinates.

Two suspensions of the diploid 11296 were irradiated with X-rays to survivals of 10% and 2% respectively. Within 10 min of the completion of the X-ray exposure, ultraviolet survival curves were run on the surviving fractions. No change in the ultraviolet sensitivity was detected. Similar results were found with suspensions of the haploid 13894 pre-irradiated to 20% survival with X-rays.

When haploid or diploid yeast suspensions were irradiated to 5% survival with ultraviolet, the X-ray resistance of a portion of the surviving fractions was increased greatly (Fig. 2). This increase may be attributed either to some alteration in the cells brought about by the action of ultraviolet radiation, or to the presence in the original population of a fraction of cells resistant to both X-ray and ultraviolet irradiation. Under this latter hypothesis, pre-irradiation with ultraviolet selects for the resistant cells by destruction of sensitives. The "break" at 4% in the haploid X-ray control curve (Fig. 1), could be due to the presence of resistant cells to the number of 7%. The haploid ultraviolet survival

[1] R. LATARJET and B. EPHRUSSI, C. r. Acad. Sci. *229*, 306 (1949).

curve, however, shows no break over the range investigated (100% to 2·5%). This must mean that the 7% of cells resistant to X-rays are normally sensitive to ultraviolet. Thus exposure of the haploid to a 5% survival dose of ultraviolet would kill 95% of the cells in both categories and subsequent exposure to X-irradiation would give a survival curve showing a break at 4% as before.

If the increase in resistance to X-irradiation of the ultraviolet survivors is due to the selection of a fraction resistant to both radiations, this fraction can be no greater than 2·5% of the original population. Therefore, under the assumption that the ultraviolet exposure which kills off 95% of the total population does not kill an appreciable number of the resistant cells, the percentage of resistant cells exposed to subsequent X-irradiation is 2·5/5% or 50%, and the break in the X-ray survival curve could not possibly occur at a higher value. Since the break obtained occurs at 90% survival, the hypothesis of a resistant fraction is not tenable for the haploid.

In the case of the diploid, the control X-ray survival curve was carried to 0·35% survival with no evidence of a break; therefore a resistant fraction could not represent more than 0·35% of a normal diploid population. Under the same assumptions as before, the break in the X-ray survival curve of cells surviving ultraviolet irradiation could not occur at a higher value than 0·35/5 or 7% survival. The observation of a break at 25% survival precludes the possibility that the X-ray resistant fraction was present prior to exposure to ultraviolet.

It is concluded that the increase in X-ray resistance must be due to an alteration in the cells brought about by ultraviolet radiation. This finding correlates with the observed suppression by ultraviolet radiation of X-ray induced chromosomal breakage in *Tradescantia*[1] and *Drosophila*[2] and suggest strongly that chromosomal aberrations are a highly significant aspect of cellular inactivation by X-rays.

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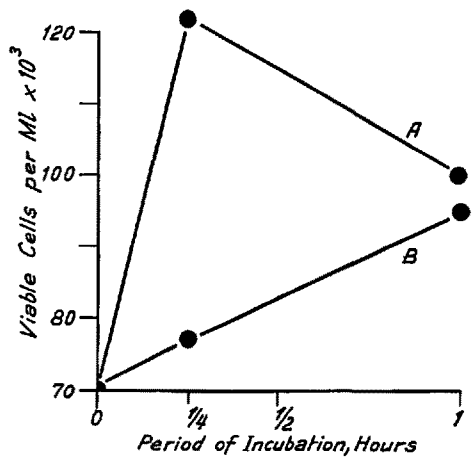
Zusammenfassung

Ultraviolettbestrahlung von Hefekulturen bewirkt, dass nachher ein Teil der Zellen gegenüber der inaktivierenden Wirkung von Röntgenstrahlen vermehrte Resistenz besitzen. Es konnte gezeigt werden, dass dies durch Zellveränderungen bedingt ist. Da bei *Tradescantia* und *Drosophila* ultraviolette Strahlen chromosomale Veränderungen unterdrücken, kann angenommen werden, dass bei der Hefe ein wesentlicher Teil der Zellinaktivierung infolge Röntgenbestrahlung auf chromosomalen Störungen beruht.

[1] C. P. SWANSON, Genetics *29*, 61 (1944).
[2] B. P. KAUFMANN and A. HOLLAENDER, Genetics *31*, 368 (1946).

Some Effects of Metabolic Inhibitors upon Survival of Ultra-Violet Irradiated *Escherichia coli*

We have recently had occasion to examine the effect of α-methyl glucoside upon the extent of survival of washed cells of the *histidineless* and *methionineless h-m-5b* strain of *Escherichia coli* following ultraviolet irradiation. In early experiments (performed at room temperatures of 20–25°C) it was found that the presence of M/250 α-methyl glucoside during the irradiation of cells taken from stationary phase cultures usually resulted in a slightly greater degree of survival than for control suspensions. On the other hand, with cells harvested in the logarithmic phase of growth the glucoside appeared to enhance the lethal effect of the irradiation. It was, therefore, decided to attempt to elucidate further the mode of action of α-methyl glucoside in enhancing the survival of stationary phase cells.



An influence of postirradiation treatment with α-methyl glucoside upon the apparent degree of survival from ultraviolet irradiation. *A* Control. *B* Treated with α-methyl glucoside
5 ml aliquots of washed cell suspension from stationary phase aerated cultures were irradiated at 4°C for 30 s. The suspensions were pooled and 4 ml portions diluted with 1 ml of (*A*) distilled water or (*B*) M/50 α-methyl glucoside. These suspensions were incubated at 37°C for varying periods of time, cooled to 4°C, washed and plated in synthetic medium containing glucose as carbon source.

Cultures were grown in a synthetic medium[1] supplemented with 50 μg of *dl* methionine and 25 μg of *l* histidine monohydrochloride per milliliter, with M/250 K gluconate (pH 7·2) as carbon source. The cultures were aerated by shaking on a mechanical agitator, and growth ceased from exhaustion of the supply of carbon source. Cells were plated in similar media containing 2% (w/v) of washed agar[2] and with appropriate carbon sources. Cells were irradiated by exposure of 5 ml aliquots of washed suspension (in distilled water) in rotating open PETRI dishes for 30 s at a distance of 13·5 cm from the centre of a Westinghouse "Sterilamp" low-pressure mercury vapour tube, stated to deliver 85% of its energy at a wavelength of 254 mμ. The cells were washed once with sterile distilled water prior to plating. All operations subsequent to the irradiation were performed under the light of amber lamps to prevent photoreactivation. Plates were counted after incubation at 37°C for 48 h and subsequently at daily intervals until the maximal number of colonies had appeared (3–5 days). The numbers recorded represent the mean value from two (or more) plates.

An attempt was made to determine whether the glucoside would affect the extent of survival when added to the cell suspension after completion of the irradiation. Unfortunately, however, the commencement of these

[1] A. W. RAVIN, J. Gen. Microbiol. *6*, 211 (1952).
[2] F. J. RYAN, *Selected methods of Neurospora research.* Methods med. Res. *3*, 51 (1950) (The Year Book Publishers Inc., Chicago.)