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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Biological Research Laboratory, Southern Illinois University, Carbondale, Ill., June 19, 1953.

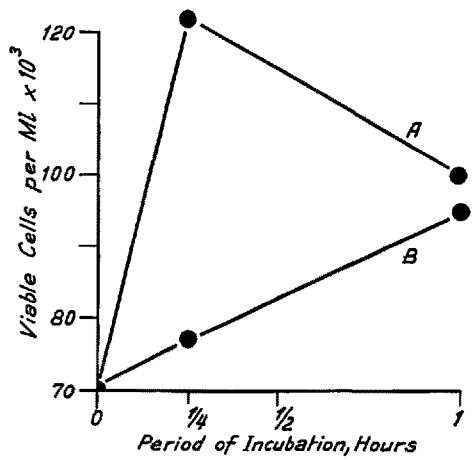
Zusammenfassung

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.)

experiments coincided with the exceptionally hot summer of 1952 and no unequivocal result was obtained when all stages of the experiment were performed at room temperature (32–35°C). The addition of α-methyl glucoside after the irradiation resulted in up to a 100-fold greater degree of survival than in control suspensions. However, there was considerable death in both irradiated and unirradiated control suspensions due to heat inactivation (arising from the poor dissipation of heat generated during centrifugation).

The Effect of Postirradiation Treatment with Iodoacetate upon the Survival of Stationary Phase Cultures

Experiment No.	Unirradiated Control Viable Cells /ml·10^7	Irradiated Control Viable Cells /ml·10^3	Irradiated Cells Treated with M/5000 Iodoacetate Viable Cells /ml·10^3
(1)	46	24	58
(2)	135	60	100
(3)	48	25	112
(4)	50	32	40
(5)	34	560	870

5 ml aliquots of washed cell suspension from stationary phase aerated cultures were irradiated at 31–34°C for 30 s. To 4 ml of irradiated suspension was added 1 ml of either water or M/1000 Na Iodoacetate (pH 7·2) and centrifugation commenced immediately in precooled centrifuge heads, the temperature of which rose to *ca.* 35°C after 15 min spinning. The cells were washed once and plated in synthetic medium with M/250 glucose as carbon source.

Experiments with cells irradiated at 4°C and subsequently incubated at 37°C prior to washing and plating did reveal an influence of post-irradiation treatment with α-methyl glucoside upon the observed degree of survival. The figure illustrates the results of one such experiment. Incubation at 37°C led to a transient recovery of some of the irradiated cells as measured by the ability to give rise to a visible colony (curve *A*), and this recovery process was inhibited by the glucoside (curve *B*). Some variation of results was obtained in different experiments. However, of nine experiments the transient recovery was observed in six, and for four of them the probability that the variations in apparent number of survivors could be attributed to sampling errors was found to be less than 0·001 by the chi-square test. Further, inhibition of the recovery was observed in all but one of these six experiments.

The demonstration of this effect of the glucoside did not, however, shed any light on the question of whether the extent of survival could be *increased* by post-irradiation treatment. α-methyl glucoside has been shown to be an inhibitor of the metabolism by *E. coli* and other microorganisms of a number of carbohydrates, and there is strong evidence suggesting that the inhibition of glucose metabolism is competitive in nature[1]. It was, therefore, decided to examine the effect of post-irradiation treatment with other inhibitors of carbohydrate metabolism. Arsenate (competitive) gave similar results to those obtained with α-methyl glucoside. On the other hand, post-irradiation treatment with M/5000 iodoacetate for approximately 15 min (the time required for centrifugation of the suspension) consistently resulted in a higher degree of survival than for control suspensions (Table).

[1] S. D. WAINWRIGHT, Biochim. Biophys. Acta *11*, 157 (1953). – F. H. JOHNSON and R. S. ANDERSON, J. Cell. Comp. Phys. *12*, 273 (1938). – J. LEIBOWITZ and S. HESTRIN, Biochem. J. *36*, 772 (1942).

Although the results presented here are preliminary in nature we believe them to be of importance in relation to hypotheses advanced to explain the action of ultraviolet light upon biological material. Our results do not warrant any detailed speculation, especially as the *h-m-5b* strain of *E. coli* is colicinogenic[1]. However, as experiments now in progress with a strain of *Streptomyces*[2] indicate that postirradiation treatment with metabolic inhibitors can change the proportion of colonies exhibiting ultraviolet induced heritable variations of character either (i) without affecting the degree of survival or (ii) as some function of an effect upon the extent of survival, we believe that the results presented above can be most readily explained as the consequences of disturbances of the intracellular balance of metabolism.

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Résumé

Le niveau apparent de survie des cellules d'*Escherichia coli* irradiées par rayonnement ultra-violet peut être modifié par «post irradiation» avec des inhibiteurs du métabolisme cellulaire.

[1] F. J. RYAN, personal communication.
[2] S. D. WAINWRIGHT and A. NEVILL, to be published.
[3] Present address: Biology Division, Atomic Energy of Canada, Ltd., Chalk River, Ontario.

Incorporation *in vitro* de glycocolle-I-$_{14}$C dans les oocytes d'Astéries

On sait que de nombreuses recherches ont été effectuées, au moyen d'acides aminés marqués, dans le but de suivre la vitesse de synthèse ou de renouvellement des protéines; elles ont porté soit sur des homogénats d'organes (HULTIN[1]) soit sur des embryons de Batraciens (BRACHET[2], FRIEDBERG et EAKIN[3]).

Il nous a semblé utile de suivre, par la méthode autoradiographique, le sort de l'acide aminé marqué au sein de cellules isolées en voie de croissance.

Nos observations ont porté sur des oocytes d'*Asterias rubens*, provenant de divers individus et se trouvant à des stades variables de la croissance.

Des lots d'une centaine d'oocytes, soigneusement lavés au préalable, ont été placés dans 5 cm^3 d'eau de mer stérile, contenant 1 μ mole de glycocolle, marqué sur le carbone du carboxyle.

Une première expérience, qui a porté sur des oocytes jeunes, avait pour but de vérifier si le glycocolle pénètre effectivement dans la cellule. L'incubation, d'une durée de 5 h, a été suivie d'un lavage soigneux dans de l'eau de mer contenant du glycocolle non marqué, puis, à deux reprises, dans de l'eau de mer stérile.

[1] T. HULTIN, Exp. Cell. Res. *1*, 376 (1950).
[2] J. BRACHET (communication personnelle).
[3] F. FRIEDBERG et R. M. EAKIN, J. exp. Zool. *110*, 33 (1949).