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Two methods of measuring rate of deoxyribonucleic acid synthesis

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Abstract

Measurement of DNA synthesis

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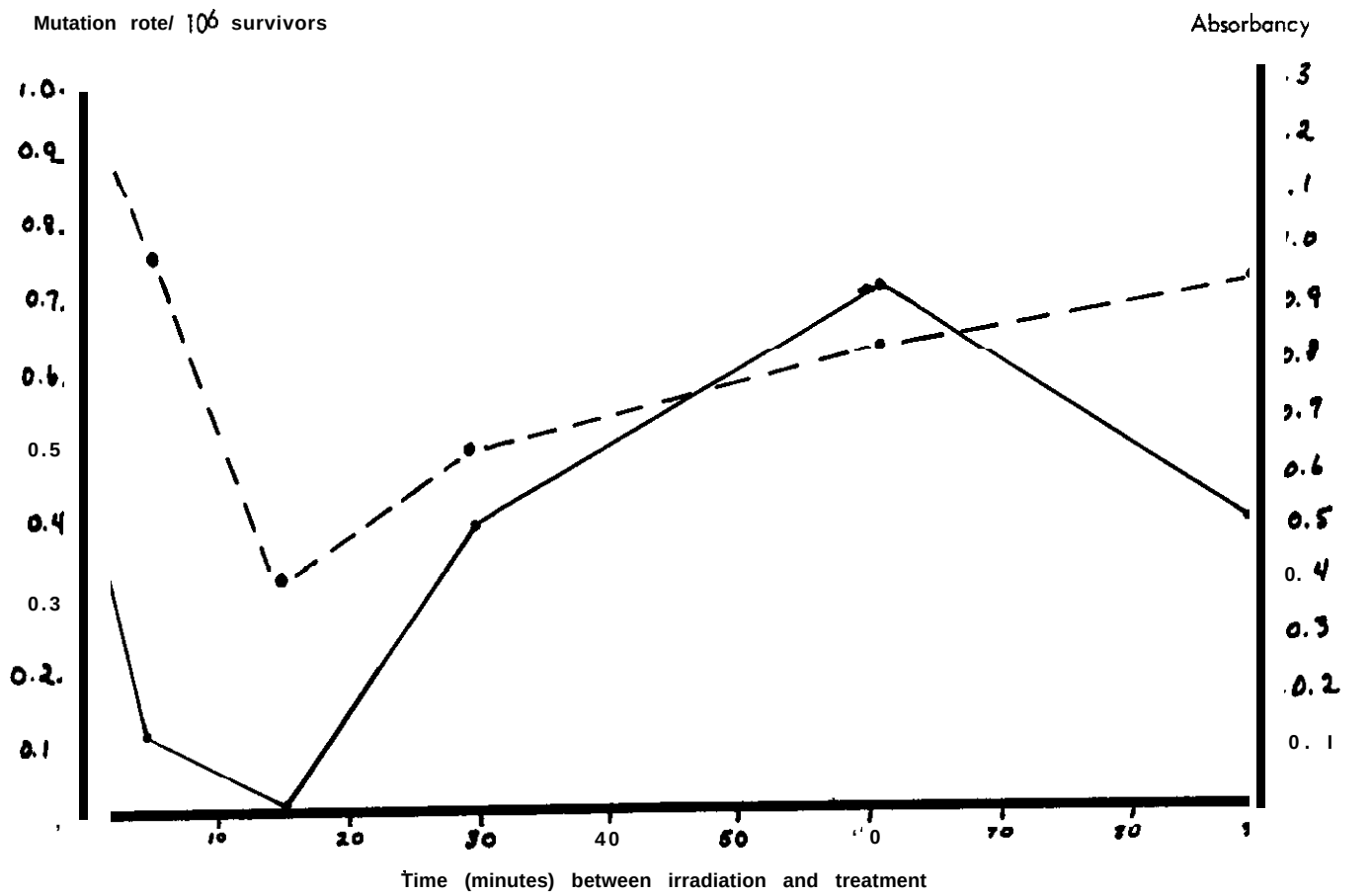
In current studies on the rate of deoxyribonucleic acid (DNA) synthesis in ultraviolet irradiated conidia of Neurospora crassa, two methods of estimating the DNA level have been developed. Spectrophotometric measurements of the DNA content of an approximated number of conidio by the diphenylamine color reaction was used, as was estimation of DNA level by sensitivity to photoreactivating light at varying time intervals after UV irradiation. Strong correlation was found between the two methods.

Photoreversal of UV-induced lesions in the DNA cannot take place if doubling of the DNA following irradiation has taken place. The mutation rate from adenine deficient to "wild-type" at each delay period was used to indicate inversely the amount of DNA already doubled, and thus protected from the mutation-blocking effect of photoreactivation. A high mutation rate indicates little photoreactivation and vice versa.

In both methods, conidio of an ad-4 mutant strain (F54) of Neurospora crassa were suspended in sterile water and adjusted to a concentration of 6.6 x 10^6 conidia/ml. by dilution. The conidia were exposed to ultraviolet light for five minutes, using a constant volume of solution, which was continually agitated. The ultraviolet source was a 15-watt Sylvania germicidal lamp at a distance of 50 cm. from the suspension. This dosage of ultraviolet was previously found to be lethal to more than 95% of the conidia of this strain.

The major obstacle to spectrophotometric studies of the conidia was the hard case surrounding the conidium. This was overcome by immediately sonifying the refrigerated suspension of irradiated conidia. Sonification for one minute at a high intensity resulted in destruction of the conidiol case on all spores. The naked conidia were hydrolysed with cold dilute (10%) trichloroacetic acid. Two ml. of diphenylamine reagent (1.5 gm. of diphenylamine in 100 ml. glacial acetic acid and 1.5 ml. of concentrated sulfuric acid with 0.5 ml. acetaldehyde added just before use) was added to each 1 ml. sample and the mixture was placed in a 30°C water both for 16-18 hours. After incubation, the sample war read on the spectrophotometer at 600 mμ. Aliquots of the conidiol suspension were sonified and treated at 0, 5, 15, 30, 60, and 90 minutes following irradiation.

In the photoreactivation study, the conidia were suspended and irradiated as indicated above. The irradiated conidia were allowed to incubate for various delay periods and were then exposed for five minutes to white light



supplied by a 15-watt Sylvania soft-white fluorescent tube. One milliliter of the treated conidial suspension was placed in each of twenty petri dishes and mixed with Neurospora minimal agar (Difco) supplemented with sorbose (8 gm./1.) to induce colony formotion. The plates were incubated at 27°C for 48 hours before reading. Survivors were counted and compared with the number of survivors an non-photoreactivated plates.

The five-minute exposure to while light was previously determined to bring about maximum photoreactivation in the strain of Neurospora used. Six treatments were used, each carried out with 1.32 x 10 conidia dispersed in each of 20 plates. The first group was treated with UV but not reactivated. The second group was treated with UV and immediately reactivated. The third was irradiated and incubated for five minutes before reactivation. The other three groups were irradiated and incubated for 30, 60, and 90 minutes respectively before photoreactivation.

The conidial samples assayed for DNA level spectrophotometrically were allowed to incubate far varying periods, as mentioned above, after irradiation and before treatment. The same periods were used for bath methods of DNA measurement. The absorbancy due to the DNA color reaction at each delay interval is compared with the mutation rate following photoreactivation at each interval in the following graph. A close correlation between DNA level as measured spectrophotometrically and by the post-photoreactivation mutation rate is demonstrated. ▪ ▪ ▪ Department of Biological Sciences, Kent State University, Kent, Ohio 44240 and Eli Lilly and Co. Research Laboratories, Indianapolis, Indiana 46206.