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 material 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 conidial 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 bath for 16-18 hours. After incubation, the sample was read on the spectrophotometer at 600 m. Aliquots of the conidial 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.

\[
\begin{array}{ccccccccccc}
\text{Time (minutes) between irradiation and treatment} & 10 & 20 & 30 & 40 & 50 & 60 & 70 & 80 & 90 & 100 \\
\text{Absorbancy} & 0.3 & 0.2 & 0.1 & 0.0 & 0.9 & 0.8 & 0.7 & 0.6 & 0.5 & 0.4 & 0.3 & 0.2 & 0.1 \\
\end{array}
\]

Vomvayanni 1965 Can. Jour. Bot. 43:765). Five such strains were shown to have resulted from a single-gene mutation. There may be more than one mutational site for resistance to these hydrocarbons as it has been shown for another ascospore (Georgopoulos and Panopoulos 1966 Con. Jour. Genet. 49:347). At least one of these sites is linked to the mating type locus and to patch (see also NN49:44). On control medium hydrocarbon resistant strains tend to sporulate less abundantly than the respective wild types.

Although patch confers no tolerance to the hydrocarbons all hydrocarbon resistant mutants “escape” the effect of L-sorbose at least as effectively as patch. On media containing sucrose and L-sorbose some of these mutants grow much better than Patch. Whether different levels of inhibition by sorbose are associated with different genes for resistance to aromatic hydrocarbons is now been investigated. - - - Department of Biology, Nuclear Research Center "Democritus", Athens, Greece.


Neurospora crassa strains STA4 (wild type) and patch (non-colonial growth an up to 1% L-sorbose) were used and were found highly sensitive to diphenyl, naphthalene, acenaphthene and other similar compounds. Resistant strains were obtained from fast growing sectors, as has been described for other fungi (Curtis et al. 1956 Am. J. Botany 43:594). Whittingham 1962 Am. J. Botany 49:866, Georgopoulos and Macris. Gene-controlled resistance to aromatic hydrocarbons in Neurospora crassa and its relationship to the inhibition by L-sorbose.

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3-Deoxy-D-arabino-heptulosonic acid 7-phosphate synthetase (DAHP synthetase) is the first enzyme of aromatic biosynthesis in micro-organisms and in E. coli has been shown to be a regulatory system of at least 3 isoenzymes (Doy and Brown 1965 Biochim. Biophys. Acta 104:377). Control is by feedback inhibition (phenylalanine and tyrosine) and repression (phenylalanine, tyrosine and tryptophan) (Brown and Day 1966 Biochim. Biophys. Acta 118:157).

DAHP synthetase has now been examined in dialysed crude extracts of wild type N. crassa. 74A, grown an Vogel's minimal medium at 25° for 48 hrs. Under the conditions stationary phase had not been reached. Extracts were made by grinding with glass and KH₂PO₄-NaOH buffer 0.1M pH 6.4 and dialysing against 0.025M of the same buffer. The supernatant was used after centrifuging the debris. DAHP synthetase was estimated essentially as described by Day and Brown.

The substrates are erythrose 4-phosphate and phosphoenolpyruvate and initial velocity measurements were determined by varying one substrate (10⁻⁵M - 2 x 10⁻³M) in the presence of excess of the other (2 x 10⁻³M). By plotting v against s, sigmoid curves were obtained which, within experimental error, had a positive initial slope. Reciprocal Plots of 1/v against 1/s show the characteristics more clearly. Parts of these data replotted as 1/v against 1/s yield a straight line as required if 1/v against 1/s is a parabola. However, it appears likely that this is fortuitous and that the present data are more consistent with the characteristics of a non-rectangular hyperbola. It is important to make this distinction.

A parabolic 1/v against 1/s curve is consistent with a model: E + S --- ES --- K₂ + P product.

$$K_1$$

$$K_2$$

$$k$$

$$E + S \overset{K_1}{\underset{ES}{\rightleftharpoons}} ES \overset{K_2}{\underset{E+S+\text{product}}{\rightleftharpoons}}$$

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