TECHNICAL PAPERS

Factors Influencing the Mutation Rate in *Neurospora*[1]

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In a previous study (*2*) concerning the production of biochemical and morphological mutations in *Neurospora* by nitrogen mustard it was observed that 7- to 12-day-old conidiospores were relatively resistant to this agent. Since this point may be of some importance in analyzing the effect of various chemicals on mutation rate, we have investigated the relative susceptibility of various stages in the life cycle of *Neurospora* to this agent (*bis*-β-chloroethylmethylamine). The data from this study are summarized in Table 1.

TABLE 1

MUTATION RATE IN *Neurospora*—SENSITIVITY OF VARIOUS STAGES TO NITROGEN MUSTARD

Nitrogen mustard conc. (%)	Stage treated	Ascospores tested	Mutations*			Mutation rate (%)
			Morphological	Identified biochemicals	Unidentified biochemicals	
0.1	7- to 11-day-old conidia	1,137	1	0	0	< 0.1
0.1	2- to 3-day-old conidia	694	8	3	6	2.5
0.1	Germinating conidia (15%)	89	4	0	1	5.6
0.1	" " (67%)	412	14	3	8	6.1
0.1	" " (90%)	624	20	6	18	7.1
0.05	" " (90%)	606	9	1	7	2.8
0.1	Hydrated conidia	411	11	4	2	4.1
0.05	" "	176	4	1	1	3.4
0.1	Protoperithecia	237	6	3	10	8.0
0.05	"	534	23	10	10	8.0

* The identified biochemicals included those strains which required the following single components for growth: methionine, adenine, adenine or hypoxanthine, proline, thiamine, leucine, *p*-aminobenzoic acid, arginine, inositol, nicotinic acid, riboflavin, threonine, isoleucine, and lysine. The riboflavin mutant is temperature sensitive, apparently similar to the one reported by Mitchell and Houlahan (*3*). The unidentified biochemicals include those which grow on complete only but not on hydrolyzed casein or a synthetic vitamin mixture, on hydrolyzed casein but not on the individual amino acids, on the vitamin mixture but not on the individual vitamins, and poor on both complete and minimal. The morphological mutants include various colonial types such as cauliflower, button, rough, etc., and the color changes such as pink, brown, albino, yellow, etc.

The conidia were suspended in acetate buffer (0.2M, pH 6.0) and the mustard was added to give the desired concentration. At the end of 30 min, the sample was centrifuged, washed, and finally suspended in water. A few drops of the treated conidial suspension were then placed on protoperithecia of the opposite sex, and subsequently single ascospores were isolated from each perithecium. The ascospores isolated were tested for possible biochemical deficiencies according to the procedure of Beadle and Tatum (*1*). Since very young conidia (2–3 days) were found to be more sensitive than older ones, it was suspected that conidia just beginning to germinate might also be more susceptible. To test this, 12-day-old conidia were suspended in normal Fries solution and incubated at 29° C. Samples of the suspension were removed at 2, 4, and 6 hrs for treatment with the nitrogen mustard. Microscopic examination revealed that at 2 hrs, approximately 15% of the conidia had begun to germinate; at 4 hrs, 67%; and at 6 hrs, 90%. The conidial samples were then centrifuged and resuspended in 0.2M acetate buffer and treated as described above. The results in Table 1 indicate that germinating conidia are far more susceptible to the nitrogen mustard than are freshly suspended conidia of the same age. When conidia showing 90% germination were treated with 0.1% mustard, a mutation rate of 7% was obtained.

As control in the above experiment, a sample of the conidial suspension was placed at 8° C for the 6-hr period and subsequently treated with the mustard. No difference could be detected microscopically between freshly suspended conidia and those which had been hydrated for 6 hrs, *i.e.* no visible germination could be detected. However, the results indicate that these hydrated conidia are likewise sensitive to the mustard, a fairly good mutation rate being obtained with both 0.05 and 0.1% solutions. It is evident that, in some way, the degree of hydration of the spores is important in the induction of mutations by the nitrogen mustard. This may be related to the penetration of the compound or to the suppressed metabolic activity of the cell resulting from the dehydration. The fact that protoperithecia are extremely sensitive to the mustard, giving the highest and most consistent mutation rate, supports the earlier

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viewpoint that cells containing actively growing or dividing nuclei are more susceptible to the mutagenic action of various agents.

Regardless of the interpretation of the above facts, the results serve to point out the necessity of controlling the age and degree of hydration of conidia in studies concerned with the production of mutations by chemical agents.

References

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The Chemical Nature and Origin of *Phaseolus* Virus 2 Crystalline Inclusions

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Isometric, crystalline inclusions were described by McWhorter (*3*) as occurring in plants infected with *Phaseolus* virus 2. In the present study, qualitative chemical tests were performed on epidermal peelings from broad bean (*Vicia faba*) plants infected with this virus. The fresh integumentary strips were examined under the microscope to find a field containing well-developed crystalline inclusions. The reagent solutions were allowed to flow under one edge of the cover slip as the original mounting material was withdrawn from the opposite edge with a blotter. Fixed material was also tested in some cases. The observations are summarized in Table 1.

In broad bean, there exists a melanin-producing system which may be initiated by wounding. This is made apparent by the discoloration of wounded tissue, which changes in color from normal green to red, and finally to black. Dark areas appear on detached broad bean leaves when they are gradually killed by soaking in physiological saline solution for 24 hrs or longer. The only microscopically visible cytological change in affected epidermal cells was the dark pigmentation of the nuclei. Mosaic-diseased broad bean leaves subjected to this treatment showed the discoloration not only in the nuclei, but also in the crystalline inclusions. The nuclei and inclusions appeared as if treated with hematoxylin. These results suggest the presence of tyrosine in both the nuclei and inclusions, since melanin is presumed to be the end-product of a tyrosine-tyrosinase system (*1*).

The solubility of the crystalline inclusions in both acid and alkali indicates an amphoteric substance. Naturally-occurring amphoteric compounds which are insoluble in fat solvents are limited largely to proteins. This fact, combined with the results obtained with picric acid, the biuret test, Millon's reagent, and the observation of melanin production, shows that the isometric crystalline inclusions of *Phaseolus* virus 2 are proteinaceous.

The crystalline inclusions are found within the nucleolus and cytoplasm only. The primary production of the inclusions within the nucleoli suggests that the crystals are either partly or entirely composed of nucleolar material. The presence of the crystals within the cytoplasm would argue against this theory, were it not for the work of Lenoir (*2*), who reported the secretion of nucleolar fragments from the dividing nucleus into the cytoplasm. It seems reasonable to conclude that the isometric crystalline inclusions of *Phaseolus* virus 2 may be the insoluble end-product of the interaction of virus material and nucleolar material. This theory is further supported by the lack of visible crystals in infected *Melilotus alba*. In this plant the nucleoli are extremely small. The small amount of one of the interacting substances (nucleolar material) would, in this case, restrict the development of the end-product (crystalline inclusions).

TABLE 1

SUMMARY OF CRYSTALLINE INCLUSION REACTIONS

Chemical	Effect
Nitric acid (conc.)	Crystalline inclusions dissolved
Sodium hydroxide (10%)	" " "
Picric acid (sat. aq.)	Nuclei and crystalline inclusions stained light yellow
Biüret test	Inclusions positive for polypeptide linkage
Millon test	Inclusions positive for phenolic group (tyrosine?)
Formaldehyde (5%)	Slight yellowing of inclusions*
Water (100° C)	No visible effect
Alcohol (95%)	No visible effect; tested on fixed material
Tertiary butyl alcohol	No visible effect; tested on fixed material
Xylol	No visible effect; tested on fixed material
Dioxan	No visible effect; tested on fixed material
Ether (10%)	No visible effect; tested on fixed material
Feulgen's reagent	Crystalline inclusions remained unstained; nuclear chromatin stained red
Acetocarmine (in 45% HAc)	Crystalline inclusions dissolved; nuclear chromatin stained red
Iodine green (10%)	Crystalline inclusions stained dull pink; nuclei stained green†

* McWhorter (*4*) found that inclusions and nuclei fixed in 5% formalin would not stain with trypan blue without previous peptonization in 10% citric acid.

† Purdy (*5*), using iodine green to stain tobacco infected with tobacco mosaic, found the amorphous inclusions stained dull pink, while the nuclei stained green.

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