Induced Reversions of Biochemical Mutants in Neurospora crassa

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INDUCED REVERSIONS OF BIOCHEMICAL MUTANTS IN NEUROSPORA CRASSA [1]

Norman H. Giles, Jr., and Esther Zimmer Lederberg [2]

ONE OF the problems connected with biochemical mutants requiring specific growth factors—such as have been induced in *Neurospora* and other microorganisms (Beadle and Tatum, 1941; Beadle, 1946)—concerns the ability of these mutant strains to regain the power of growth in a medium lacking the substance originally required. "Adaptation," as this phenomenon is usually designated, has been observed in a number of instances (Ryan, 1946). The question immediately arises whether adaptations represent some kind of non-genetic change presumably involving cytoplasmic components, or whether they are the result of genetic changes involving reverse-mutations of specific genes to their wild-type alleles, thus restoring the synthetic capacity of the organism. It has been shown in an extensive study of a leucineless mutant of *Neurospora crassa* that spontaneous adaptations in this strain do involve reverse-mutation (Ryan and Lederberg, 1946; Ryan, 1946). Thus it seemed of interest to investigate the possibility of reverse-mutations in other *Neurospora* mutants. Further, it appeared especially desirable to determine whether the rates of such reversions could be increased by various treatments. The techniques for detecting changes involving the restoration of synthetic capacity for specific substances essential for growth are much simpler than those for changes involving losses of such capacities. Thus in instances where adaptations are found to result from reverse mutations, it should be possible to utilize these techniques to obtain data on the mutability of numerous genes with a variety of agents and under many different conditions.

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EXPERIMENTAL METHODS.—For most of the experiments multiple biochemical mutant stocks obtained by recombinations of single mutants were utilized. The use of such stocks makes it possible to compare the effect of a single uniform treatment on two or more genetic loci. Further, the additional mutant genes serve as useful genetic markers. In most stocks the marker gene for colorless conidia (albino-2) was also present, making possible the visible detection of possible contamination by wild type conidia.

The procedure in a typical experiment (exp. 12, table 1) was as follows: a conidial suspension of

the multiple mutant stock #G37a was prepared, filtered through absorbent cotton to remove mycelial fragments, and the concentration of conidia determined with a haemocytometer (in this experiment the conidial concentration was 5.7×10^6/ml.). This stock, originally synthesized as a genetic tester stock, has the following mutant loci, all on different chromosomes: albino-2 15300 [al], inositolless 37401 [inos], pantothenicless 5531 [pan], tryptophaneless 10575 [tpt], riboflavinless 51602 [rbl]. The numbers following the mutant name in this and all subsequent stocks used refer to the original Stanford culture numbers (Beadle and Tatum, 1945), while the abbreviations in brackets refer to the growth factor required. The normal (wild) type will be indicated by adding "+". These symbols will be used throughout. Half of the suspension was retained for use in controls; the other half was subjected to ultra-violet radiation from a Hanovia quartz mercury-arc lamp for 2 min. at 15 cm. from the lamp hood. Dilution platings from the control and treated suspensions were made to determine conidial viability (approximately 100 per cent) and percentage survival after treatment (approximately 50 per cent). The use of a limiting inositol concentration (0.3γ/ml.) results in a colonial type of growth rather than the typical spreading type (Beadle, 1944), and facilitates colony counts in agar. Samples from the control and irradiated suspensions were then dispensed into a series of flasks (flasks 1–5 received 0.3 ml. and 6–15, 0.1 ml. each of suspension) containing 40 ml. of minimal medium supplemented with all but one of the substances required for growth (*e.g.*, for tests of adaptations at the inositolless locus, flasks received minimal plus 2γ/ml. Ca-pantothenate, 10-γ/ml. (1–) tryptophane, and 5γ/ml. riboflavin). Tests for riboflavinless adaptations were run at 35°C., since this is a temperature-sensitive mutant (Mitchell and Houlahan, 1946); all other flasks were kept at 25°C. For the mutants used little or no growth normally occurs in the absence of the required substance, and adaptations are detected as cultures showing normal growth and conidiation in the deficient medium. The experiment was maintained for 15 days.

It is evident from table 1 that the four mutants used show three different types of behavior when control and ultra-violet treated conidial suspensions are compared: (a) the inositolless and riboflavinless mutants do not grow in the controls, but exhibit a striking response to irradiation, with adaptations resulting in almost all flasks; (b) the pantothenicless mutant shows no adaptations in any flasks, whereas (c) the tryptophaneless mutant adapts in all flasks. It should be noted that in general the adaptations in the flasks of treated conidia appeared sooner than those from non-treated conidia; further, the adaptations also appeared earlier in flasks with the greater amount of inoculum.

Investigations with the inositolless mutant (37401).—Since the inositolless mutant (37401)

Table 1. *Effect of ultra-violet irradiation of a conidial suspension of multiple mutant #G37a in inducing adaptations at four loci.*

Mutant locus being tested for adaptation	Control (no irradiation)		Irradiated	
	Number of flasks	Number of flasks showing adaptation	Number of flasks	Number of flasks showing adaptation
Inositolless (37401)	15	0	15	15
Pantothenicless (5531)	15	0	15	0
Tryptophaneless (10575)	15	15	15	15
Riboflavinless (51602)	4	0	4	3

showed the most marked effect of the treatment and is easy to manipulate experimentally, particular attention was focussed on this mutant in subsequent experiments.

Effects of ultra-violet irradiation on adaptation of the inositolless mutant.—The results of some additional experiments with ultra-violet on the inositolless locus are shown in table 2. The effect of the irradiation in increasing the frequency of adaptations (inositol-independent strains) is obvious. In all controls to date, involving experiments with ultra-violet as well as other treatments, no adaptations of the inositolless mutant with a wild-type growth habit have been obtained in media lacking inositol. In exp. 13, in which irradiated conidia were dispersed into agar plates without inositol, it is possible to obtain an idea of the frequency of induced adaptations per conidium by counting the number of colonies which appear. The average number of colonies per plate was approximately sixteen; test platings on limiting inositol indicated control conidial viability of about 100 per cent, and survival rate after ultra-violet irradiation of 60 per cent. Thus the frequency of adaptations is approximately 1 per 130,000 surviving conidia.

Because of the normally spreading growth habit of *Neurospora*, colony counts are not easily made. Consequently, it was decided to use a colonial mutant (YM2-9.2), obtained in an experiment with nitrogen mustard, combined with inositolless to facilitate such counts. With this mutant the effect of increasing doses of ultra-violet on the frequency of adaptations was also investigated (in experiments performed in the Biology Division, Clinton National Laboratory, Oak Ridge, Tenn.) The mutant stock used was colonial—albino-2—pantothenicless—inositolless. After treatment, platings were made into minimal agar supplemented with Ca-pantothenate. The results, shown in table 3, demonstrate that the frequency of adaptations increases with the ultra-violet dose. The fact that the macroconidia are multinucleate and also difficult to separate completely in conidial suspensions, makes the

TABLE 2. *Effect of ultra-violet irradiation of conidial suspensions in inducing adaptations of the inositolless mutant (37401). (40 ml. of liquid minimal plus necessary supplements in each flask; 15 ml. supplemented minimal agar (1.5%) per plate.)*

Experiment number	No. of conidia added per flask (or plate)	Control: No. flasks (or plates) tested	Control: No. showing adaptation	Irradiated: No. flasks (or plates) tested	Irradiated: No. showing adaptation
10[a]	3.9×10^6	4	0	6	6
16[b]	3.3×10^5	10	0	10	10
13[a]	2.2×10^6	20 (plates)	0	20 (plates)	20

[a] Mutant stock used = #G37a (15300 — 37401 — 5531 — 10575 — 51602).
[b] Mutant stock used = inositolless (37401) — methionineless (4894) — a.

determination from these data of survival and mutation frequencies in terms of individual inositolless nuclei very difficult. Consequently, it is not yet possible to state whether the adaptation frequency increases directly with dose, or whether some other relationship holds, as the present data suggest. Attempts are being made to obtain more information on this point by utilizing a stock (Y-8743; Tatum, Barratt, and Garnjobst, unpublished) in which inositolless is combined with a double mutant which has a colonial type of growth and produces microconidia.

In recent plating experiments with certain stocks of 37401 (in which greater dilutions of conidial suspensions have been used) small colonies have appeared on minimal agar in both treated and control plates. At least some of these appear to be inositol-independent when tested on fresh media and all apparently retain their colonial growth habit on either minimal or inositol-supplemented media. The problem of the origin of these unexpected types is being investigated further.

Proof that the induced adaptations result from genetic reversions at the inositolless locus.—It remains to be determined whether the adaptations with wild-type growth habit induced by ultra-violet are non-genetic in nature or actually represent reverse mutations of the inositolless gene. Crosses of adapted cultures were made to inositolless strains of the opposite mating type. Random ascospore isolations from F_1 gave both inositolless and inositol-independent cultures, but simple 1:1 ratios were not obtained, the inositolless nuclei being considerably in excess in most instances in which macroconidia were irradiated. Such results indicated that the adapted cultures were probably heterocaryons (Beadle and Coonradt, 1944) with inositolless nuclei predominant. This situation might well be expected, since the multinucleate condition of the treated macroconidia makes it probable that any induced change to inositol-independence, if nuclear in character, will affect only one of the nuclei, resulting in a mixture of nuclear types. However, if such is the case, random spore isolations fail to give adequate data on the ratio of the two nuclear types, nor do they furnish a direct demonstration of segregation ratios. To demonstrate segregation ratios directly, all the spores of an individual ascus must be isolated and the resulting progeny tested. Further, since present evidence (Grant, 1945) indicates that ordinarily all the asci in a single perithecium are derived from the descendants of a single pair of haploid nuclei, it is therefore necessary to analyze a complete single ascus per perithecium from several perithecia to determine the ratio of nuclear types present in a heterocaryon. Table 4 gives the results of such an analysis with an adapted strain, R-3, crossed to inositolless (37401).

The regular 1:1 ratios of inositolless and inositol-independent cultures obtained from asci III, VII, and VIII indicate that inositol-independence in the adapted strain is due to a chromosomal and not a non-Mendelian cytoplasmic factor. Further, the ratio of whole asci yielding only inositolless cultures (22) to those yielding both types (8), indicate that the adapted strain R-3 is a heterocaryon with a ratio of inositolless:inositol-independent nuclei of approximately 3:1. Segregation of the other charac-

TABLE 3. *Frequency of induced adaptations of inositolless (37401) in relation to ultra-violet dosage. Platings of colonial (YM2-9.2)—albino-2—pantothenic less-inositolless into minimal agar supplemented with Ca-pantothenate.*

Ultra-violet irradiation time (in seconds)	No. of macroconidia tested after treatment	Per cent macroconidial survival	Total number of adaptations	No. of adaptations per 10^6 surviving macroconidia
0	48.0×10^6	100	0	0.0
15	45.6×10^6	53.8	216	8.8
30	60.0×10^6	53.8	584	18.3
45[a]	22.4×10^6	4.5	63	63.0
60	134.4×10^6	2.0	376	139.4

[a] Data for 45 sec. treatment obtained in an independent experiment.

TABLE 4. *Characteristics of F_1 cultures obtained from a cross of an adapted (inositol-independent) strain, R-3 (derived from ultra-violet irradiation of multiple mutant, 15300-5531-37401-A) with inositolless, 37401-a. Ascospores isolated in order from whole asci, one per perithecium.*

Spore no.	Ascus number							
	I	II	III	IV	V	VI[b]	VII[b]	VIII[b]
1	inos pan^{+} al^{+}	inos pan^{+} al^{+}	$inos^{+}$ pan^{+} al^{+}	inos pan al^{+}	inos pan al^{+}	inos al^{+}	[a]	inos al
2	inos pan^{+} al^{+}	inos pan^{+} al^{+}	$inos^{+}$ pan^{+} al^{+}	inos pan al^{+}	inos pan al^{+}	[a]	$inos^{+}$ al^{+}	[a]
3	inos pan al	inos pan^{+} al	$inos^{+}$ pan^{+} al	inos pan al^{+}	inos pan al	inos al	inos al^{+}	$inos^{+}$ al^{+}
4	inos pan al	inos pan^{+} al	$inos^{+}$ pan^{+} al	inos pan al^{+}	inos pan al	inos al	inos al^{+}	$inos^{+}$ al^{+}
5	inos pan al	inos pan al	inos pan al	[a]	inos pan^{+} al	inos al^{+}	[a]	inos al^{+}
6	inos pan al	inos pan al	[a]	inos pan^{+} al	inos pan^{+} al	inos al^{+}	inos al	inos al^{+}
7	inos pan^{-} al^{+}	inos pan al^{+}	inos pan al^{+}	inos pan^{+} al	inos pan^{+} al^{+}	inos al	$inos^{+}$ al	$inos^{+}$ al
8	inos pan^{-} al^{+}	inos pan al^{+}	inos pan al^{+}	[a]	inos pan^{+} al^{+}	[a]	$inos^{+}$ al	$inos^{+}$ al

[a] Spore did not germinate. [b] Pantothenic requirement not determined in resulting spores.

Total whole asci analyzed—one per perithecium (including those in order) 30
Number asci yielding only inositolless cultures 22
Number asci yielding both inositolless and inositol-independent cultures 8

ters, albino and pantothenicless, are as would be expected from a cross of the indicated parentage, and show that these genes are present and unchanged in both the inositolless and inositol-independent nuclei of the heterocaryon.

Finally, it is necessary to demonstrate that the inositol-independence of the adapted strain is due

TABLE 5. *Origin and number of single-ascospore cultures from crosses of three inositol-independent F_1 cultures with wild-type. All cultures were found to be inositol-independent.*

Cross of	× wild-type	No. of asci analyzed	No. of spores isolated at random
R-3.23			
(al^{+} $inos^{+}$ pan).....	15300-a	10	128
R-3.21			
(al $inos^{+}$ pan)......	15300-a	0	167
R-1.1			
(al^{+} $inos^{+}$ pan).....	15300-A	0	84

to an induced reversion of the original inositolless gene, and not to a mutation at some other locus. If another locus has mutated to give inositol-independence, crossing an extracted F_1 adapted strain to the original wild-type strain should yield a recombination class which is inositolless, whereas if the adapted strain is actually a reverse mutation at the original inositolless locus, only wild-type cultures are expected from such a cross. In table 5 are tabulated the ascospores isolated from whole asci or at random from crosses involving three different inositol-independent cultures—obtained by single ascospore isolations from the F_1 of crosses of adapted strains R-1 (from ultra-violet irradiation of strain 5531-37401-A) and R-3 with inositolless. When the cultures produced from these ascospores were tested, they all proved to be inositol-independent. As in previous crosses, regular expected segregations for other genetic markers present, such as albino and pantothenicless, were observed. Since no recombinations were found in any of the cultures tested, it follows that the genes involved are probably alleles.

Physiological studies with induced reversions of inositolless.—Growth rate comparisons were made of the reverted inositol-independent strains and wild-type (table 6). The procedure was essentially that described by Ryan, Beadle and Tatum (1943). The use of unwashed Difco Noble (3 per cent) agar probably accounts for the somewhat lower average growth rates obtained. In exp. 1 the growth rates of the original adapted and F_1 extracted inositol-independent strains, R-1 and R-1.1, were essentially similar to those of wild-type both with or without added inositol. This is further evidence that a reverse mutation to the normal wild-type allele of inositolless had been induced by the ultraviolet treatment. Even though R-3, as demonstrated previously (table 4), is genetically a heterocaryon,

Table 6. *Comparison of growth rates (mm./hr. at 25°C.).*

Strain	Origin	Exp. 1 A: 2.0γCa panto-thenate/ml.	Exp. 1 B: as in A, plus 4.0γ inositol/ml.	Exp. 2 C: 2.0γCa panto-thenate/ml.	Exp. 2 D: as in C, plus 16γ inositol/ml.	Exp. 2 E: as in D, plus 0.5% yeast extract
		Minimal agar supplemented with:				
1-A	wild type	3.7[a]	3.7	3.7	..	3.7
15300-5531-37401	al pan inos (parental)	0.0	3.5	0.0	3.8	3.6
R-1	U.V. induced reversions of inositolless	4.0	4.0	..	..	..
R-3	U.V. induced reversions of inositolless	3.5	3.7	3.3	3.7	3.4
R-1.1	extracted F_1 cultures	3.5	3.7	..	..	..
R-3.21	extracted F_1 cultures	2.1	2.1	2.0	2.2	1.8
R-3.23	extracted F_1 cultures	2.4	2.4	..	..	..

[a] Each rate given represents the average of measurements in two growth tubes.

including a high proportion of the inositolless nuclei, yet essentially wild-type growth rate is obtained both on minimal and inositol-supplemented media.

The growth rates obtained with strains R-3.21 and R-3.23 were much less than those for wild-type. At first it was thought that these slow-growing types might represent new alleles at the inositol locus—controlling inositol synthesis, but in amounts less than those required for optimal growth. The evidence from the B part of exp. 1 as well as from exp. 2 rules out this hypothesis, however, since the addition of inositol, even in amounts well above those required for maximal growth of the inositolless strain, has no effect on the reverted strains. It has further been shown that the restricted growth rate of R-3.21 is not due to diffusible growth factors other than inositol, since in part E of exp. 2, the addition of 0.5 per cent yeast extract has no stimulatory effect.

The problem of the relation of these slow-growing reversions to the inositol locus still remained. It was necessary to determine whether the slow-growth character segregated in a regular Mendelian fashion and, if so, what was the relation of this character to the inositol locus. In crosses of both R-3.21 and R-3.23 to strains with wild-type growth rates, approximately 1:1 segregations for slow and normal growth rates were obtained. In addition, backcrosses of strain R-3.21 to the parental inositolless stock (15300–37401–5531) were made and segregations for inositol-dependence and growth-rate type studied (table 7). Here also the slow-growth rate character shows a regular Mendelian segregation, indicating that a single major gene is responsible. Further, although most of the resulting cultures have the same character combinations as the parental types, it is possible to obtain inositol-independent strains with normal growth rates, as in spore No. 7. The present data thus suggest that a single gene, *s*, is responsible for the characteristic growth rate of R-3.21, and that this gene may be linked with the inositol locus, but can be separated from it by crossing over. There are at least two possibilities as to the origin of gene *s*. It may have been induced by the ultra-violet treatment at the same time as the inositolless reversion in strain R-3, where its effect would not be immediately detectable because of the heterocaryotic nature of this strain, or the gene may have been present in one of the wild-type stocks used in crossing, where its presence would again be obscured if wild-type nuclei were also present.

Table 7. *Characteristics of cultures derived from random single ascospore isolations of a cross of the F_1 slow-growing inositol-independent (reverted) strain R-3.21 with inositolless (15300-37401-5531).*

Spore no.	Inositol requirement[a]	Growth rate (in mm./hr.)[b]
1	+	2.4
2	+	2.4
3	+	2.3
4	+	2.3
5	+	2.2
6	—	3.9
7	+	3.8
8	—	4.2
9	—	3.6
10	—	3.9

[a] + indicates inositol-independence, — inositol required.
[b] One growth tube measurement on minimal 3% agar plus 4γCa-pantothenate and 8γinositol/ml. at 25°C.

Spontaneous reversions of inositolless.—Since reverse mutations occurred only in the treated conidia in all previous experiments, the question naturally arose whether such mutations ever occur spontaneously. Consequently, experiments were set up utilizing larger populations of inositolless nuclei over longer periods of time than when conidial suspensions were used to test this point. Five series of flasks containing 30 ml. each of minimal medium

plus 2.0 γ Ca-pantothenate/ml. were supplemented with increasing amounts of inositol to permit increasing mycelial growth and thus larger populations of inositolless nuclei. The highest concentration of inositol used, 1.2γ/ml., still gives much less than maximal growth, and thus any adaptations can be readily detected. The flasks were inoculated with the inositolless stock 15300–5531–37401-a; twenty flasks of each series were kept at 25°C. for 30 days, and ten at 30°C. for 57 days. The results of these experiments are shown in table 8. It is evident that spontaneous adaptations do occur, but at a very low rate. Genetic tests of the type discussed previously indicate that these adaptations also result from reverse mutations of the inositolless gene.

The results of these experiments are of further interest in showing that the frequency of spontaneous reverse mutations increases with increased inositol concentration (and consequently with increasing populations of inositolless nuclei, as would be expected). This evidence suggests that selection against reverse-mutations to inositol-independence in the presence of increasing amounts of inositol (and thus increasing populations of inositolless nuclei) does not occur. Thus the behavior of inositolless reversions is different from that found in leucine reversions (Ryan and Lederberg, 1946), where such negative selection does occur. Further evidence against negative selection is obtained from observations with adapted cultures which are heterocaryons containing both inositolless and reverted nuclei. The nuclear ratios in such heterocaryons, as obtained by crossing tests, do not seem to be appreciably modified when they are grown on inositol-supplemented as compared with minimal media. It thus appears that when a mutation to inositol-independence occurs it will be regularly selected for in the absence of inositol and an adaptation will result. This mutant should thus provide favorable material for a study of mutation rates and for a comparison of the effectiveness of various mutagenic agents, especially when uninucleate conidia are used.

Effects of mutagenic agents other than ultra-violet in inducing reversions of inositolless.—Preliminary tests have been made of the effects of three agents in addition to ultra-violet in inducing reversion of the inositolless gene in macroconidia. These results are given in table 9.

In the x-ray experiments separate conidial suspensions of multiple mutant stock #G37a were exposed to 25,000 and 50,000r respectively using a tube operating at 50 KV and 25 ma with a tungsten target at an intensity of 1200r/min.

For the nitrogen mustard experiments (Auerbach *et al.*, 1947; Tatum, 1946) conidia were exposed to the indicated concentrations of tris (β-chloroethyl) amine hydrochloride in phosphate buffer at *p*H 6.2, centrifuged and resuspended in *Neurospora* liquid minimal twice and then transferred to the test flasks of supplemented minimal medium.

The radiophosphorus used was the separated isotope, p32 (half-life:14.3d, β-radiation:1.69 Mev, obtained from the Clinton Laboratories at Oak Ridge). The concentration (activity) of the radiophosphorus solution when obtained was approximately 0.58 millicuries/ml. Appropriate amounts of this solution were added directly to suspensions of conidia in minimal medium to give the final test concentrations of radiophosphorus indicated. The conidia remained in the radiophosphorus solutions throughout the course of the experiment. Additional control experiments in which inositol was added to conidial suspensions in flasks containing radiophosphorus concentrations as high as 0.29 mc./ml. indicated that normal growth occurs and is maintained for a considerable period even in the presence of continuous radiation of the intensity indicated.

At least one adapted culture produced by each of these types of treatment has been tested to show that an actual genetic reversion is involved. These experiments thus demonstrate that reversions at the inositolless locus can be induced by other mutagens—both chemical and physical. Although none of the agents tested was as effective under the indicated experimental conditions as ultra-violet radiation, it is not yet possible to make adequate quantitative comparisons of the relative mutagenic efficiencies of the various treatments.

INVESTIGATIONS WITH MUTANTS OTHER THAN INOSITOLLESS.—Attention has been centered principally on the behavior of the inositolless mutant (37401), but data have been obtained for adaptations of several other mutants as well. In table 1, the results indicate no effect of ultra-violet treatment on pantothenicless (5531). This mutant appears to be extremely stable, as no adaptations have been obtained either spontaneously or with any of the several treatments tried. This stability of the pantothenicless mutant may represent a case of an extremely low reverse mutation rate, or, perhaps more likely, may result from a chromosomal deletion—a genetic change which is presumably irreversible. The riboflavinless mutant (51602) appears to behave more like inositolless in response to ultra-violet and other treatments. It is more difficult to

TABLE 8. *Numbers of spontaneous reversions of the inositolless mutant (37401) at different levels of inositol concentration. Mutant strain used: 15300-5531-37401-a. 30 ml. of liquid minimal medium plus 2.0γ/ml. of Ca-pantothenate/flask. Figures in parentheses indicate days after start of experiment on which reversions were first detected.*

	25°C.[a]		30°C.[b]	
γ inositol per ml.	No. of flasks	No. of reversions	No. of flasks	No. of reversions
0.00	20	0	10	0
0.15	20	0	10	0
0.30	20	0	10	0
0.60	20	1 (16)	10	2 (24,41)
1.20	20	3 (22,24,25)	10	1 (17)

[a] Experiment maintained for 30 days.
[b] Experiment maintained for 57 days.

TABLE 9. *Effect of various treatments of conidial suspensions on reversion of the inositolless mutant (37401). Multiple mutant stock #G37a used .40 ml. liquid minimal plus necessary supplements per flask. 30°C.*

Treatment		No. of conidia added per flask	Approx. % killed	Control		Treated		Duration of exp. in days
				No. of flasks	No. of reversions	No. of flasks	No. of reversions	
X-ray	25,000r	3.3×10^6	60	10	0	6	1	21
	50,000r		85			15	3	
Nitrogen mustard	0.001% 10 min.	3.7×10^6	15	5	0	5	0	39
	0.01% 30 min.	1.5×10^6	68	10	0	10	4	30
Radiophosphorus	5.8×10^{-5}mc./ml.					10	1	
	5.8×10^{-4}mc./ml.	7.2×10^6	..	10	0	15	2	25
	5.8×10^{-3}mc./ml.					10	3	

work with, however, because it is a temperature-sensitive mutant (Mitchell and Houlahan, 1946) and requires more precisely controlled conditions for its expression. One of the ultra-violet induced riboflavin adaptations has been shown to be a reverse mutation by the appropriate genetic tests. The tryptophane-requiring mutant (10575) adapts as frequently without treatment as with, both in ultra-violet and other experiments. Some tests have been made to determine whether this regular adaptation is genetic. From crosses of three spontaneously adapted cultures with the original tryptophaneless, only tryptophaneless progeny have been recovered in 154 ascospore isolations made at random. From a similar cross involving an ultra-violet treated adapted culture, three tryptophane-independent strains have been recovered from a total of thirty-eight random ascospore isolations. These results suggest that the spontaneous adaptations are non-genetic in nature, whereas those adaptations resulting after ultra-violet treatment may represent a combination of genetic and non-genetic effects.

Other multiple mutant strains have been utilized in similar preliminary experiments to determine the effect of irradiation on adaptation of additional biochemical mutants (table 10). In the two instances cited, the treatment greatly increases the frequency of adaptations, though these also occur spontaneously, but appear much later than in the flasks of treated conidia. Genetic analyses have not yet been made of these adapted strains, but the evidence obtained in the previously described experiments with other mutants, strongly suggests that they represent reverse mutations.

SUMMARY

Studies have been made of the effects of various mutagenic agents in inducing adaptations of a number of biochemical mutants in *Neurospora crassa.* The frequency of adaptation of certain mutants, such as inositolless (Stanford mutant number 37401), cholineless (34486), methionineless (4894), and riboflavinless (51602) can be greatly increased by ultra-violet radiation; for other mutants, such as pantothenicless (5531), no adaptations have been produced by any treatment yet attempted. Genetic and physiological tests, particularly with the inositolless mutant, indicate that these induced adaptations represent reverse mutations to the original wild-type allele. X-rays, radiophosphorus, and nitrogen mustard have also proved effective in inducing reversions of inositolless. The frequency of reverse mutation has been demonstrated to increase with increasing radiation dose (for ultra-violet treatment) for the inositolless mutant in experiments utilizing a plating colony-count technique with a colonial-inositolless mutant.

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TABLE 10. *Effect of ultra-violet irradiation of conidial suspensions in inducing adaptations of two biochemical mutants. In all experiments four flasks received 0.5 ml. of conidial suspensions, three, 0.2 ml., and three, 0.05 ml. Figures in parentheses indicate days after start of experiment when adaptations were first detected. Other experimental conditions as described for exp. 12 (table 1).*

Mutant locus being tested	Original conidial concentration/ml.	Control (no irradiation)		Irradiated	
		No. of flasks	No. of adaptations	No. of flasks	No. of adaptations[a]
Cholineless (34486)	45.0×10^6	10	1(9)	10	9
Methionineless (4894)	26.6×10^6	10	2(10)	10	8

[a] All adaptations in irradiated flasks appeared within 4 days after treatment.

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THE ISOLATION, GROWTH, AND METABOLISM OF BLASTOCLADIA IN PURE CULTURE [1]

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For conclusive investigation of any microorganism, pure cultures are not only extremely desirable but usually absolutely essential. Whether interest is focussed upon structure or metabolism, systematic position or reproductive behavior, controlled study permitting reproducible observations nearly always depends on the use of pure cultures. Without them, many variables remain almost entirely uncontrolled. And yet much effort, even in recent years, has been and still is being devoted to observation and description of contaminated growths of many of the key genera of those interesting lower Phycomycetes included in the general category of "water-molds." Certain of the descriptive and even monographic studies of the past few decades could have been of more lasting value had more effort been made to obtain and maintain pure cultures of the fungi concerned, and less to the description of often doubtfully new species.

This point is convincingly illustrated by a glance at present knowledge of the phycomycetous family Blastocladiaceae (Sparrow, 1943; Quantz, 1943; Teter, 1944; Hatch and Jones, 1944; Jones, 1946; Shoup and Wolf, 1946; and references cited therein). For simplicity we may consider three genera, *Blastocladia, Allomyces,* and *Blastocladiella,*[3] first described in 1878 (Reinsch), 1911 (Butler), and 1937 (Matthews) respectively. Despite its relatively recent discovery, much reliable information has been gathered on the structure, taxonomy, reproduction, and nutritional requirements of *Blastocladiella.* Three different life cycles, exactly paralleling those of *Allomyces,* and two types of sexuality are known. Most if not all of the seven or eight described species have been grown in pure culture and appear to be quite distinct entities. For *Allomyces* we have a large number of reliable data on morphology, systematic relations, variability under controlled conditions, sexual behavior, alternation of generations, and nutrition, as well as a start on certain phases of genetics and metabolism. Nearly all exact knowledge of this extremely interesting genus relates finally to the isolation and maintenance of all strains and species in pure culture.

Our understanding of the oldest genus of the family is in striking contrast. While Blackwell (1940) has described a reduced life history in *Blastocladia Pringsheimii,* we must admit that we have no complete picture of the reproductive cycles of the genus as a whole. No form of sexual reproduction has been discovered (Blackwell, 1939). The diagnoses of half of the twelve or more described species are admittedly indistinct (*cf.* Sparrow, 1943). Virtually nothing is known about the nutrition and metabolism of these organisms which are found in nature on suggestively limited types of substrata. Since Reinsch's original discovery of *B. Pringsheimii* 70 years ago, a number of unsuccessful attempts have been made to obtain pure cultures of *Blastocladia* (Kanouse, 1927; Crooks, 1937; Lloyd, 1938; Indoh, 1940). Despite his unique success with *Rhipidium* and *Araiospora,*

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[3] *Sphaerocladia* Stüben (1939) is included here in accordance with the interpretation of Couch and Whiffen (1942).