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Induction of Mitotic Recombination by Chemical Agents in *Aspergillus nidulans*

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*Aspergillus nidulans*에 있어서 體細胞 再組合의 誘發에 化學物質이 미치는 影響

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ABSTRACT

Germinating conidia of *Aspergillus nidulans* diploid heterozygous for color and other genetic markers were used to detect and distinguish genetic events such as mutation, mitotic crossingover and nondisjunction in a single test after treatment with N-methyl-N′-nitro-N-nitrosoguanidine (NG), mitomycin C (MC), and chloral hydrate (CH).

The following results were obtained:

1. NG reduced the survival of conidia and increased the frequencies of mitotic segregants about sevenfold over the control; among the mitotic segregants the predominant genetic event was mitotic crossingover. NG also produced many abnormal colonies, which appeared to be of the types caused by induced semi-dominant lethals or chromosomal aberrations, and the aneuploid types found spontaneously.
2. After treatment with MC the survival of conidia was reduced but few abnormal colonies were produced. The frequencies of mitotic segregants were increased about threefold over the control; in the mitotic segeregants the induced genetic event was mitotic crossingover.
3. CH gave no apparent effect on the survival of conidia and the frequencies of mitotic segregants. However, CH generated abnormal colonies, very greatly, which turned out to be of the aneuploid types. This result suggests that CH interferes with the normal distribution of chromosomes in mitosis.

INTRODUCTION

Heterozygous diploid strains of various fungi have been extensively used to study chemically induced mitotic recombination. For such analyses it is important to have genetic markers available so that induced

mitotic recombination can be easily recognized visually or by genetic test. Morpurgo and Sermonti(1959) observed increases in mitotic recombination due to chemical agents in some heterozygous diploid strains of *Penicillium chrysogenum*. In diploid strain of *Aspergillus nidulans* an increase in the frequencies of mitotic recombination was induced by formaldehyde (Fratello *et al.*, 1960). A variety of chemical agents have been found to increase mitotic recombination in Aspergillus (Morpurgo, 1963; Beccari *et al.*, 1967; Bignami *et al.*, 1974; Kappas and Georgopoulos, 1974; Kappas *et al.*, 1974; Harsanyi *et al.*, 1977), in Saccharomyces (Yost *et al.*, 1967; Chanet *et al.*, 1975; Davies *et al.*, 1975), in Penicillum (MacDonald, 1971), in Ustilago (Esposito and Holliday, 1964), and in Coprinus (North, 1977).

For the genetic analysis of mitotic recombination induced by chemical agents, diploid strains of *Aspergillus nidulans* are well suited (Kaffer *et al.*, 1976). It is possible through the use of these diploid strains to recognize the presence of any member of the eight pairs of linkage groups. Furthermore, these diploid strains make it possible to detect and distinguish all types of induced mitotic recombination because extensively marked linkage groups I and II permit discrimination among the genetic events of mutation, mitotic crossingover and nondisjunction.

In several species of fungi the frequency of mitotic crossingover was increased by NG(Zimmerman *et al.*, 1966; Shanfield *et al.*, 1971). MC has been shown to induce mitotic recombinationin in Ustilago and Saccharomyces under conditions in which the antibiotic is not mutagenic (Holliday, 1964). Although CH has been shown to prevent the formation of mitotic spindles and to destroy those previously formed in *Aspergillus nidulans* (Mercer and Morris, 1975), its mechanism of action are not clearly known.

The purpose of this experiment was to investigate the effects of different types of chemical agents (NG, MC, and CH) in germinating conidia of a heterozygous diploid of *Aspergillus nidulans*. The effects of these chemical agents on mitotic recombination were determined by identifying the colored mitotic segregants and testing the biochemical markers. At the same time, the genetic activity and the effects on chromosome segregation were evaluated by an examination of the percentage survival after treatment with chemical agents and from the frequency and type of abnormal colonies.

MATERIALS AND METHODS

1. Strains

The haploid strains of *Aspergillus nidulans* No. 154 and No. 159 were derived from the Fungal Genetic Stock Center, FGSC. Strain No. 154 had white conidia but carried the hypostatic gene for chartreuse conidia, while strain No. 159 had yellow conidia. All genetic markers were recessive except the dominant gene for acriflavine resistance (acrA). The genotypes of the two strains are shown in Figure 1, using the gene symbols suggested by Clutterbuck (1974). These two haploid strains were used to synthesize a heterozygous diploid which had green conidia. This diploid was used in all the experiments.

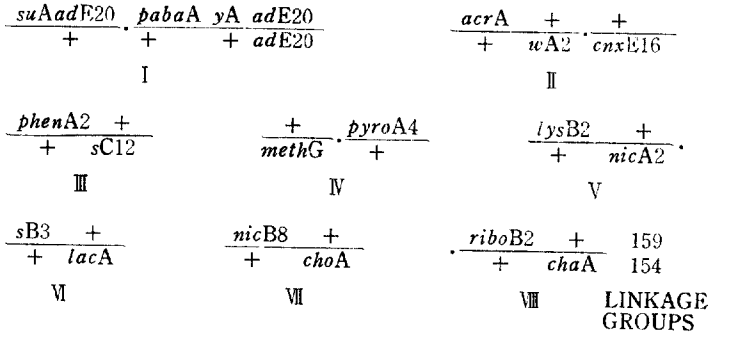

Fig. 1. Genotype of diploid strain (synthesized from the two haploids No. 154 and No. 159) with markers on linkage groups I—VIII. Conidial color markers: *yA*, yellow; *wA2*, white; *cha*A, chartreuse. Markers determining requirements: *ad*E20, adenine; *cho*A, choline; *lys*B5, lysine; *meth*G, methionine; *cnx*E16, nitrite; *nic*A2 and *nic*B8, nicotinic acid; *paba*, *p*-aminobenzoic acid; *phen*A2, phenylalanine; *pyro*A4, pyridoxine; *ribo*B2, riboflavine; *s*B3 and *s*C12, sulfite. Suppressor: *su*A*ad*E20, suppressor of *ad*E20. Resistance marker: *acr*A, acriflavine. (All unnumbered alleles have isolation number 1.)

2. Media

The minimal medium, modified from Harsanyi *et al.* (1977), consisted of sodium nitrate, 6 g; magnesium sulfate, 0.52 g; potassium dihydrogen phosphate, 3.04 g; potassium chloride, 0.52 g; ammonium molybdate, 0.08 mg; zinc chloride, 8.31 mg; manganese chloride ($4H_2O$) 0.16mg; cupric sulfate ($5H_2O$), 0.69mg; ferric chloride ($6H_2O$), 2 mg;dextrose, 10 g; agar (Difco), 15 g; and distilled water, 1 l; it was adjusted to pH 6.5. The complete medium has been described by Harsanyi *et al.*, (1977).

3. Chemical treatment

For all chemical treatments, conidia were suspended in 0.85% saline with 0.02 % Tween 80. Conidia were resuspended and germinated in the appropiate treatment solution. After treatment conidia were replated after appropriate dilution on complete medium.

NG treatment: Conidia suspended in saline were germinated for $4\frac{1}{2}$ hr in liquid minimal medium at 37°C. These germinating conidia were incubated with NG at a concentration of 0.5mg/ml in 0.1M disodium citrate buffer at pH 5.5. Treatments were terminated after 3 min by diluting 100-fold into pH 7.0 phosphate buffer (Chang and Tuvesun, 1967). Control suspensions were treated identically, except the NG was omitted.

MC treatment: Conidia were incubated in liquid minimal medium at 37°C until the time at which germ tubes were formed. Control suspensions of conidia needed 6 hr incubation at this temperature, while experimental suspensions containing 400 μg/ml-MC (Holliday, 1964) required 8 hr.

CH treatment: Treatments with CH were similar to those with MC. Conidia were incubated in liquid minimal media containing 0.01 M-CH for 12 hr—the time at which germ tubes were formed.

4. Genetic analysis

After 3 day's incubation at 37°C, survival was calculated as a percentage of the corresponding values obtained from control (which is set at 100%). All colonies were first classified into three groups: normal, abnormal, and normal with colored sectors. Abnormals were recognized

poor and/or poor conidiation and invariably a characteristic crinkled appearance, in contrast to the densely conidiating, relatively flat, rapidly growing normal colonies. Abnormal colonies produced many rapidly growing and fully condiating sectors which appeared on the original plates, as well as after subculture of abnormal conidia from the center of the abnormal colonies onto normal complete media. All sectors were isolated by restreaking and transferred to master plates, which were then replicated onto the various test media.

The normal colonies with colored sectors were classified according to colors of the sectors. White, yellow, and chartreuse sectors were found to originate from the center of the colony or from the periphery of the colony. Sectors originating from the periphery of the colony were found to be equal frequency among experimental and control samples; therefore, only those sectors originating from the center of the colony are included in these results.

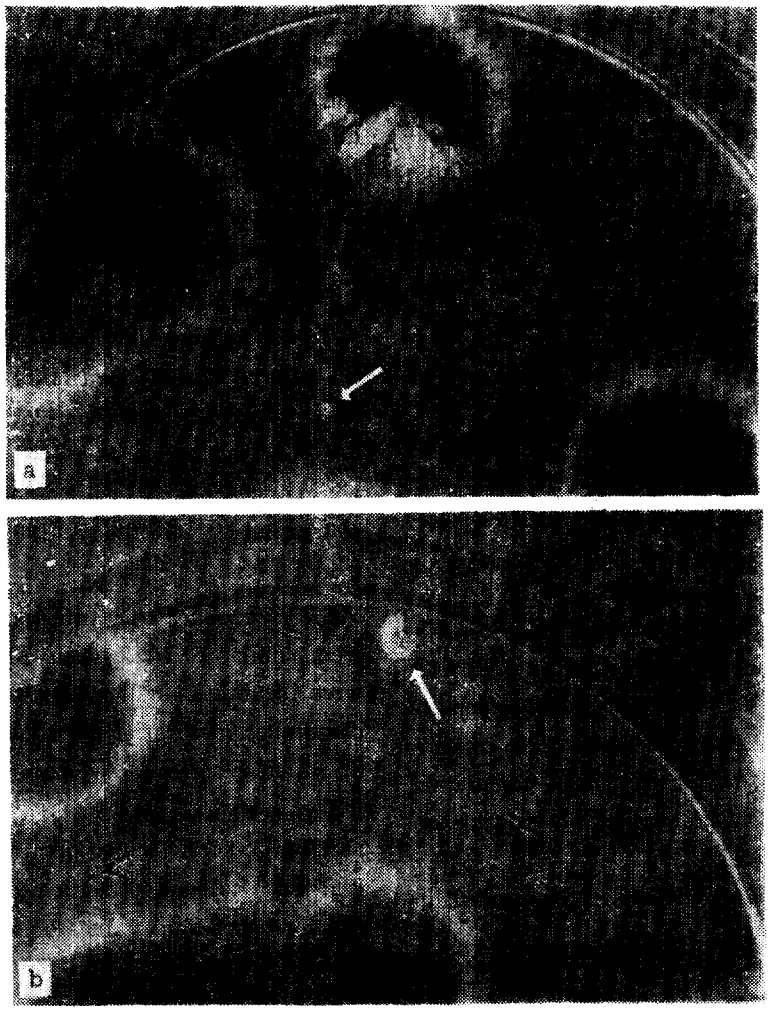

Fig. 2. Colonies surviving after treatment with NG. (a) Normal colony with white sector, normal colonies, and nonsectoring abnormal colony (arrow). (b) Sectoring abnormal colony (arrow) and large normal colonies.

The colored sectors arising from normal colonies could usually be identified as diploids or haploids by carring out one or more alternative identification procedures, namely (a) appearance and presence of heterozygous markers; (b) measurement of conidiospore diameter (Pontecorvo *et al.*, 1953); (c) somatic instability and production of haploid sectors on prolonged incubation of the Benlate test plates (Upshall *et al.*, 1977). A diploid sector could be caused owing to mutation, mitotic crossingover, or nondisjunction. These three genetic events can be distinguished in the case of linkage groups having many heterozygous markers, preferably on both arms; namely, linkage groups I and II, which also carried the color markers.

RESULTS

1. Effect of NG treatment

Genetic activity: Figures 2a and 2b show examples of the three groups of colonies. In Figure 2a a normal colony with white sector, normal colonies, and a nonsectoring abnormal colony are seen. In Figure 2b an abnormal colony is seen to produce normal sectors. Table 1 gives the frequencies of mitotic segregants and abnormal colonies after NG treatment. These are compared with the corresponding control values. Of the 63.36% which survived, 13.73% were abnormals and 13.98% were normals with sectors. The ratio of diploids to haploids of colored sectors was approximately 11:1. In comparison, the control colonies consisted of 0.92% abnormals, and 2.14% normals with sectors, of which the ratio of diploids to haploids was 2:1.

Table 1. Frequencies of mitotic segregants and abnormal colonies after treatment with NG

	Control		NG treatment	
	Number	%	Number	%
Survival		(100)*		63.36
Total number of colonies	655		415	
Abnormals	6	0.92	57	13.73
Normals	635	96.94	299	72.29
Normals with sectors	14	2.14	58	13.98
Diploids	9	1.37	53	12.77
Haploids	5	0.77	5	1.21

*The control value of 655 was taken to be 100% survival.

Diploid segregants: In Table 2 the frequencies and types of diploid segregants are presented. The genetic event producing each genotype in the white and yellow segregants, but these events could not be distinguished phenotypically in the case of the chartreuse segregants. After treatment with NG mitotic crossingover was the predominant event leading to white and yellow segregants, appearing six times as frequently as nondisjunction(4.57% vs 0.72%). Compared with controls, mitotic crossingover was more frequently induced about six times (4.57% vs 0.76%). The increase in nondisjunction was twofold(0.72% vs 0.31%).The frequencies of all diploid segregants were over ninefold higher than control values (12.77% vs 1.31%).

Among the diploid segregants, some were found to have additional genetic events. An additional event was recognized as a homozygosity for any one of the recessive markars. The homozygosity could have been due to any one of the genetic events leading to original diploid segregants. The frequencies of the additional events after NG treatment were very greatly augmented in comparison with the control values (4.58 % vs 0.15%).

Table 2. Frequencies and types of diploid mitotic segregants among NG treated and control colonies

Genotype	Genetic event	Treatment	Sectors with event alone		Sectors with additional event		Total sectors	
			Number	%	Number	%	Number	%
*acr*A⁺*w*A2*cnx*E16⁺ / *acr*A⁺*w*A2*cnx*E16	C.O.[a]	Control[c]	1	0.15	0	0	1	0.15
		NG[d]	3	0.72	2	0.48	5	1.20
*acr*A⁺*w*A2*cnx*E16 / *acr*A⁺*w*A2*cnx*E16	NDJ[b]	Control	0	0	0	0	0	0
		NG	0	0	1	0.24	1	0.24
*su*A*ad*E20*paba*A*y*A*ad*E20[e] / *su*A*ad*E20⁺*paba*A*y*A*ad*E20	C.O.[f]	Control	4	0.61	0	0	4	0.61
		NG	9	2.17	5	1.20	14	3.37
*su*A*ad*E20*paba*A*y*A*ad*E20 / *su*A*ad*E20*paba*A*y*A*ab*E20	NDJ	Control	1	0.15	1	0.15	2	0.31
		NG	2	0.48	0	0	2	0.48
*ribo*B2⁺*cha*A[g] / *ribo*B2⁺*cha*A	Not determined	Control	2	0.31	0	0	2	0.31
		NG	20	4.82	11	2.65	31	7.47
Total		Control	8	1.22	1	0.15	9	1.37
		NG	34	8.19	19	4.58	53	12.77

[a] C.O. (Crossingover). [b] NDJ (Nondisjunction). [c] Percent based on 655 total control colonies.
[d] Percent based on 415 surviving NG treated colonies.
[e] Or the alternative crossover genotype $\frac{su\text{A}ad\text{E}20paba\text{A}y\text{A}ad\text{E}20}{su\text{A}ad\text{E}20^{+}paba\text{A}^{+}y\text{A}ad\text{E}20}$
[f] Or due to mutation.
[g] Or the phenotypically indistinguishable $\frac{ribo\text{B}2^{+}cha\text{A}}{ribo\text{B}2cha\text{A}}$

Haploid segregants: The frequencies and types of haploid segregants are presented in Table 3. An increase in the frequencies of all haploid segregants is less marked. Although additional genetic events were also found among the haploid segregants, the frequencies of these additional events did not increase.

Abnormal colonies: Table 4 lists the types of abnormal colonies after treatment with NG. The number of abnormal colonies in which at least one of the segregants was associated with corssingover or nondisjunction in one of the eight linkage groups was investigated. First abnormal colonies were categorized as nonsectoring and sectoring colonies. Of the 21 which were nonsectoring colonies, 6 could not be characterized genetically, because they did not conidiate. The 15 which could be tested were found to be diploids with reduced conidiation. Six of these were homozygous for the nutritional markers. The sectoring colonies were classified on the basis of the ploidy of the produced sectors. Thus 7 of the 57 abnormal colonies, produced only haploid segregants, while 6 produced both haploid

Table 3. Frequencies and types of haploid mitotic segregants among NG treated and control colonies

Genotype	Treatment	Sectors with event alone		Sectors with additional event		Total sectors	
		Number	%	Number	%	Number	%
*su*A*ad*E20*pabaA*y*A*ad*E20	Control[a]	1	0.15	0	0	1	0.15
	NG[b]	0	0	0	0	0	0
*acr*A$^+$*w*A2*cnx*E16	Control	2	0.31	2	0.31	4	0.61
	NG	2	0.48	1	0.24	3	0.72
*ribo*B2$^+$*cha*A[c]	Control	0	0	0	0	0	0
	NG	2	0.48	0	0	2	0.48
Total	Control	3	0.46	2	0.31	5	0.77
	NG	4	0.97	1	0.24	5	1.21

[a] Percent based on 655 total control colonies.
[b] Percent based on 415 surviving NG treated colonies.
[c] Included is the genotype *cha*A*y*A, which is phenotypically cream colored.

and diploid segregants. Only diploid segregants were by 23 abnormal colonies. Hence, the center of colony in the last two catagories had to have been at least diploid. In a word, 29 abnormal colonies were able to raise diploid segregants, whereas no diploid segregants were recovered from 7 abnormal colonies. Some segregants arising from abnormal colonies gave rise to additional segregants which were found to be haploid or diploid. These segregants were either of hyperhaploid (mostly n+1, n+2) or hyperdiploid (mostly 2n+1, 2n+2).

All the sectors arising from the abnormal colonies are analyzed in Table 5. A total of 67 diploid and 23 haploid sectors was generated by 36 abnormal colonies. Among the diploid segregants, 17 events of crossingover and one event of nondisjunction were found in linkage groups I and II. The predominant gen-

Table 4. Number and distribution of abnormal colonies induced by NG

Type of abnormal colony	Number of colonies in which genetic event was found						
	Total	C.O./ I[a]	NDJ/ I	C.O./ II	NDJ/ II	C.O. or NDJ/ III—VIII	None
Non-sectoring							
Not characterized	6	—	—	—	—	—	—
Diploid	15	0	0	0	0	6	8
Sectoring							
Haploids	7	0	—[b]	1	—	0	6
Diploids and haploids	6	4	0	2	0	2	4
Diploids	23	3	1	3	1	15	6
Total	57	7	1	6	1	23	24

[a] C.O. (Crossingover) or NDJ (Nondisjunction) /I, II, or III—VIII (Specific linkage group).
[b] Events of nondisjunction cannot be detected in these haploids.

Table 5. Number and distribution of sectors arising from NG-induced abnormal colonies

Genetic event	C.O.[a]	NDJ[b]	C.O.	NDJ	C.O. or NDJ		Total events		
Linkage group	I	I	II	II	III—VII	VIII	single	additional	none
Number of diploid sectors	13	1	4	0	20	18	41	15	11
Number of haploid sectors	3	—[c]	3	—	0	0	6	0	17

[a] C.O., crossingover.
[b] NDJ, nondisjunction.
[c] Events of nondisjunction cannot be detected in haploids.

etic event, therefore, was mitotic crossingover. Among the 56 diploids which were found to have a genetic event, 15 (26.8%) had additional event. The figures are meaningless for haploids because the events of nondisjunction cannot be detected in them.

2. Effect of MC treatment

Genetic activity: The frequencies of mitotic segregants and abnormal colonies after MC treatment are presented in Table 6. Of the 85.97% which survived, the 6.71% were normals with sectors, which had an approximately 6 : 1 ratio of diploids to haploids. In comparison, the control colonies consisted of 2.72% normals with sectors, of which there were twice as diploids as haploids. On the other hand, the frequencies of abnormal colonies after MC treatment were almost equal to those of control.

Table 6. Frequencies of mitotic segregants and abnormal colonies after treatment with MC

	Control		MC treatment	
	Number	%	Number	%
Survival		(100)*		85.97
Total number of colonies	884		760	
Abnormals	9	1.02	8	1.05
Normals	851	96.26	701	92.24
Normals with sectors	24	2.72	51	6.71
Diploids	16	1.81	44	5.79
Haploids	8	0.91	7	0.92

*The control value of 884 was taken to be 100% survival.

Diploid segregants: Results in Table 7 show the frequencies and types of diploid segregants. After treatment with MC mitotic crossingover was the predominant event in the generation of white and yellow segregants, appearing over 13-fold higher than nondisjunction (3.55% vs 0.26%). As compared with controls, mitotic crossingover was found to occur about four times more frequently (3.55% vs 1.02%). The frequencies of nondisjunction did not increase. The frequencies of all diploid segregants were threefold higher than control values(5.79% vs 1.81%). However, an increase in the frequencies of the additional events after MC treatment was less indicated.

Haploid segregants: Table 8 presents the frequencies and types of haploid segregants. The frequencies of all the ha-

ploid segregants after MC treatment were almost equal to those of control. The frequencies of the additional events did not increase.

Table 7. Frequencies and types of diploid mitotic segregants among MC treated and control colonies

Genotype	Genetic event	Treatment	Sectors with event alone		Sectors with additional event		Total sectors	
			Number	%	Number	%	Number	%
*acr*A⁺*w*A2*cnx*E16⁺ / *acr*A⁺*w*A2*cnx*E16	C. O. [a]	Control[c]	2	0.23	0	0	2	0.23
		MC[d]	8	1.05	0	0	8	1.05
*acr*A⁺*w*A2*cnx*E16 / *acr*A⁺*w*A2*cnx*E16	NDJ[b]	Control	0	0	1	0.11	1	0.11
		MC	0	0	0	0	0	0
*su*A*ad*E20*paba*A*y*A*ad*E20[e] / *su*A*ad*E20⁺*paba*A*y*A*ad*E20	C. O. [f]	Control	7	0.79	0	0	7	0.79
		MC	19	2.50	0	0	19	2.50
*su*A*ad*E20*paba*A*y*A*ad*E20 / *su*A*ad*E20*paba*A*y*A*ad*E20	NDJ	Control	2	0.23	0	0	2	0.23
		MC	0	0	2	0.26	2	0.26
*ribo*B2⁺*cha*A[g] / *ribo*B2⁺*cha*A	Not determined	Control	3	0.34	1	0.11	4	0.45
		MC	14	1.84	1	0.13	15	1.99
Total		Control	14	1.58	2	0.23	16	1.81
		MC	41	5.39	3	0.39	44	5.79

[a] C. O. (Crossingover). [b] NDJ (Nondisjunction).
[c] Percent based on 884 total control colonies.
[d] Percent based on 760 surviving MC treated colonies.
[e] Or the alternative crossover genotype $\frac{su\text{A}ad\text{E}20paba\text{A}y\text{A}ad\text{E}20}{su\text{A}ad\text{E}20^{+}paba\text{A}^{+}y\text{A}ad\text{E}20}$
[f] Or due to mutation.
[g] Or the phenotypically indistinguishable $\frac{ribo\text{B}2^{+}cha\text{A}}{ribo\text{B}2cha\text{A}}$

Table 8. Frequencies and types of haploid mitotic segregants among MC treated and control colonies

Genotype	Treatment	Sectors with event alone		Sectors with additional event		Total sectors	
		Number	%	Number	%	Number	%
*su*A*ad*E20*paba*A*y*A*ad*E20	Control[a]	2	0.23	0	0	2	0.23
	MC[b]	2	0.26	0	0	2	0.26
*acr*A⁺*w*A2*cnx*E16	Control	3	0.34	3	0.34	6	0.68
	MC	4	0.53	1	0.13	5	0.66
*ribo*B2⁺*cha*A[c]	Control	0	0	0	0	0	0
	MC	0	0	0	0	0	0
Total	Control	5	0.57	3	0.34	8	0.91
	MC	6	0.79	1	0.13	7	0.92

[a] Percent based on 884 total control colonies.
[b] Percent based on 760 surviving MC treated colonies.
[c] Included is the genotype *cha*A*y*A, which is phenotypically cream colored.

3. Effect of CH treatment

Genetic activity: In Table 9. the frequencies of mitotic segregants and abnormal colonies after CH treatment are given. The survival of conidia was not reduced and few normals with sectors were induced. On the other hand, the frequencies of abnormal colonies were very augmented (34.26% vs 1.01%).

Table 9. Frequencies of mitotic segregants and abnormal colonies after treatment with CH

	Control		CH treatment	
	Number	%	Number	%
Survival		(100)*		99.75
Total number of colonies	395		394	
Abnormals	4	1.01	135	34.26
Normals	380	96.21	247	62.69
Normals with sectors	11	2.78	12	3.05
Diploids	7	1.77	7	1.78
Haploids	4	1.01	5	1.27

* The control value of 395 was taken to be 100% survial.

Table 10. Frequencies and types of diploid mitotic segregants among CH treated and control colonies

Genotype	Genetic event	Treatment	Sectors with event alone		Sectors with additional event		Total sectors	
			Number	%	Number	%	Number	%
*acr*A⁺*w*A2*cnx*E16⁺ *acs*A⁺*w*A2*cnx*E16	C.O.[a]	Control[c]	1	0.26	0	0	1	0.25
		CH[d]	2	0.51	0	0	2	0.51
*acr*A⁺*w*A2*cnx*E16 *acr*A⁺*w*A2*cnx*E16	NDJ[b]	Control	0	0	0	0	0	0
		CH	0	0	0	0	0	0
*su*A*ad*E20*paba*A*y*A*ad*E20[e] *su*A*ad*E20⁺*paba*A*y*A*ad*E20	C.O.[f]	Control	3	0.76	0	0	3	0.76
		CH	0	0	0	0	0	0
*su*A*ad*E20*paba*A*y*A*ad*E20 *su*A*ad*E20*paba*A*y*A*ad*E20	NDJ	Control	1	0.25	0	0	1	0.25
		CH	2	0.51	0	0	2	0.51
*ribo*B2⁺*cha*A[g] *ribo*B2⁺*cha*A	Not determined	Control	2	0.51	0	0	2	0.51
		CH	2	0.51	1	0.25	3	0.76
Total		Control	7	1.77	0	0	7	1.77
		CH	6	1.52	1	0.25	7	1.78

[a] C.O. (Crossingover). [b] NDJ (Nondisjunction). [c] Percent based on 395 total control colonies.
[d] Percent based on 394 surviving CH treated colones.
[e] Or the alternative crossover genotype $\frac{su\text{A}ad\text{E}20paba\text{A}y\text{A}ad\text{E}20}{su\text{A}ad\text{E}20^{+}paba\text{A}^{+}y\text{A}ad\text{E}20}$
[f] Or due to mutation.
[g] Or the phenotypically indistinguishable $\frac{ribo\text{B}2^{+}cha\text{A}}{ribo\text{B}2cha\text{A}}$

Diploid segregants: The frequencies and types of diploid segregants are presented in Table 10. After treatment with CH the frequencies of mitotic crossingover leading to white and yellow segregants were the same as those of nondisjunction. In comparison with controls, a difference in the frequencies of mitotic crossingover and nondisjunction was less marked. The frequencies of all diploid segregants were equal to those of control. Few additional events were arisen.

Haploid segregants: Results in Table 11. show the frequencies and types of haploid segregants. An increase in the frequencies of all haploid segregants and the additional events after CH treatment was indicated.

Table 11. Frequencies and types of haploid mitotic segregants among CH treated and control colones

Genotype	Treatment	Sectors with event alone		Sectors with additional event		Total sectors	
		Number	%	Number	%	Number	%
*su*A*ad*E20*paba*A*y*A*ad*E20	Control[a]	1	0.25	0	0	1	0.25
	CH[b]	1	0.25	0	0	1	0.25
*acr*A$^+$*w*A2*cnx*E16	Control	2	0.51	1	0.25	3	0.76
	CH	1	0.25	1	0.25	2	0.51
*ribo*B2$^+$*cha*A^c	Control	0	0	0	0	0	0
	CH	1	0.25	1	0.25	2	0.51
Total	Control	3	0.76	1	0.25	4	1.01
	CH	3	0.17	2	0.51	5	1.27

[a] Percent based on 395 total control colonies.
[b] Percent based on 394 surviving CH treated colonies.
[c] Included is the genotype chaAyA, which is phenotypically cream colored.

Abnormal colonies: The types of abnormal colonies after treatment with CH are listed in Table 12. The 9 of the 27 which did not produce sectors, could not be characterized genetically. The 18 which could be tested were found to be diploids with reduced conidiation. Eight of these were found to be homozygous for the nutritional markers. Of the 135 abnormal colonies 40 generated only haploid segregants, while 35 generated both haploid and diploid segregants. From 33, only diploid segregants were raised. In summary, 68 abnormal colonies could produce diploid segregants, whereas no diploid segregants were produced by 40 abnormal colonies. Many abnormal colonies raised hyperhaploid or hyperdiploid segregants. The hyperhaploid segregants were mostly n+1 or n+2 types, and the hyperdiploid segregants were mostly 2n +1 or 2n+2 types.

An analysis of all the sectors arising from the abnormal colonies is summerized in Table 13. A total of 138 haploid and 135 diploid sectors arose from 108 abnormal colonies. Among the diploid segregants the predominant genetic event was mitotic crossingover. In linkage groups I and II, 19 events of corssingover

and 6 events of nondisjunction were found. Among the 103 diploids which were found to have a genetic event, 24 (20.3%) had additional evnt. On the other hand, few haploid segregants were found to have any genetic event.

Table 12. Number and distribution of abnormal colonies induced by CH

Type of abnormal colony	Number of colonies in which genetic event was found						
	Total	C.O./ I [a]	NDJ/ I	C.O./ II	NDJ/ II	C.O. or NDJ/ III—VIII	None
Non-sectoring							
Not characterized	9	—	—	—	—	—	—
Diploid	18	0	0	0	0	8	10
Sectoring							
Haploids	40	8	—[b]	8	—	1	33
Diploids and haploids	35	7	0	5	2	22	31
Diploids	33	6	3	5	0	21	5
Total	135	21	3	18	2	52	79

[a] C.O. (Crossingover) or NDJ(Nondisjunction) / I, II, or III—VIII (Specific linkage group).
[b] Events of nondisjunction cannot be detected in these haploids.

Table 13. Number and distribution of sectors arising from CH-induced abnormal colonies

Genetic event	C.O.[a]	NDJ[b]	C.O.	NDJ	C.O. or NDJ		Total event		
Linkage group	I	I	II	II	III—VII	VIII	single	additional	none
Number of diploid sectors	17	4	12	2	62	6	79	24	32
Number of haploid sectors	11	—[c]	17	—	0	1	29	0	109

[a] C.O., crossingover.
[b] NDJ, nondisjunction.
[c] Events of nondisjunction cannot be detected in haploids.

DISCUSSION

This experiment has been shown that the treatment with chemical agents can induce two types of phenotypic changes, normal colonies with colored sectors and abnormal colonies. The abnormal colonies subsequently produced normal segregants. The mechanism inducing two types of phenotypic changes may or may not be the same. First, the treatment with chemical agents leads to some damage. Next, the damage produces an abnormal phenotype. Finally, the damage is eliminated, whereupon normal segregants can be produced. It is clear this damage is associate with genetic changes. These genetic changes leading to normal segregants are found to include mitoic crossingover and nondisjunction. If a chromosome breakage, terminal deletion, or semidominant lethal is induced, such genetic damage might be eliminated by mitotic crossingover; then the frequency of mitotic crossingover would be increased.

Mitotic crossingover has been induced in *Saccharomyces cerevisiae* by ethyl methane sulphonate(Davies *et al.*, 1975). In *Aspergillus nidulans* mitotic crossing-

over has been induced by the one-armed alkylating agents ethylene oxide and chlorotriethylamine HCl, and of the two-armed diepoxybutane and methyl-bis (β-chloro-ethyl) amine (Morpurgo, 1963). The frequency of intergenic recombination was strongly increased by fluorodeoxyuridine and fluorouracil (Beccari *et al.*, 1967). Nondisjunction and crsosing-over have been raised by different pharmaceutical drugs, particulary quinolines, sulfonamides, thiazides (Bignami *et al.*, 1974). Mitotic instabilities have been caused by benlate(methyl-1-(butylcarbamoyl)-2-benzimidazol carbamate) (Harstie, 1970), benzimidazol, and thiophanate fungicides (Kappas *et al.*, 1954). Other investigators have been shown that chromosome distribution can be disrupted by ethyl alcohol in Aspergillus (Harsanyi *et al.*, 1977). Disruption of chromosome distribution has been caused by *p*-fluorophenylanine and griseofulvin in Aspergillus, (Lhoas, 1961; 1968; Kappas and Georgopoulos, 1974), in Penicillium (Ball, 1971), in Schizosaccharomyces (Gutz, 1966), in Ustilago (Day and Jones, 1971), in Coprinus (North, 1977). Of the chemical agents used in this experiment, NG was found to be the potent recombinogenic agent, producing a sevenfold increase in mitotic segregants over the control values. NG not only increased the frequencies of abnormals resulting from semidominant lethals or chromosome aberrations, but apparently also produced aneuploids. These results agree with previous evidence by Shanfield and Kafer (1971).

With MC, an increase in the frequencies of mitotic segregants was only about threefold over the control values. However, since it produced few abnormal colonies, MC appears to be specifically recombinogenic agent. This agrees with earlier evidence that MC induces mitotic recombination in Ustilago ana Saccharomyces under conditions in which the antibiotic is not mutagenic (Holliday, 1964)

With CH, the frequencies of mitotic segregants were equal to those of control. On the other hand, it produced abnormal colonies very greatly. The CH-induced abnormal colonies, for the most part, appeared to be of the aneuploid types found spontaneously. This result suggests that CH interferes with the normal distribution of chromosomes in mitosis as *p*-fluorophenylalanine and griseofulvin. However, that CH might induce some chromosome damage has not been ruled out because the genetic change leading to normal segregants from some abnormal colonies were found to be mitotic crossingover.

Simple examination of colonies grown from conidia treated with chemical agents will be sufficient to obtain information about the effects of chemical agents in *Aspergillus nidulans*. This heterozygous diploid strain of *Aspergillus nidulans* makes possible the study of mitotic recombination as well as the action of chemical agents at the gene and chromosome levels.

적 요

3종의 열성포자색 유전자 지표를 뿐만 아니라 모든 염색체에 유전자 지표들을 가지고 있는 이형이배체 *Aspergillus nidulans*의 발아포자에 N-methyl-N′-nitro-N-nitrosoguanidine (NG), mitomycin C (MC) 및 chloral hydrate (CH) 같은 화학물질이 각기 처리되었다.

그 결과 아래와 같은 사실들이 관찰되었다.

1. NG는 포자생존을 감소시켰으며 체세포 분리개체들의 수를 대조구보다 7배 이상 증가시켰다. 이러한 체세포 분리개체들의 대부분은 체세포 교차에 의한 것으로 밝혀졌다. 또한 NG는 많은 이상군체를 생성하였는데 이들은 염색체 이상과 자연발생의 이수성에 의하여 유발된 것으로 분석되었다.

2. MC처리후 포자생존은 감소되었지만 이상군체는 거의 생성되지 않았다. 또한 MC는 체세포 분리개체들의 수를 대조구보다 3배이상 증가시켰다. 이러한 체세포 분리개체들의 대부분은 체세포 교차에 의한 것으로 판명되었다.

3. CH는 포자생존과 체세포 분리개체들의 수의 증가에 거의 영향을 미치지 않으면서 많은 이상군체를 생성하였고 이들은 대부분 이수성에 의해 일어난 것으로 분석되었다. 이런 사실로 CH는 체세포 분열시 염색체의 정상분열을 방해하는 것으로 생각된다.

REFERENCES

1. Ball, C., 1971. Haploidization analysis in *Penicillium chrysogenum*. *J. Gen. Microbiol.* **66**, 63~69.
2. Beccari, E., P. Modigliani and G. Morpurgo, 1969. Induction of inter-and intragenic mitotic recombination by fluorodeoxyuridine and fluorouracil in *Aspergillus nidulans*. *Genetics* **56**, 7~12.
3. Bignami, M., G. Morpurgo, R. Pagliani, A. Carere, G. Conti and G. di Gioseppe, 1974. Nondisjunction and crossingover induced by pharmaceutical drugs in *Aspergillus nidulans*. *Mutation Res.* **26**, 159~170.
4. Chanet, R., C. Izard and E. Moustacchi, 1975. Genetic effects of formaldehyde in yeast. I. Influence of the growth stages on killing and recombination. *Mutation Res.* **33**, 179~186.
5. Chang, L. and R.W. Tuveson, 1969. Ultraviolet-sensitive mutants in *Neurospora crassa*. *Genetics* **56**, 801~810.
6. Clutterbuck, J., 1974. *Aspergillus nidulans*, in R.C. King(ed.) Handbook of Genetics. Vol. 1, Plenum Press, N.Y. 447~510.
7. Davies, P.J., W.E. Evans and J.M. Parry, 1975. Mitotic recombination induced by chemical and physical agents in the yeast *Saccharomyces cerevisiae*. *Mutation Res.* **29**, 301~314.
8. Day, A.W. and J.K. Jones, 1971 *p*-Fluorophenylalanine-induced mitotic haploidization in *Ustilago violacea*. *Genet. Res.* **18**, 299~309.
9. Esposito, R.E. and R. Holliday, 1964 The effect of 5-fluorodeoxyuridine on genetic replication and mitotic crossing-over in synchronized cultures of *Ustilago maydis*. *Genetics* **50**, 1009~1017.
10. Fratello, B., G. Morpurgo and G. Sermonti, 1960. Induced somatic segregation in *Aspergillus nidulans*. *Genetics* **45**, 785~800.
11. Gutz, H., 1966. Induction of mitotic segregation with *p*-fluorophenylalanine in *Schizosaccharomyces pombe*. *J. Bacteriol.* **92**, 1567~1568.
12. Harsanyi, Z., I.A. Granet and D.W.R. Mackenzie, 1977. Genetic damage induced by ethyl alcohol in *Aspergillus nidulans*. *Mutation Res.* **48**, 51~74.

13. Hastie, A.C., 1970. Benlate-induced instability of Aspergillus diploids. *Nature* **226**, 771.
14. Holliday, R., 1964. The induction of mitotic recombination by mitomycin C in Ustilago and Saccharomyces. *Genetics* **50**, 323~335.
15. Kafer, E., P. Marshall and G. Cohen, 1976. Well-marked stralns of Aspergillus for tests of environmental mutagens: Identification of induced mitotic recombination and mutation. *Mutation Res.* **38**, 141~146.
16. Kappas, A., S.G. Georgopoulos and A. C. Hastie, 1974. On the genetic activity of benzimidazole and thiophanate fungicides on diploid *Aspergillus nidulans*. *Mutation Res.* **26**, 17~27.
17. Kappas, A. and S.G. Georgopoulos, 1974. Interference of griseofulvin with the segregation of chromosomes at mitosis in diploid *Aspergillus nidulans*. *J. Bacteriol.* **119**, 334~335.
18. Lhoas, P., 1960. Mitotic haploidization by treatment of *Aspergillus niger* diploids with para-fluorophenylalanine. *Nature* **190**, 744.
19. Lhoas, P., 1968. Growth rate and haploidization of *Aspergillus niger* on medium containing *p*-fluorophenylalanine. *Genet. Res.* **12**, 305~315.
20. MacDonald, K.D., 1971. Segregants from a heterozygous diploid of *Penicillium chrysogenum* following different physical and chemical treatments. *J. Gen. Microbiol.* **67**, 247~250.
21. Mercer, B. and N.R. Morris, 1975. The effect of chloral hydrate upon mitosis in *Aspergillus nidulans*. *J. Gen. Microbiol.* **88**, 197~199.
22. Morpurgo, G., 1963. Induction of mitotic crossingover in *Aspergillus nidulans* by bifunctional alkylating agents. *Genetics* **48**, 1,259~1,263.
23. North, J., 1977. The effects of griseofulvin on diploid strains of *Coprinus lagopus*. *J. Gen. Microbiol.* **98**, 529~534.
24. Pontecorvo, G., J.A. Roper, L.M. Hemmons, K.P. MacDonald and A.W. J. Bufton., 1953. The genetics of *Aspergillus nidulans*. *Advan. Genet.* **5**, 141~238.
25. Shanfield, B. and E. Kafer, 1971. Chemical induction of mitotic recombination in *Aspergillus nidulans*. *Genetics* **67**, 209~219
26. Upshall, A., B. Gidding and I.D. Mortimore, 1977. The use of benlate for distinguishing between haploid and diploid strains of *Aspergillus nidulans* and *Aspergillus terrus*. *J. Gen. Microbiol.* **100**, 413~418.
27. Yost, H. T., R. S. Chalett and J.P. Finerty, 1967. Induction of mitotic recombination in *Saccharomyces cerevisiae* by ethylmethanesulfonate. *Nature* **215**, 660~661.
28. Zimmermann, F.K., R. Schwaier and U. von Laer, 1966. Mitotic recombination induced in *Saccharomyces cerevisiae* with nitrous acid, diethylsulfate, and carcinogenic alkylating amines. *Z. Vererbl.* **98**, 230~246.