INDUCED SOMATIC SEGREGATION IN *ASPERGILLUS NIDULANS*

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HETEROZYGOUS diploid strains of *Aspergillus nidulans* spontaneously produce segregant clones belonging to three classes: diploid segregants which have undergone mitotic crossing over; haploid segregants with random redistribution of whole chromosomes (PONTECORVO, TARR GLOOR and FORBES 1954); and "nondisjunctional" segregants with all the markers on both branches of one chromosome in the homozygous condition, while the other chromosomes are still in the heterozygous condition (PONTECORVO and KAUFER 1958). IKEDA, ISHITANI and NAKAMURA (1957) observed increases in mitotic segregation due to ultraviolet irradiation in some diploid strains of *Aspergillus oryzae*. MORPURGO and SERMONTI (1959) increased the frequency of mitotic segregation in some heterozygous diploid strains of *Penicillium chrysogenum* by the use of irradiation and chemical mutagenic agents.

It does not appear from the work published so far what types of chromosomal rearrangement are induced by mutagenic agents in diploid molds. This paper describes a preliminary investigation of the rearrangements produced by three mutagenic agents, formaldehyde, nitrogen mustard, and ultraviolet irradiation, on a heterozygous diploid of *Aspergillus nidulans*, marked on six out of the eight chromosomes so far identified (KAUFER 1958).

MATERIAL AND METHODS

The cultures and microbiological techniques adopted as routine by PONTECORVO and his colleagues (1954) are used throughout, except where otherwise indicated.

Strain: The strain used was a diploid strain of *Aspergillus nidulans* kindly supplied by MR. E. FORBES of the University of Glasgow. The diploid is green and prototrophic, and its genotype is as follows:

$$\begin{array}{lllll} \text{I} & \frac{su_1 ad_{20}}{+} \bigcirc \frac{\gamma ad_{20} +}{+ + bi} ; & \text{II} & \frac{Acr_1 +}{+ w} ; & \\ \text{III} & \frac{phen_2 +}{+ s_{12}} ; & \text{IV} & \frac{pyro_4 +}{+ orn_4} ; & \text{V} & \frac{lys_5 +}{+ nic_2} ; \\ \text{VII} & \frac{nic_8}{+} . & & & & \end{array}$$

PONTECORVO and KAUFER's (1958) symbols for the mutant alleles and also their numbering of the chromosomes have been used. The symbol system and the

method of tabulating data adopted by PONTECORVO and KAUFER (1958) have been followed as far as possible in the present paper in order to simplify the comparison between the results now obtained concerning induced mitotic recombination and those of the earlier writers concerning spontaneous mitotic recombination. The symbol for mutant su_1ad_{20} (ad_{20} suppressor) will be abbreviated to su ; marker Acr_1 was not considered. All the other markers are nutritional requirements, except those referred to as γ and w , yellow and white conidium color respectively (w is epistatic to γ). Marker nic_2 was not distinguished from nic_s (nicotinamide requirements).

Medium: The minimal medium (Czapek-Dox) and the complete medium (corn-steep-sucrose) were adopted as standard in this laboratory (SERMONTI 1957). Three gm/litre of yeast extract were added to the complete medium.

Mutagenic agent treatment: Three mutagenic agents were used: formaldehyde (HCHO), methyl-bis(β -chloroethyl)amine (HN-2), and ultraviolet irradiation (U.V.). The most extensively used was HCHO, which in the majority of the experiments was added to the complete agar. Its action was continuous throughout colonial growth. HN-2 and U.V. were used on freshly collected conidia. The use of these two agents has been described in earlier papers (SERMONTI 1956).

Selection and classification of somatic segregants: Somatic segregants were selected for yellow or white color as sectors or macroscopic spots visible on the green colonies of the heterozygous diploid and in some cases as whole yellow or white colonies. Not more than one yellow and one white segregant were collected from each colony. The segregant clones were purified and then tested for nutritional requirements. Every colony tested which failed to develop on a dish lacking a particular nutrient was considered to have a requirement for that nutrient.

Classification criteria

Determination of ploidy of segregants

The possibility of stable aneuploid conditions was disregarded in accordance with the views of PONTECORVO and KAUFER (1958); isolated and purified segregants have, therefore, been classified as either diploid or haploid. This assumption cannot be proved or disproved by the analytical method used in this paper. In particular, in all the segregants which will here be regarded as diploids, it is not determined whether the segregant region is in the homozygous or hemizygous state.

Haploid segregants: Segregants with the markers of one or other of the members of each of the chromosome pairs in both parents were considered haploid. As appears from Table 1, two thirds of the identifiable haploid segregants express phenotypically the markers of only four chromosomes, and one third those of five chromosomes. The segregation of chromosome VII was not taken into account since its marker, nic_s , mimics nic_2 on chromosome V. In half the cases the segregation of chromosome I is masked because w is epistatic to γ , and in half the cases the segregation of chromosome II was not detected because marker Acr_1 was ignored. Where there is no evidence that a chromosome pair has failed to segregate, all the chromosomes are assumed to have segregated. Such segregants

will be called *genomic*, in line with the terms used for the classes considered below.

Diploid segregants: Segregants showing markers located on only one chromosome were regarded as diploid. One of the first pair of chromosomes has markers on both its arms, *su* on the left and γ and *ad*₂₀ on the right. The latter two will be regarded as being located at a single locus, being only 0.2 units apart (PONTECORVO and KAUFER 1958). Simultaneous segregation of all the markers on chromosome I produces yellow prototrophic segregants, whereas segregation of the markers on the right arm only gives yellow adenineless segregants. The first type will be called *chromosomal segregants* and the second type *subchromosomal segregants* throughout. According to PONTECORVO and KAUFER (1958), the first type derives from nondisjunction, the second from mitotic crossing over. We prefer to use a formal designation of the two types of segregants, without reference to the processes from which the segregants arise, since the somatic segregants we are dealing with are of induced origin, and processes different from those described by PONTECORVO and KAUFER (1958) may also produce the same segregant phenotypes. In particular it is not possible to phenotypically distinguish the results of mitotic crossing over from those of terminal chromosome deletions, and the latter are to be expected frequently after mutagenic treatments. Segregants showing markers from two different pairs of chromosomes are also regarded as diploid, but those showing markers from more than one pair are counted as "aberrant" (or classified as haploid segregants) if they fulfill the requirements shown above for such classification.

Other segregants: These include segregants showing markers from three chromosome pairs—those showing markers from both members of the same pair, and others whose origin can only be explained on the assumption that different segregation events have taken place simultaneously. They will be discussed in the next section.

RESULTS AND DISCUSSION

Effect of formaldehyde treatment

Treatment of conidia: In some experiments, conidia of the heterozygous diploid were suspended in distilled water containing HCHO at a concentration of 0.05 percent (v/v) for periods from one to 13 minutes, giving survival rates of from 30 percent to 0.04 percent of the conidia. In none of the conditions tried did any increase of somatic segregation appear in the surviving colonies.

Treatment of growing colonies on agar: A more extensive study was made of the effect of HCHO on colonies grown on complete agar containing the mutagenic agent in concentrations of 0.05 percent, 0.04 percent, or 0.03 percent (Figure 1). At the highest concentration (experiments 1336, 1305) only 1–2 percent of the conidia seeded produced colonies; at the lowest concentration (experiment 1331) more than half the conidia produced colonies. In the absence of HCHO the macroscopically visible segregants show up as narrow strips comprising a few tens of white or yellow heads, detectable in about a tenth of the colonies. In the presence

FIGURE 1.—Color segregation in colonies growing on agar containing 0.05 percent formalin.

of HCHO, the pattern changes as the concentration is increased. At low concentrations, the majority of the colonies grew in the presence of HCHO not appreciably slower than normal; only a small percentage of ("anomalous") colonies grew poorly, remaining small in size and producing practically no conidia; the appearance of the sectoring colonies was essentially the same as in the absence of HCHO, but they were more numerous. At the highest concentrations, "anomalous" colonies appeared in large numbers; it was not possible to classify these as sectoring or nonsectoring. On transferring them to fresh medium without HCHO, practically all these colonies produced colored sectors. These "anomalous" colonies constituted more than 50 percent of the whole at the highest concentrations (experiment 1336). Alongside these there were normally growing colonies with wide colored sectors or broad stripes, and completely white or yellow colonies, generally characterized by a dense conidiumless central zone of a deep brown color on the back.

The sectors, and the white or yellow segregant areas, were suitably purified and classified according to the criteria already described for phenotype and ploidy. Eight experiments have been arranged into four groups (Table 2) according to the survival rates of the conidia. These will be taken hereafter as measure of the intensity of the effect of the mutagenic agent. Four increasing "doses" of HCHO—from one to four—will be considered, corresponding to the four groups of decreasing survival rates. The "doses" are roughly proportional to the actual concentrations of HCHO in agar (Table 2).

Haploid (or genomic) segregants: Their relative frequency increases in proportion with the increasing of the mortality of the conidia plated. (Table 2 and

TABLE 2
Experiments in formalin-induced somatic segregation

Experiments* (code no.)	Formalin conc. in agar (percent, v/v)	HClO "dose" (percent)	Survival (percent)	Colonies observed (no.)	Colonies exhibiting segregation (percent)†	Classification of segregants						
						Color	Diploid					
							Segregation in one chromosome (adenine requir.)	Segregation in two chromosomes (no.)	Totals (no.)	Haploid Aberrant§ (no.)	Total (no.)	
1336-1305	0.05	4	1.1-1.8	892	93.1‡	yellow	{ + ad	15 11 23	10 2 14	25 13 37	39 15 28	92 17 167
						white						
						totals		49	26	75	141	43
1356a-1351a	0.04	3	15-18	75	91.5	yellow	{ + ad	5 5 8	2 0 2	7 5 10	3 2 4	17 21 21
						white						
						totals		18	4	22	10	6
1351b-1330	0.03-0.04	2	38-40	265	50.0	yellow	{ + ad	7 25 20	6 1 3	13 26 23	10 2 4	51 44 44
						white						
						totals		52	10	62	27	6
1356b-1331	0.03	1	49-59	340	34.9	yellow	{ + ad	2 32 16	1 4 1	3 36 17	4 0 9	43 28 28
						white						
						totals		50	6	56	13	2

* The experiments have been grouped according to the survival rate.
† Percent of scorable colonies. "Anomalous" colonies not considered.
‡ Data from experiment 1336.
§ Unclassifiable as haploid or diploid (see text).

Figure 2, left). At the highest dose of HCHO (experiments 1336, 1305) the genomic segregants represent more than 50 percent of all tested segregants, as against less than 20 percent at the lowest dose (experiments 1356b, 1331).

FIGURE 2.—Graphical representation of some frequency values deduced from Table 2. (Left) Frequencies of different classes of segregant percent color segregants tested. The circles joined by the broken line give the frequencies of chromosome segregants ($\gamma + ad$) percent yellow diploid segregants tested. (Right) Frequencies of white segregants percent haploid, diploid or total segregants tested. The dotted line is the expected value for the haploid segregants ($2/3$).

Markers located on different chromosomes recombine at random (Table 3). For each pair of chromosomes, the frequency of the recombinant classes is almost equal to the frequency of the parental classes, i.e., to half the total frequency of

TABLE 3

Mitotic recombination (percent) in formalin-induced haploid segregants between markers of different linkage groups

Linkage groups	I		II		III	IV	VII
	Actual	Corrected*	Actual	Corrected†			Corrected‡
V	55.0	52.8	45.0	46.9	42.9	54.4	47.1
IV	36.2	47.4	66.0	55.2	53.4	...	...
III	45.5	43.3	45.0	45.8	...	...	...
II	...	58.0§	...	...	...	...	...

* The member of chromosome pair I marked with $su \gamma ad_{20}$ occurs in two thirds of the selectable haploid segregants, and the member with bi in one third (Table 1). The figures have been corrected by doubling the frequency of the biotine requiring segregants.

† The member of chromosome pair II marked with w occurs in two thirds of the selectable haploid segregants and the member with Acr_1 in one third. The figures have been corrected by doubling the frequency of the non w (yellow) haploids.

‡ Only the recombinant class is detectable ($lys+^{n^{10}}$). The recombinant frequency has been obtained by doubling the frequency of this class and dividing by the total haploids.

§ Two parental classes are selectable and only one recombinant class. The frequency of the latter has therefore been doubled to calculate the recombination frequency.

the selected haploids. This of course does not apply to the recombinant classes between the first and second chromosome, which carry the markers (γ and w) for which the selection was made. Only three of the four combinations (YW , γW , Yw , γw) can be selected, one recombinant (γw) and the two parental (γW and Yw) (Table 1). Among the haploid segregants the number of yellow segregants (γW) is always about half of the number of white segregants ($\gamma w + Yw$) (Table 2 and Figure 2, right).

Diploid segregants: Diploid segregants form a smaller proportion of all the segregants tested as the dose of HCHO increases (Table 2): from about 80 percent at the lower concentrations (experiments 1356b, 1331) to 34 percent at the highest concentrations (experiments 1336, 1305). As already mentioned in the section headed "Classification criteria," there are two distinct classes of diploid segregants showing markers in coupling with γ in chromosome I—chromosomal (*su γ ad_{20}*) and subchromosomal (*γ ad_{20}*). The frequency of chromosomal segregants among yellow diploids increases very greatly as the HCHO concentration in the agar increases, until at the highest concentrations they are in the majority, whereas they are less than ten percent at the lowest concentrations (Figure 2, left).

A proportion of the diploid segregants express markers segregating on a second chromosome besides the one with the selected marker (Table 2). Since none of the chromosomes without selective markers is marked on both branches, it is impossible to distinguish between chromosomal and subchromosomal segregation of the unselected markers. The same is true of the chromosome carrying the w marker.

The coincidence of segregation on two chromosomes varies from about ten percent of diploid segregants in the cultures treated with dose one of HCHO to 35 percent in those treated with dose 4 (Table 2).

Aberrant phenotypes: Some segregants deviate from the phenotypes classifiable as haploid by having one marker from each member of one pair of chromosomes, alongside one marker from either member of the other chromosome pairs. These segregants can be classified with certainty as haploids with one crossover chromosome carrying both the markers of the parent chromosomes.

Other segregants deviate from the haploid phenotypes (Table 1) by the absence of one or two markers. Assuming not more than one crossing over simultaneous with haploidization, and not more than three chromosomes segregating simultaneously in segregant diploids, these can be interpreted as falling into three classes (Table 4):

1. Diploids segregating for three chromosomes;
2. Haploids with one crossover chromosome (phenotype deviating from the haploid phenotypes with evidence of segregation on five or six chromosomes (see Table 1), the markers of one or of two chromosomes respectively being absent);
3. Optional, i.e., possibly belonging to either of the above classes (phenotype deviating from the haploid phenotypes with evidence of segregation on four chromosomes (see Table 1), the markers of one chromosome being absent).

TABLE 4

Aberrant phenotypes obtained after formalin treatment (experiment 1305)

(1) (Diploid?) segregants for three chromosomes Phenotypes (no.)		(2) Haploid segregants with one crossover chromosome Phenotypes (no.)		(3) Optional segregants Phenotypes (no.)	
bi w phen	1	w pyro lys nic*	2	w s nic	1
		bi w s nic	1	w phen nic	1
		bi w phen pyro	1	w pyro nic	2
		bi w phen lys	1	w pyr lys	1
		bi w phen nic	1	y s nic	2
		bi w pyro lys*	1	y s lys	1
		bi w <i>phen</i> s pyro nic †	1	y phen lys	1
		bi w <i>phen</i> s pyro lys nic†	2	y phen nic	1
		bi w <i>phen</i> s lys nic†	1		

* Recessive markers from both members of chromosome III (*phen*, *s*) absent.† Recessive markers from both members of chromosome III (*phen*, *s*) present.

In the latter haploids (classes 2 and 3) the crossover chromosome is assumed to carry the wild alleles of the markers of both parental chromosomes. The aberrant haploids with one marker in excess may be classified with those of class 2 (Table 4).

The most frequent crossover chromosome among aberrant phenotypes is one carrying the markers *phen*₂ and *s*₁₂. There are eight of these (4.7 percent of haploids) among all the HCHO-induced segregants, and both markers are missing in 13 of the haploid or optional segregants (classes 2 and 3). Probably some of this last type are haploid, carrying the crossover chromosome complementary to *phen*₂-*s*₁₂ and some diploids segregant on three chromosomes not including chromosome III.

Effect of nitrogen mustard and ultraviolet irradiation treatment

As in the case of the HCHO treatments, the experiments have been set in groups, according to the survival rates. Only two groups for each mutagenic agent were set due to the small crop of data so far collected. They were considered as treated with dose 1 (higher survival) or dose 2 (lower survival) of the corresponding agent (Table 5).

HN-2: No evaluation was made of the frequency of anomalous colonies, which in this case do not form a clearly defined class. The frequency of sector forming colonies is related to the total of surviving colonies. In the experiment 1373a (Table 5) it was over 53.6 percent. This figure is not comparable with the frequency obtained after formalin treatment, in view of the different conditions of treatment. The percentage of haploid segregants is higher at the highest dose (2) of *HN-2*. At this dose the number of chromosomal segregants becomes greater than that of subchromosomal segregants (see "Classification criteria" and the section on the action of formalin); the coincidence of segregation of two chromosomes was 32 percent. The segregation pattern at the highest *HN-2* dose was very similar to that at the highest dose of formalin, but there is no pattern correspond-

TABLE 5
Experiments in nitrogen mustard and ultraviolet irradiation induced and spontaneous somatic segregation

Experiments* (code no.)	Survival (percent)	Colonies observed (no.)	Colonies exhibiting segregation (percent)†	Classification of segregants						Total (no.)
				Color	Diploid				Haploid Aberrant‡ (no.)	
					Segregation in one chromosome (adenine requir.) (no.)	Segregation in two chromosomes (no.)	Totals (no.)			
1373a, 1370, 1373b	0.6, 1.8, 4.0	163	46.0	(1) Nitrogen mustard (HN-2)						46
				yellow	{ + 8 ad 5 }	4 12 }	18	13 15	455	
				white	8	5 13	38 4			
				totals	21 10 31	51 19	101			
1389	23.0	218	28.4	yellow	{ + 5 ad 8 }	1 6 }	16	7 2	25	
				white	2 2 4	19 1	24			
				totals	15 5 20	26 3		49		
				(2) Ultraviolet irradiation (U.V.)						37
1379a, b,	2.3, 2.5	356	18.5	yellow	{ + 2 ad 3 }	0 2 }	5	7 2	14	
				white	5 1 6	13 4	23			
				totals	10 1 11	20 6		39		
				1379a, b,	7.5, 14.0	196	21.0		yellow	{ + 3 ad 10 }
white	4 0 4	10 1	15							
totals	17 2 19	18 2						44		
(3) Spontaneous									88	
1364, 1374 1389, 1402		690	12.6	yellow	{ + 3 ad 32 }	0 3 }	35	8 1		44
				white	23 4 27	17 0	44			
				totals	58 4 62	25 1		88		

* The experiments have been grouped according to the survival rate.
† Percent of colonies observed.
‡ Unclassifiable as haploid or diploid.

ing to that at the lower doses of formalin. At dose 1 (survival 23.0 percent) only four percent of the surviving colonies had subchromosomal segregants, the same percentage as in spontaneous segregation. Thus, there is as yet no experimental evidence to show that HN-2 increases the rate of subchromosomal segregation. Out of a total of 22 aberrant phenotypes, only one had the crossover chromosome *phen-s* while seven lacked both these markers.

U.V. rays: The data obtained are hardly conclusive. The pattern seems not to differ from that obtained with nitrogen mustard. At equal lethality the stimulation of segregation would seem to be much less effective. This is shown by the lower rate of colonies showing segregation and by the lower coincidence.

Evaluation of segregation frequencies

The mutagenic action of formalin was exerted on nuclei in division (not on resting nuclei), and it is difficult to establish a parameter by which to calculate the segregation rate. As an index of the intensity of segregation the frequency of colonies exhibiting yellow or white sectors has been adopted (the "anomalous" colonies have been disregarded—see Table 2). Since this frequency is equal to 12.6 in the untreated colonies (Table 5), the observable increase in segregation cannot exceed eightfold (100:12.6). This is actually 7.4 fold with the highest dose of formalin. Apart from the limitation of the method, this value is certainly conservative, since in the presence of formalin the number of sectors per sectoring colony is much higher than in the absence of the drug. This number, however, cannot be safely estimated. Evaluation of the frequency of segregation induced by HN-2 (or U.V.) is simpler than with HCHO induced segregation. Since the material treated consists of single resting uninucleate conidia, the effect can easily be evaluated as percent of affected colonies among those surviving the treatment. In experiment 1377a more than 50 percent exhibited symptoms of the action of HN-2 on their chromosomal integrity or nuclear kinetics. HN-2 has to a large extent a delayed effect, resulting in the formation of late sectors. U.V. action is less pronounced than that of HN-2. The observed effects are parallel to those reported by MORPURGO and SERMONTI (1959) in induced somatic segregation in *Penicillium chrysogenum* (Table 6). In that species the frequencies of the segregating colonies after treatment with the same three mutagenic agents dealt with in this paper are comparable with the frequencies obtained in *Aspergillus nidulans*, but the increase compared with the controls was much higher in some cases, macroscopically observable segregation being practically absent in the untreated colonies. The use as "control" of the rate of mitotic segregation in untreated colonies has only limited validity. What is called "spontaneous" segregation would be better described as segregation due to uncontrolled causes. It has been shown that the rate of spontaneous segregation may depend on genetic (MORPURGO and SERMONTI 1959; SERMONTI 1957) and environmental (SERMONTI and MORPURGO 1959b) factors. Rather it should be regarded as a background whose intensity determines the amount of information obtainable about induced segregation.

TABLE 6

Frequency of spontaneous and induced yellow segregants in Aspergillus nidulans and Penicillium chrysogenum

Mutagenic agent	<i>Aspergillus nidulans</i>				Strain	<i>Penicillium chrysogenum</i> *			
	Surviving conidia (percent)	Colonies observed (no.)	Colonies exhibiting yellow segregants			Surviving conidia (percent)	Colonies observed (no.)	Colonies exhibiting yellow segregants	
			(no.)	(percent)				(no.)	(percent)
.....	(100)	690	87	12.6	XXXIV	(100)	1,103	44	4.0
					XXXIV S	(100)	623	0	0.0
Formalin (HCHO)	15-18	75	43	57.8	XXXIV	2.5	146	47	32.8
Nitrogen mustard (HN-2)	0.6	84	45	53.6	XXXIV	0.5	208	34	16.2
					XXXIV S	0.2	145	35	24.1
Ultraviolet rays (U.V.)	2.0-2.5	356	66	18.5	XXXIV	0.8	220	16	7.3

* After SERMONTI and MORPURGO (1959b).

One criterion for evaluating the increase in rate of diploid segregants after mutagenic agent action arises from consideration of the rate of coincident segregation of markers located on different chromosomes. In other words, the rate of segregation in the other chromosomes can be determined in segregants selected for segregation on one chromosome on the assumption of random simultaneous occurrence of the events considered. On this basis the increase in diploid segregants is about sixfold after HCHO or HN-2 treatment and barely detectable after U.V. treatment.

Figure 3 shows the frequencies as percent of colonies observed of the various classes of segregant sectors induced by increasing "doses" of HCHO. At zero dose the values shown are deduced from the figures for spontaneous segregation. Each frequency value in Figure 3 is calculated by multiplying the relative frequency (percent tested) of the various classes of segregant (Figure 2, left) by the frequency (percent observed) of colonies exhibiting color segregation (see Table 2 and Table 5, untreated).

The various types of segregant will be considered separately in relation to the action of HCHO.

Genomic (haploid) segregants: The frequency of genomic segregants is only slightly increased at HCHO doses sufficient to inactivate about half the conidia (dose 1). At higher doses it increases rapidly. As already noted, the number of white haploid segregants at all doses of HCHO is about double the number of yellow segregants (see Figure 2, right).

Chromosomal (nondisjunctional) segregants: The frequency of these is taken as equal to the frequency of colonies having yellow diploid segregants without adenine requirement (*su y ad₂₀*). The first part of the curve expressing this frequency follows the curve for the frequency of genomic segregation. This agrees with PONTECORVO and KAUFER's (1958) proposal of a similar mechanism of origin for both types of segregant in spontaneous segregation. At higher HCHO doses there is a drop in the number of nondisjunctional sectors percent of surviving colonies, which may be related to the corresponding sharp increase in haploid

FIGURE 3.—Frequencies of colonies exhibiting color segregant sectors belonging to different classes. The values were obtained by multiplying the values given in Figure 2 (left) by the percent of colonies exhibiting segregation (Table 2, sixth column). The values for "dose" 0 (no HCHO in the agar) were calculated from Table 5 (spontaneous).

segregants; the process of irregular distribution of the chromosomes may tend, at higher doses of HCHO, to lead to haploidization rather than nondisjunction.

The relative increase in chromosomal segregation shows up in the relation between the frequencies of yellow and white diploid segregants. This should remain at about 2:1 so long as the only segregation process involved is crossing over (or other subchromosomal segregation process), since the γ marker is about twice as far from the centromere as the w marker (meiotic distances; KAUFER 1958). This ratio is actually found (2.29:1) at lower concentrations of HCHO (see Figure 2, right). As the concentration increases, the ratio comes closer to unity as is to be expected since the segregation frequency of the two markers in segregations involving whole chromosomes (nondisjunctional) is independent of the distances of the markers from the centromeres of their respective chromosomes. Since both genomic and chromosomal segregation produce more white segregants than subchromosomal processes, the over-all increase in white segregants as compared to yellow (see Figure 2, right) can be used as an index for the displacement of the segregation processes towards those due to irregular distribution of whole chromosomes, i.e., to aberration in centromere behavior. In other words, the extent of the excess of white over yellow segregants is diagnostic of disturbances in chromosome kinetics.

Subchromosomal segregants: The frequency of this class is taken as equal to the frequency of colonies showing yellow diploid segregants with an adenine requirement (γad_{20}). This is highest at the lowest HCHO concentrations (dose 1), at which the increase in the frequency of other types of segregant is negligible.

As the HCHO dose increases (doses 2, 3, 4), the frequency of subchromosomal segregants decreases. This decrease is probably due to the reduced probability of collecting subchromosomal segregants as a consequence of the increase in other types of segregant and the precaution observed against collecting more than one segregant of the same color from any colony. This behavior suggests, however, that the processes leading to subchromosomal segregation are already stimulated by quite light treatment, while more drastic treatment produces aberrations affecting whole chromosomes. In comparing different classes of segregant sector it must be borne in mind that the ratio between detectable and undetectable segregants is very different for the various classes. Three quarters of the possible haploids are detectable. Only one chromosomal segregant of the 16 possible ones is characterizable. Detectable subchromosomal segregation would occur in only 20 meiotic units (from γ to centromere) of a total of more than 1,000 (KAFFER 1958). The different patterns of the three classes are therefore comparable, while the comparison between slopes is of no value.

It remains an open question whether or not subchromosomal segregation is due to somatic crossing over. There is at present no means of distinguishing between segregations due to crossing over, terminal deletions, and other breaches of chromosomal integrity. A point of some interest is the presence among the aberrant phenotypes of haploid segregants with the crossover chromosome showing two recessive markers which were originally in repulsion (Table 4). The *phen-s* segregants have already been considered. There were eight of these against 191 haploid HCHO-induced segregants without crossover, i.e., four percent. This figure gives an indication of the coincidence of somatic crossing over in the region concerned with haploidization. In these cases intrachromosomal recombination has certainly taken place. No crossover chromosomes were observed in the spontaneous haploid segregants. A comparison of somatic crossover frequencies in haploids and diploids, assuming subchromosomal segregation to be due to mitotic crossing over, cannot be made at present without too many other arbitrary assumptions.

Coincidence of different segregation events: The coincidence of different segregation events has already been considered in connection with segregation on two chromosomes and with haploidization accompanied by crossing over. One point which still has to be discussed is the possibility of segregation on more than two chromosomes. As already remarked, segregants for three chromosomes are not phenotypically distinguishable in many cases from haploid segregants with one crossover chromosome (see Table 4). The number of such segregants which have been detected with certainty is very small, but it remains possible that they may be quite frequent, since the majority of the aberrant segregants described as "optional" could belong to this class. This coincidence can only be regarded for the moment as a possible occurrence which will have to be borne in mind in future work.

Comparison of action of different agents: The segregation pattern produced by HN-2 (apart from the frequency of the induced segregation which is not comparable) corresponds to the "strong" effect of HCHO. There is the same lack of

evidence of any increase in subchromosomal segregation and the same almost complete absence of demonstrable crossover chromosomes in the haploid segregants (one *phen-s* chromosome out of 75 haploids).

The effect of U.V. is of the same type as that of HN-2, but the U.V. treatment is less effective at equal lethality levels. This is evident from both the small number of colonies with segregation and the small number of segregants with segregation on two chromosomes.

CONCLUSION

Emphasis should be laid upon the considerable amount of information obtainable from heterozygous diploid molds on the action of mutagenic agents at the chromosome level, and the convenience of this type of material for large-scale research. Microorganisms, including molds, have in the past been regarded as ideal material for the study of point mutations, but ill-adapted for the observation of chromosomal aberrations induced by mutagenic agents. The occurrence of heterozygous diploid molds and of induced somatic segregation make possible the use of microorganisms in the study of chromosomal mutations as well, and permit parallel research on the action of mutagenic agents at the gene and chromosome levels.

SUMMARY

The authors have studied the action of three mutagenic agents—formalin (HCHO), nitrogen mustard (HN-2), and ultraviolet rays (U.V.)—on somatic segregation processes in a heterozygous diploid of *Aspergillus nidulans*.

The following points are emphasized:

1. HCHO and HN-2 produced a sharp increase in the frequency of somatic segregation; this increase is less marked in the case of U.V.
2. HCHO treatment sufficient to give a survival rate of 50 percent produced a particularly notable increase in the frequency of subchromosomal segregants, possibly due to mitotic crossing over or terminal deletions. With increasing doses of HCHO increasing frequencies were obtained of chromosomal (nondisjunctional) and genomic (haploid) segregants, but no further increasing in the rate of subchromosomal segregants was observed.
3. The segregation patterns induced by HN-2 and U.V. treatment resemble that obtained at higher concentrations of HCHO; there is no indication of an increase in subchromosomal segregants.

This work illustrates the possibility of analysing genetically induced chromosomal rearrangements in a group of microorganisms.

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