*EVIDENCE FROM TETRAD ANALYSIS FOR BOTH NORMAL AND ABERRANT RECOMBINATION BETWEEN ALLELIC MUTANTS IN NEUROSPORA CRASSA**

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Evidence accumulated during the past few years indicates that aberrant recombination occurs in crosses of allelic mutants in organisms such as *Neurospora* and yeast in which the presence of chromosomes exhibiting regular meiotic behavior leads to the expectation of conventional genetic mechanisms. Early observations in *Neurospora* suggesting atypical behavior came from studies by Giles[1, 2] on the origin of prototrophs obtained in random ascospore platings from crosses between allelic inositol mutants carrying linked markers on either side of the inositol locus. Although the origin of inositol-independent isolates exhibited a positive correlation with crossing over between adjacent markers—the majority of such isolates occurring in one crossover class—the reciprocal crossover class and the two parental classes with respect to the markers were also represented in the progeny. Evidence that atypical segregation rather than negative interference is involved in a similar situation in *Neurospora* has been provided by Mitchell[3, 4] on the basis of an analysis of tetrads from a cross of two pyridoxine mutants. These results indicated that asci containing pyridoxine-independent isolates did not contain the reciprocal double mutant expected on the basis of conventional crossing over. Similar results from tetrad studies have been reported in yeast by Lindegren[5, 6] and by Roman,[7] and this phenomenon has been referred to by Lindegren[5] as "gene conversion."

Although the phenomenon of "gene conversion" appears well established in at least some of these cases, the possibility clearly remains that both conventional crossing over as well as atypical segregation may occur in such crosses. In an apparently comparable situation in *Aspergillus*[8] the results are different from those in *Neurospora*. In this instance, from a cross of two adenine mutants, the expected double mutant has been recovered (although not in tetrads, but by mitotic rather than by meiotic recombination), even though the distribution of adjacent markers in adenine prototrophs from random ascospore isolations from the same cross suggests aberrant recombination. Thus it would appear significant to determine from tetrad data whether both "gene conversion" and conventional crossing over can occur in a cross of the same two mutants. Evidence on this problem is provided in the present studies from tetrad analyses of crosses of two pantothenic acid-requiring mutants in *N. crassa* carrying appropriate linked markers.

Materials and Methods.—The pantothenicless mutants used in these studies were obtained by means of several filtration-concentration experiments employing the technique of Woodward *et al.*[9] and utilizing untreated, X-irradiated, or ultraviolet-irradiated macroconidia of three wild-type strains of *N. crassa:* 74A (obtained from Dr. Patricia St. Lawrence), 5.5A, and 3.1a—the latter two strains having originated from further inbreeding experiments with 74A. As will be indicated

in detail later, these pantothenicless mutants are physiologically (by heterocaryon tests), genetically (by crossing analyses), and biochemically (on the basis of precursor utilization) different from the previously known pantothenicless mutant 5531 (Barratt *et al.*[10]). Hence the mutant locus in strain 5531 (located in linkage group IV) is now termed the *pan-1* locus (also designated as "group A pantothenicless mutants" in this paper), and these new pantothenicless mutants (which have been located in linkage group VI) are designated as mutants at the *pan-2* locus (also referred to herein as "group B pantothenicless mutants"). In later discussions of these mutants, the shortened designation "pan mutants" will be employed. The other mutant strains used in the genetic analyses were *ad-1* (adenine—3254), *tryp-2* (tryptophan—75001), and *ylo* (yellow).[10]

The growth medium used throughout was Fries minimal[11] with appropriate biochemical supplementations. Crosses were made on supplemented Westergaard's synthetic crossing medium,[12] or on a modified low-sulfate Westergaard's with $MgSO_4$ replaced by 0.4 gm/l $MgCl_2$.[13] The only sulfate source in this medium is adenine sulfate (used at a concentration of 50 μg/ml). The technique described by Newmeyer[14] was used in all random ascospore platings. Heterocaryon tests were made with conidial suspensions of the strains on minimal agar Petri plates in the manner described by de Serres.[15]

Biochemical Relationships of the Mutants.—The previously known *pan-1* mutants in *Neurospora* were found to be blocked in the terminal reaction in pantothenic acid synthesis in which β-alanine and pantoic acid are coupled.[16] Preliminary tests with the *pan-2* mutants indicated that growth would occur on pantoyl lactone (the lactone of pantoic acid) as well as on calcium pantothenate. Since the *pan-2* mutants differed biochemically from the *pan-1* mutants, their position in pantothenic acid synthesis had to be determined.

On the basis of the scheme for pantothenic acid synthesis in *Escherichia coli* described by Maas and Vogel,[17] growth-stimulation tests utilizing the probable precursors of pantothenic acid—ketovaline, ketopantoic acid, and pantoic acid—were made with *pan-2* mutants. The results indicate that all the *pan-2* mutants are blocked in the same step in pantothenic acid biosynthesis—the conversion of ketovaline to ketopantoic acid—since all mutants will grow on ketopantoic acid, pantoic acid, and pantothenic acid, but not on ketovaline.

Intra-group Heterocaryon Relationships of the Pan-2 Mutants.—The assumption was made in early investigations with *Neurospora* that complementation between two phenotypically similar biochemical mutants resulting in growth of a heterocaryon on unsupplemented media indicates non-allelism, whereas non-complementation establishes two mutants as alleles.[18] All the 37 *pan-2* mutants show a positive heterocaryon response with a genetically and biochemically different mutant (mutant *A1*) at the *pan-1* locus, and it was anticipated on the basis of their biochemical similarity that *pan-2* mutants would exhibit no complementation with one another. However, preliminary tests with all possible combinations by twos of the first 7 *pan-2* (group B) mutants indicated that two of the mutants, *B3* and *B5*, formed a pantothenic acid–independent heterocaryon (bicaryon[19]). Additional heterocaryon responses with 7 more B mutants were found in tests employing all the *pan-2* mutants in all possible combinations by twos. Such heterocaryons formed between mutants assumed to be allelic are termed "intra-group hetero-

caryons." The pattern of complementation of the mutants established three major groups: (1) mutants *B3*, *B7*, *B19*, *B20*, *B25*, and *B36*, which complement with (2) mutants *B5*, *B23*, and *B28*, and (3) most of the remaining mutants, which exhibit no complementation responses. Several of these combinations (*B3* with *B23* and *B28*; *B7* with *B23* and *B28*) are partially temperature-sensitive in exhibiting reduced growth at 35° C. In addition, the heterocaryon between *B3* and *B5* is completely temperature-sensitive, in that it does not grow on minimal at 35° C. This fact has been particularly useful in the subsequent genetical analyses.

Genetic Relationships of the B Mutants.—The biochemical and heterocaryon studies just described indicated that the B mutants were distinct from the previously known *pan-1* mutants in *Neurospora*. Despite peculiarities in the pattern of heterocaryon complementation, the biochemical results suggested that the B mutants were probably allelic in the conventional sense. However, it was necessary to provide genetic evidence on this point.

To establish the linkage relationships of the B mutants, one mutant, *B3*, was crossed to markers in each of the seven linkage groups. Random isolations indicated that *B3* was associated with *ylo*, *ad-1*, and *tryp-2* in linkage group VI. All the *pan-2* mutants were then crossed to a common stock, *ad-1 tryp-2*, and by random ascospore platings were found to be between these two markers. There were a few instances in which abnormal linkage with one of the markers was noted. Additional tests established that the position of the *pan-2* locus with respect to the three other loci utilized in linkage group VI is as follows: *ylo*, *ad-1*, *pan-2*, *tryp-2*. These results showed that all 37 *pan-2* mutants were located in the same general region of linkage group VI but also indicated that a more detailed genetic analysis was necessary to establish their precise relationships.

Tetrad Analyses of B3 × B5 Crosses.—In the initial studies with the first seven B mutants, only *B3* and *B5* formed an intra-group heterocaryon. Additional heterocaryon tests with the other thirty mutants indicated that most of the mutants which gave an intra-group heterocaryon response could be classified as being generally similar to either *B3* or *B5*. Hence these two complementing mutants were selected for further study. This study consisted of tetrad analyses to obtain information on the inheritance of the heterocaryon complementation pattern and possible evidence for exceptional behavior in segregation or in recombination at the *pan-2* locus.

Two marked crosses of similar genotypes (*ylo ad-1*$^+$ *B3 tryp-2* × *ylo*$^+$ *ad-1 B5 tryp-2*$^+$) were utilized in the tetrad analyses. However, in the first cross the *B3* parent was not heterocaryon-compatible with the B mutant testers, and hence segregation for *B3* and *B5* could not be examined. A more extensive analysis was made in the second cross, since in this cross both parental stocks were heterocaryon-compatible with the original *B3* and *B5* mutants. Thus segregation of *B3* and *B5* could be followed in the second cross by testing the heterocaryon response of the isolates.

Seventy-two complete tetrads (at least one of each spore pair germinating) were obtained in the first cross (out of 100 asci isolated), and 856 complete tetrads (encluding 11 exceptional asci to be discussed later) from 1,299 asci isolated in the second cross.

Since more complete data are available from the second cross, the linkage rela-

tionships of the *pan-2* locus with the adjacent markers as determined from "normal asci" (those having two 2 *B3* and 2 *B5* spore pairs) in this cross are given in Table 1.

TABLE 1

RESULTS OF TETRAD ANALYSES FROM 856 NORMAL COMPLETE ASCI
(Second Cross: *ylo ad*$^+$ *B3 tryp* × *ylo*$^+$ *ad B5 tryp*$^+$)

CLASSIFICATION OF ASCI	NO. ASCI WITH RECOMBINATION IN THE INTERVALS			
	ylo-ad	*ad-centromere*	*centromere-pan*	*pan-tryp*
Parental ditype	826	851	827	723
Non-parental ditype	0	1	0	1
Tetratype	30	4	29	132
Per cent crossing over	1.7	0.2	1.7	7.7

The results indicate the order and map distances of the mutants to be as follows: *ylo* 1.7 *ad-1* 0.2 centromere 1.7 *pan-2* 7.7 *tryp-2*. All complete asci in the second cross were tested for their heterocaryon responses with *B3* and *B5* testers of the appropriate sex. In all but three asci (to be discussed later) having only mutant isolates, a regular 2:2 segregation of *B3*:*B5* heterocaryon-complementing types was observed. Furthermore, a given type of complementation exhibited linkage with adjacent markers as anticipated on the basis of prior crosses of both *B3* and *B5* mutants with *pan-2*$^+$ strains carrying linked markers. These results indicate that the pattern of heterocaryon response is characteristic of each *pan-2* mutant and that this response cannot be attributed to genetic modifiers at other loci, unless these are exceedingly closely linked to the *pan-2* locus.

Analysis of Asci with Exceptional Segregation Patterns.—On the basis of conventional genetic mechanisms, it was anticipated that all asci from these two crosses would exhibit segregation such that two *B3* and two *B5* spore cultures would be present. However, exceptional segregations were noted in eleven asci (one from the first cross and ten from the second). The genotypes of these asci are shown in Table 2, and the over-all tetrad results with respect to segregation types are summarized in Table 3. The *pan-2* genotype—whether *B3*, *B5*, or *B5 B3*—was determined initially by heterocaryosis and later confirmed by backcrossing to both the *B3* and the *B5* parents.

In two asci, Nos. 21 and 1142, there is an irregular order of isolates, indicating either nuclear passing or, probably more likely, especially in the case of ascus 21, isolation errors. The constitution of ascus 365 is more difficult to interpret because spore cultures 365.7 and 365.8 do not agree in genotype. It appears probable that spore culture 365.8 does not belong with this ascus and constitutes an isolation error, and such will be assumed in further discussion of this ascus. The possibility that the wild-type spore (culture 365.7) arose as a result of post-meiotic reversion was tested by plating conidia. The failure to recover *pan-2* isolates may be taken as evidence against this possibility.

In two of the asci (Nos. 400 and 1167—type 1 of Tables 2 and 3) a wild-type culture (in both instances of the same crossover type with respect to the linked markers) was present, with the reciprocal crossover type testing as a double *B5 B3* mutant. In addition, one of these asci (1167) contained the two single mutant parental types. The second ascus (400) had one parental type present, and it appears reasonable to assume that the spore pair missing in this ascus was the other parental type. Thus these two asci appear to be instances of conventional cross-

TABLE 2

GENOTYPES OF ISOLATES FROM EXCEPTIONAL ASCI AS DETERMINED BY HETEROCARYON AND CROSSING TESTS*

DESIGNATION	ASCUS No.	DISTAL 1	ORDER OF SPORES IN ASCI 2	3	4	5	6	7	PROXIMAL 8
Type 1.....	400	y a	y a	...	...	ad B5 B3 t A	ad B5 B3 t A	y B3 t A	y B3 t A
	1167	y B3 t A	y B3 t A	y A	y A	ad B5 B3 t a	ad B5 B3 t a	ad B5 a	ad B5 a
Type 2.....	21†	y B3 t A	ad B5 a	ad B3 t a	ad B3 t a	y A	y A	ad B5 a	y B3 t A
	145	y A	y A	y B3 t A	y B3 t A	ad B3 t a	ad B3 t a	ad B5 a	ad B5 a
	1142	y B3 A	y t A	y B3 A	y t A	ad B5 a	ad B5 a	ad B3 t a	ad B3 t a
	1200	ad B5 a	ad B5 a	ad B3 t A	ad B3 t A	y A	y A	y B3 t a	y B3 t a
Type 3.....	291	ad B5 a	ad B5 a	...	...	y B5 a	...	y t A	...
	365	ad B5 a	ad B5 a	ad B5 a	...	y B3 t A	y B3 t A	y t A	y B3 t A‡
Type 4.....	1029	ad B5 A	ad B5 A	ad B3 A	ad B3 A	y B3 t a	y B3 t a	y B3 t a	y B3 t a
	1109	ad B3 A	ad B3 A	ad B5 a	...	y B3 t A	y B3 t A	y B3 t a	y B3 t a
	1208	y B3 t A	y B3 t A	y B3 t a	y B3 t a	ad B3 A	ad B3 A	ad B5 a	ad B5 a

* Key: y = ylo; ad = adenineless; t = tryptophanless; A or a = mating type; B3 or B5 = *pan-2* B mutants.
† This ascus from the first cross, the other ten from the second cross.
‡ This isolate presumably does not belong with this ascus; see text for further discussion.

ing over at the four-strand stage in which two parental non-crossover and two reciprocal crossover products result. On the basis of these two asci the linear order of *B3* and *B5* with respect to the adjacent markers is indicated as *ylo, ad-1, B5 B3, tryp-2*. The distance between *B5* and *B3* is 0.1 crossover unit (2 asci in 939).

In contrast to the two preceding asci, there was no evidence in the six additional asci containing wild-type spore cultures for the expected reciprocal double *pan-2* mutant. Rather, in these asci, either one or the other parental type was represented twice. In four asci having two *B3* mutants (type 2), crossing over had occurred between the markers such that in each instance the wild-type spore culture represented either a single or a double (ascus 1142) crossover chromatid. In the two asci having two *B5* mutants (type 3), crossing over had occurred between the markers in ascus 291 on the proximal side of the *pan-2* locus, but the wild-type spore culture was a non-crossover type, while in ascus 365 there was no evidence of crossing over. However, it should be noted that a two-strand double crossover on either side of the *pan-2* locus would not be detectable in either of these asci.

The remaining exceptional asci (1029, 1109, and 1208—type 4) were distinct from the other eight in lacking wild-type spore cultures. Although all spore cultures were pantothenic acid-requiring, the segregations were irregular, in that each of the three asci contained one *B5* and three *B3* types. In each instance two of the *B3* cultures and one *B5* culture were non-crossover types, while the third *B3* was a double crossover.

These exceptional asci were further characterized by checking their heterocaryon responses with other intra-group testers and by backcrossing to *B3* and to *B5*. The isolates which tested as *B3* or *B5* in the original heterocaryon tests of the asci responded in the same manner with the other group *B3* and group *B5* testers. Also the two *B5 B3*

double mutants, originally identified as heterocaryon-negative with both *B3* and *B5*, tested similarly with the other B group testers. Additional tests of the apparent double mutants indicated no complementation with either *B3* or *B5* even in forced heterocaryons.

TABLE 3

SUMMARY OF TYPES OF SEGREGATIONS IN CROSS OF *pan-2* MUTANTS *B3* × *B5*

Types of Asci	No. Asci
Normal segregation	
2 *B3*:2 *B5*	856
Exceptional segregations	
Type 1	
1*B5*:1 *B3*:1 WT:1 *B3 B5*	2
Type 2	
1 WT:1 *B5*:2 *B3*	4
Type 3	
1 WT:1 *B3*:2 *B5*	2
Type 4	
1 *B5*:3 *B3*	3

Genetic analyses of the isolates from exceptional asci consisted of backcrosses of such isolates to both *B3* and *B5* parental types carrying appropriate linked markers. These crosses were tested by random ascospore platings for the recovery of true wild types (pantothenic acid–independent isolates). True wild types could be easily distinguished from pseudo-wild types, which also occur, since the latter give rise to temperature-sensitive heterocaryons which do not grow in the absence of pantothenic acid at 35° C. Wild types were obtained only from crosses involving isolates testing phenotypically as *B3* × *B5*. No wild types were obtained from presumptive selfings of either *B3* or *B5* or from crosses of the presumptive double *B5 B3* isolates with either *B3* or *B5*. Furthermore, the distribution into crossover and non-crossover categories (with respect to adjacent linked markers) of the wild types derived from these backcrosses was, in general, similar to that obtained in the original *B3* × *B5* cross (Table 4). Further genetic tests in which one *B5 B3*

TABLE 4

CLASSIFICATION OF PAN PROTOTROPHS OBTAINED IN RANDOM ASCOSPORE ISOLATIONS*

TYPE OF CROSS	TOTAL VIABLE COLONIES	NO. PAN PROTOTROPHS	PER CENT PAN PROTOTROPHS IN FOUR CLASSES			
			Non-crossover		Crossover	
ylo ad$^+$ *B3 tryp* (= P1) × *ylo*$^+$ *ad B5 tryp*$^+$ (= P2)			P1	P2	*ylo*$^+$	*ad tryp*
B3 × *B5* (parental cross)	129,256	212	38.8	13.8	40.0	7.1
B3 × 145.7	70,806	185	28.6	21.6	41.6	8.1
B3 × 1208.7	125,100	162	27.1	25.3	40.1	7.4
ad B3 tryp (= P1) × *ad*$^+$ *B5 tryp*$^+$ (= P2)			P1	P2	*ad* $^+$	$^+$*tryp*
21.4 × *B5*	34,268	55	21.8	34.5	38.1	5.4
ylo B3 tryp (= P1) × *ylo*$^+$ *B5 tryp*$^+$ (= P2)			P1	P2	*ylo* $^+$	$^+$*tryp*
145.4 × *B5*	76,698	180	27.3	22.9	41.3	8.3

* Data for the parental cross and for representative crosses (to either *B3* or *B5*) of pan isolates from the exceptional asci.

double was crossed to wild type indicated that it is possible to reisolate from such a cross both *B3* and *B5* single mutants. Thus all these crossing results serve to confirm by genotypic tests the phenotypic determinations derived from the heterocaryon tests and substantiate the conclusion that four types of exceptional asci have been obtained in the tetrad analyses.

Discussion.—The evidence from tetrad analyses of crosses of the pan mutants (*B3* × *B5*) supports the view that both conventional and aberrant recombination can occur between these two mutants.

The simplest interpretation of the two asci of type 1 is that these have resulted from conventional reciprocal crossing over in the four-strand stage between the two pan mutants (which may be represented as *B5 B3*$^+$ and *B5*$^+$ *B3*), giving rise to one pan-independent (*B5*$^+$ *B3*$^+$) and one reciprocal double mutant isolate (*B5 B3*), accompanied by the two non-crossover parental single mutants. The recovery in a single chromosome of a double mutant identified as such both phenotypically and genotypically (and from which it has been possible to recover by subsequent recombination both the two original single mutants) clearly establishes the separability of the two *pan-2* mutants by the criterion of recombination. Furthermore, the marker relationships in these two asci permit the establishment of a linear order of the two mutants, with *B5* proximal to *B3*.

The additional separability of these two mutants on a functional basis is indicated by their biochemical complementation, which results in growth in the absence of pantothenic acid when the two mutants are tested in a heterocaryon. Present evidence indicates that both mutants are blocked in the same step in pantothenic acid synthesis and presumably lack the same enzyme. Hence, although the enzymatic evidence has not yet been obtained, the probability appears high that these mutants are similar to mutants which lack adenylosuccinase but exhibit heterocaryon complementation associated with restored enzyme activity.[19] Thus on functional evidence these two pantothenic mutants may be considered to have resulted from independent mutations in two distinct "cistrons."[20] On this basis, the occurrence of conventional recombination between them is not surprising.

However, the three additional exceptional types of tetrads indicate that apparently aberrant recombination between these two mutants may also occur. Before attempting to interpret such tetrads in terms of novel genetic mechanisms, the possibility must be considered that such tetrads may be explained on the basis of conventional, albeit unusual, meiotic behavior. The most likely explanation of this type for the exceptional segregations observed appears to involve the possible occurrence of triploid, or especially of trisomic, nuclei at meiosis. However, preliminary cytological studies of asci from *B3* × *B5* crosses indicate the regular occurrence of seven bivalents at MI of meiosis and of only seven chromosomes at all first post-meiotic mitoses examined. Additionally, there is strong genetic evidence against a heteroploidy hypothesis. In particular, the segregation of adjacent linked markers in all the asci, both normal and exceptional, has been regular rather than aberrant. Also, there are no instances of 4:0 or 0:4 segregations for markers or for the pan mutants, as would be anticipated if extra chromosomes were segregating. Despite this general evidence against heteroploidy, it is still theoretically possible to explain the exceptional segregations on the basis of trisomic

behavior. However, such interpretations require apparently highly improbable sequences of trivalent crossing over, non-disjunction at AI, and post-AI chromosome loss. On the basis of all the above evidence the heteroploidy hypothesis has been discarded as an explanation for the aberrant tetrads.

Unequal crossing over has been considered as another conventional mechanism whereby the last three types of aberrant tetrads might arise. However, selfings of either *B3* or *B5* do not give rise to pan prototrophs. In addition, in a genetic analysis of the wild types from one type 2 ascus, crosses were made between the original wild-type culture and the reciprocal crossover *B3* culture from the same ascus. In the latter intra-ascus cross, heterocaryon tests of a substantial number of pan isolates failed to give any evidence that *B5* types could be recovered. This result appears to eliminate the possibility that such wild types, although phenotypically wild, may contain the *B5* element in an unusual arrangement detectable only by such an intra-ascus cross.

The absence of evidence for conventional, even though unusual, genetic mechanisms makes it probable that the three types of aberrant tetrads have resulted from novel recombination mechanisms which have been referred to by such terms as "gene conversion,"[5] "copy-choice,"[21] "transmutation,"[22] and "transreplication."[23] The present study differs from any previous ones in that in this instance it is possible to follow the simultaneous segregation of two close-linked mutants separable by conventional crossing over and phenotypically distinguishable when alone or together. On this view, either the proximal *B5* or the distal *B3* or both mutants simultaneously can exhibit atypical 3:1 segregations.

Although conventional reciprocal crossing over in a diploid nucleus cannot account for the aberrant tetrads, the evidence from the adjacent linked markers indicates that the occurrence of such types is positively correlated with crossing over in the vicinity of the *pan-2* locus. In four of the six asci having a pan-independent isolate with no accompanying reciprocal double mutant (types 2 and 3), this isolate is a crossover type. This result agrees with data on pan prototrophs obtained from random ascospore isolations from *B3* × *B5* crosses, which also indicate a marked positive correlation between the origin of such types and adjacent marker recombination.

In the present tetrad data the types of aberrant segregations may be interpreted even more specifically in terms of the pattern of adjacent crossing over. Thus, in all four asci of type 2 (1WT:2*B3*:1*B5* and hence segregating 3*B5*$^+$: 1*B5*, but 2*B3*:2*B3*$^+$ on the basis of two separable mutants) crossing over has occurred between the *pan-2* locus and the centromere, thus adjacent to *B5*, the mutant exhibiting "conversion." (In one ascus, No. 1142, crossing over has also occurred between the *pan-2* locus and the *tryp-2* marker such that this pan prototroph is actually a double crossover.) The two asci of type 3 (1WT:2*B5*:1*B3*, and hence 2*B5*$^+$:2*B5*, but 1*B3*:3*B3*$^+$) are apparently non-crossover types. However, these two asci may also be interpreted as two-strand double crossover tetrads in the *pan-2–tryp-2* interval, with one crossover occurring closely adjacent to *B3*, the mutant exhibiting "conversion." Support for this interpretation comes from the fact that the distance between *B3* and the adjacent distal marker (*tryp-2*) is over four times that between *B5* and the adjacent proximal marker (the centromere). Additionally, "conversion" of *B3* associated with a single crossover event in the

B3-tryp-2 interval has been obtained in a cross of a *B5 B3* double with wild type (Case, unpublished).

Finally, the three asci of type 4 must be considered. These asci contain no pan-independent isolates but rather have 3*B3*:1*B5* isolates. Thus they appear to be instances in which two simultaneous "conversions" took place—in one instance of *B5* to *B5*$^+$, and in the other of *B3*$^+$ to *B3*. With respect to markers, in all three asci two of the *B3*'s and the one *B5* are non-crossovers, whereas one *B3* is a double crossover, one crossover having occurred in the centromere-*pan-2* interval, and the other in the *pan-2–tryp-2* interval. Although it is impossible in these asci to determine which *B3* isolate contains the "converted" mutants, a reasonable interpretation appears to be that this isolate is, in each instance, the double crossover type and hence that two simultaneous crossovers, one adjacent to *B5* the other to *B3*, have occurred in association with these "conversion" events.

The preceding discussion suggests a precise positive relationship between adjacent conventional crossing over and aberrant segregations. Such a relationship does not, however, provide a mechanism to explain these segregations. Aberrant tetrad ratios may be interpreted on the basis of either of two principal hypotheses. One of these involves "gene conversion" in the sense of directed gene mutation occurring at meiosis.[2, 5, 6] The other involves a "copy-choice" or "transreplication" mechanism, in which one of the two newly formed chromatids is assumed to replicate, in the region showing aberrant segregation, using its homologue as a template.[21] The present results are taken to favor the second of these two hypotheses for the following reasons: (1) In this study only 3:1 or 1:3 and no 4:0 or 0:4 segregation ratios have been obtained. The latter ratios would presumably be anticipated on the "conversion" hypothesis, since directed mutation might affect a given strand either before or after replication, whereas, on the simplest copy-choice mechanism, the two parental mutants types should always be present. (2) The *B3* mutant is very stable in conidia and has not been shown to be capable of back-mutating to wild type either spontaneously or following irradiation. Despite this fact, *B3* reversions occur at meiosis, suggesting an origin other than mutational. (3) In all instances of conversion, the resulting isolates, whether wild type (type 2 and 3) or mutant (*B3* mutants in type 4) have been indistinguishable from the original types (either the pan *B3* parental type or 74A wild type) by all available criteria (e.g., on the basis of heterocaryon complementation patterns and mutational stability). Although these results are not incompatible with a mutational hypothesis, provided that such mutations are very specifically directed, they appear more reasonably interpreted on a copy-choice mechanism. (4) The occurrence of exceptional asci of type 4 (3*B3*:1*B5*) appears to provide especially strong evidence for a copy-choice rather than a mutational mechanism. If mutation is involved, these asci require for their origin the simultaneous occurrence in one *B5* mutant of two mutations, one from mutant to wild type (the *B5* element mutating to *B5*$^+$), the other from wild type to mutant (the *B3*$^+$ element mutating to *B3*). On a copy-choice mechanism, however, it is sufficient to assume that the replication of the *B5* strand involves a copying of the two adjacent elements (*B5*$^+$ and *B3*) from the *B3* strand as a "single event."

The possibility has also been considered that the observed aberrant segregations may involve a phenomenon similar to transduction. On this interpretation, a

small segment (including either the *B5*$^+$, the *B3*$^+$, or the entire *B5*$^+$ *B3* region) of one parental chromosome (presumably originating by an extra replication) is subsequently incorporated, after synapsis with its homologous region in the other parental-type chromosome, by a mechanism equivalent to a two-strand double crossover. On this view, unless incorporation occurs almost immediately after meiosis, the resulting phenotypically wild-type cultures should give rise to heterocaryons in which mutant nuclei are present. In addition, such wild types should be temperature-sensitive, as is the *B3 B5* heterocaryon. However, no mutant nuclei have been detected in such wild-type cultures either by conidial platings or by crossing, nor are the wild types temperature-sensitive.

The possibility of a prolonged synaptic association without incorporation, resulting in the maintenance of a homocaryotic wild-type phenotype, was also considered. Evidence against this situation was obtained from radiation experiments, which indicated that such wild-type cultures behave essentially like the original wild-type strain both quantitatively and qualitatively with respect to the induction of new pan mutants.

Additional general evidence against any transduction hypothesis involving heterogenote synapsis[21] comes from the existence of asci of type 4 (which do not contain any wild-type cultures but exhibit 3:1 segregations for both mutants), since the association of a *B5*$^+$ *B3* segment with a *B5* chromosome, as would be initially required in the origin of such types, should give a culture phenotypically wild type rather than mutant.

If a type of copy-choice mechanism is indeed responsible for the observed aberrant segregations, the relationship of this process to adjacent conventional crossing over remains to be determined. It may well be that synapsis typically occurs in localized short chromosomal segments in which several recombinational events can occur.[8] If this is so, however, the present evidence indicates that these are not all reciprocal recombinational events but that both reciprocal and nonreciprocal mechanisms are involved. Indeed, both types may be part of a general recombination mechanism, the details of which remain to be elucidated. For example, any such general mechanism will have to reconcile copy-choice interpretations with recent evidence[24] that chromosome duplication (on the basis of DNA measurements) has occurred prior to the initiation of meiosis. On the simplest view, this evidence appears to require that synapsis and copy-choice mechanisms operate prior to meiosis. How such a requirement can be made compatible with conventional views regarding the sequence of events at meiosis is not clear at this time.

With reference to the problem of "negative interference," the present results indicate clearly that this phenomenon, as usually interpreted,[8, 25] is not associated with the origin of pan-independent wild types in these tetrads. In only two asci (type 1) is there evidence for conventional recombination (on the basis of the simultaneous recovery of the reciprocal double mutant), and in both of these the resulting pan-independent isolates are single crossovers. In other asci containing pan-independent isolates (types 2 and 3) the absence of the reciprocal double is taken to indicate that such types have not arisen by conventional crossing over. As has been indicated, however, these asci furnish evidence for an association between orthodox crossing over in the vicinity of the *pan-2* locus and the origin of pan proto-

trophs. Thus there is a positive correlation between adjacent reciprocal and apparently non-reciprocal recombination.

The hypothesis developed earlier assumed that a copy-choice event involving either one or the other of the pan mutants (to give a pan prototroph) is always associated with either proximal or distal adjacent reciprocal crossing over. On this basis, all pan prototrophs should be single crossover types indistinguishable with respect to markers from similar types arising by conventional reciprocal crossing over between the two *pan-2* mutants (*B5* being proximal to *B3*). However, the tetrad data themselves indicate that this is not always the case, since in the two asci of type 3, the pan prototrophs are non-crossovers. Hence it follows either that the hypothesis just mentioned is not correct in assuming that a copy-choice event is always associated with adjacent conventional crossing over or that multiple conventional crossover events (negative interference) tend to occur with an unexpectedly high frequency, at least in the *pan-2–tryp-2* interval. The evidence from random ascospore isolations indicates also, on the basis of much larger numbers of pan prototrophs, that, although the expected single crossover class is the most frequent, both parental classes are found, as is the other single crossover class. Thus negative interference may be involved in the origin of the parental classes and the second crossover class. At present, however, the simpler assumption appears to be that a copy-choice event is not always associated with adjacent crossing over. A decision between these alternatives may be possible if more closely adjacent markers become available, especially on the distal side of the *pan-2* locus.

The problem of interpreting the relative frequencies of the four classes of pan prototrophs with respect to adjacent markers still remains. In the tetrad results, pan prototrophs occurred in only two classes—three in the *B3* parental and 5 in one crossover class. In random spore data, all four classes are represented, but there, is, in general, a marked asymmetry such that the two classes found in the tetrad analysis are in excess. In the random spore platings the asymmetry of the two parental classes may arise from an inherently greater probability that one of the two mutants (*B3*) will be involved in a copy-choice event unassociated with adjacent crossing over. An additional possibility involves associated multiple crossover events, as has already been pointed out. The asymmetry of the two crossover classes is presumably due in part to the fact that only one of these contains pan prototrophs arising by conventional crossing over. On the basis of the tetrad results, however, this fact alone cannot account for the magnitude of the observed excess in this class. An additional factor appears to be one already suggested—a positive correlation between adjacent conventional crossing over and the occurrence of a copy-choice event. It should be noted that this asymmetry permits the utilization of the two crossover classes from random data in establishing a linear order for the two mutants, despite the fact that the majority of individuals in the class in excess presumably have not arisen by conventional crossing over between the two mutants. It is evident, however, that the over-all frequency of pan prototrophs determined from random spore data does not give a correct estimate of the linear map distance between these two closely linked mutants.

There are clearly many similarities between the present results and those obtained in other cases where random spore analysis has been employed.[2, 3, 8, 25, 26] No attempt will be made at this time to interpret all such situations on the basis

of a common mechanism. It is evident, however, that if the present tetrad data have been correctly interpreted as indicating that the majority of pan prototrophs arise by a mechanism other than conventional crossing over, a similar situation may well exist in these cases. Thus it would appear to be extremely difficult, if not impossible, to arrive at a satisfactory interpretation of the mechanism of recombination in such organisms on the basis of random spore isolation data alone.

Summary.—A new group of 37 pantothenic acid-requiring mutants has been obtained from control, X-irradiated, and ultraviolet-irradiated macroconidia of *N. crassa* by means of the filtration-concentration technique. These mutants are all blocked in the same step in pantothenic acid biosynthesis—the conversion of ketovaline to ketopantoic acid. All 37 mutants are located in linkage group VI between the *ad-1* and the *tryp-2* loci and can be considered as alleles at the *pan-2* locus.

Despite their biochemical and genetical similarities, certain mutants can complement to form pantothenic acid–independent heterocaryons. A tetrad analysis has been performed on a cross of two such mutants (*B5* × *B3*) carrying linked markers on either side of the *pan-2* locus. In a total of 939 complete tetrads, exceptional behavior of the pan mutants was noted in 11 asci. On the basis of both heterocaryon and genetic tests, four different types of exceptional asci were obtained having the following isolate ratios: (1) two asci—1 wild type: 1*B3*:1*B5*: 1 double *B5 B3;* (2) four asci—1 wild type:1*B5*:2*B3;* (3) two asci—1 wild type:1*B3*:2*B5;* and (4) three asci—1*B5*:3*B3*. In the two asci of type 1 the adjacent linked markers indicate that the *B3* and *B5* isolates are non-crossover (parental) types, while the wild types and the *B5 B3* doubles are reciprocal single crossover types. This evidence is taken to indicate that the *pan-2* "locus" can be separated into two regions on the basis of genetic (crossover) as well as functional (heterocaryon) tests.

Possible explanations for the three additional types of exceptional tetrads—those with one wild type but no double *B5 B3* or with a 3:1 ratio of mutant types—have been considered. Cytological and genetical evidence argues against an explanation based on heteroploidy. The origin of the three aberrant tetrad types is attributed to some kind of "copy-choice" mechanism which results in irregular segregations for either the *B5*, the *B3*, or both the *B5* and *B3* regions simultaneously. Such "copy-choice" events appear to be correlated with specific patterns of conventional crossing over in the immediate vicinity of the *pan-2* locus.

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*MODIFICATION OF ULTRAVIOLET-INDUCED MUTATION FREQUENCY AND SURVIVAL IN BACTERIA BY POST-IRRADIATION TREATMENT**

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A hypothesis has been advanced that nucleic acid precursors altered *in vivo* by ultraviolet radiation (U.V.) constitute chemical intermediates in U.V.-induced mutation.[1] This theory is based on observations that supplementation of the immediate pre-irradiation growth medium of *Escherichia coli* strain B with certain purines and pyrimidines leads to increases in the mutation frequency subsequently induced by U.V. These studies and those of Witkin[2] indicate that the induction process is related to post-irradiation protein synthesis. It was therefore suggested that the process of U.V.-induced mutation involves post-irradiation synthesis of nucleic acid from radiation-modified precursors; and this process is dependent on *concurrent* protein synthesis.[1]

The experiments reported here were designed to investigate the immediate post-irradiation processes which influence mutation in *E. coli* strain B and the tryptophan-requiring strain of *E. coli* used by Witkin. In addition, considerable information has been accumulated on the post-irradiation conditions influencing survival of this organism following U.V. exposure.

Materials and Methods.—The mutations of *E. coli* strain B studied were those giving aberrant colonial color response on Difco eosin–methylene blue agar (EMB) after 2 days' incubation at 37° C.[3] This particular class of mutants is advantageous for studies in which the surviving fraction is small, since both survival and mutation frequency are determined with the same medium and on the same plates. The basal growth medium was a salts-glucose medium (hereinafter called "*M* medium").[1] *M* medium was supplemented with various metabolites or antimetabo-