THE EFFECT OF A SUPPRESSOR ON ALLELIC INOSITOLLESS MUTANTS IN NEUROSPORA CRASSA*

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Extensive analyses of spontaneous and radiation-induced reversions in a series of independently induced allelic inositolless mutants of *Neurospora crassa* have revealed that most such inositol-independent types are the result of reverse mutation.[1, 2] Furthermore, evidence was obtained that the inositolless mutants, which are phenotypically and biochemically indistinguishable,[1, 3] can be separated into several groups on the basis of quantitative differences in their spontaneous or induced reverse mutation rates. In addition to typical revertants to inositol-independence by reverse mutation, occasional phenotypically atypical revertants were recovered which, on crossing to wild type, segregated inositolless cultures in random ascospore isolation tests, indicating the probable occurrence of occasional suppressor mutations. The present paper deals with a more detailed genetic analysis of one such suppressor type. In addition, tests of the effect of this suppressor on other inositolless mutants have been made. These studies demonstrate that the suppressor is highly specific, since it apparently does not suppress any of the other mutants. Consequently, these results serve to establish a further difference between the suppressed inositolless mutant and all other inositolless mutants tested to date.

Origin, Genetic Analysis, and Biochemical Characteristics of the Suppressor Mutant.—The mutant described in these studies appeared following platings on minimal (inositol-free) medium of microconidia of inositolless strain 37401 (Stanford number) which had been exposed to ultraviolet. The mutant was initially noted as growing more slowly on minimal than the usual reverse mutation types. Even though the reversion had arisen in a microconidial strain, the mutant was next back-crossed to mutant 37401 and an inositol-independent isolate recovered in order to be certain that all subsequent tests would be performed with a homocaryotic strain. This extracted inositol-independent culture was then crossed to a wild-type inositol-independent strain and serial ascospore isolations made and tested, with the results indicated in table 1. The recovery of inositolless types as indicated by the presence of 4:4 and 6:2 ascus segregations demonstrates that the mutant being studied did not arise from reverse mutation, but rather was the result of mutation at some other locus. Such types in Neurospora have been designated, following the Drosophila terminology, suppressor mutants.[4]

The recovery of the three segregating types indicated in table 1 pro-

vides material for comparing the growth characteristics of the suppressor with the original inositolless mutant and with wild type (inositol-independent). These also furnish cultures in which the suppressor is present with the wild type allele at the inositolless locus. Growth tests of a series of such cultures have been carried out (at 25°C.) in 125-ml. Erlenmeyer flasks, using minimal medium and minimal supplemented with 4 μg. inositol per ml. Pads were removed at 3, 6, and 8 days for dry weight determinations, three flasks being used to provide an average value for each determination. Growth curves for asci exhibiting 6:2, 8:0, and 4:4 segregations are presented in figures 1, 2, and 3, respectively. The most instructive segregation is that shown in figure 1, the 6:2 type. In all instances the indicated genotypes were confirmed by appropriate crossing tests. It is immediately clear that all four genotypes are phenotypically distinguishable. It is also evident from the growth response in the culture from spore 16.3 that the suppressor only partially suppresses the effect of the inositolless mutation in 37401, since growth of the suppressor in the absence of inositol is considerably less than that obtained in the inositol-independent wild type. Further, added inositol markedly stimulates the

TABLE 1

RESULTS OF A CROSS OF REVERSION (HOMOCARYOTIC CULTURE EXTRACTED FROM A BACK CROSS TO 37401) WITH WILD TYPE (INOSITOL-INDEPENDENT).

ASCUS SEGREGATION PATTERN— INOSITOL-INDEPENDENT : INOSITOLLESS	NUMBER OF COMPLETE ASCI TESTED
4:4	2
6:2	8
8:0	2

growth of the suppressor. The growth response of the suppressor tends to be rather variable when different stocks are compared in simultaneous experiments (e.g., compare spore cultures 6.1, 6.3, and 16.3), perhaps due to segregating modifiers. However, there is also a rather considerable variability when the same stock is used in successive experiments. But in no instance has the growth of a suppressor strain closely approached that of wild type.

In addition to the suppressor which is segregating with inositolless mutant 37401 in spore 16.3, two additional cultures of the expected types, inositolless 37401 alone (spore 16.5) and inositol-independent (spore 16.7), are present. The fourth culture, from spore 16.1, in which the suppressor is present with wild type, is phenotypically distinct and exhibits a growth pattern which could not have been predicted in advance. It is evident, however, from culture 16.1 as well as from cultures 15.5 and 15.7 (figure 3), that the presence of the suppressor results in a marked delay in the growth on minimal of the wild type strain and that this delay is not overcome by the addition of inositol.

Microbiological assays have indicated that the suppressor culture is actually synthesizing *meso*-inositol, but apparently at a much reduced rate compared with the inositol-independent wild type. Quantitative assays have been performed with mutant 37401 of *Neurospora crassa* and with *Ashbya gossypi*, for the first of which a marked specificity of response to *meso*-inositol apparently exists.[5] Following 21 days' growth on minimal,

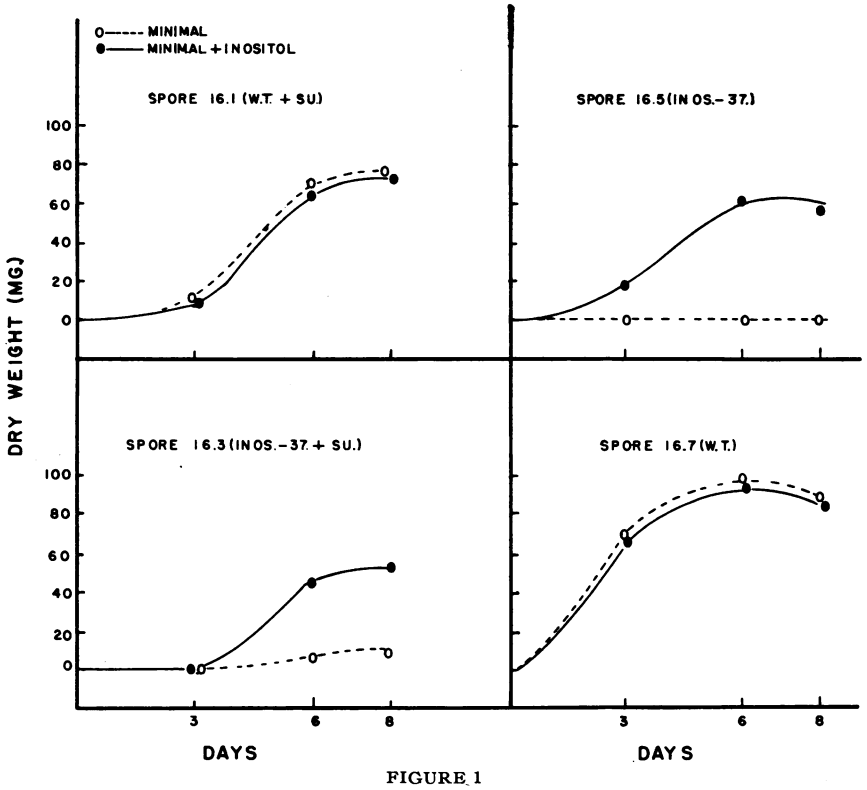

FIGURE 1

Figures 1–3 show comparative growth curves obtained in liquid minimal and minimal supplemented with 4.0 μg. inositol per ml. (20 ml. in 125-ml. Erlenmeyer flasks) for cultures from complete asci from a cross of the suppressor of inositolless 37401 (inos.-37 + Su.) with wild type (W. T.). Segregations of inositol-independent: inositolless cultures are as follows: figure 1, 6:2; figure 2, 8:0; figure 3, 4:4.

the mycelium of a suppressor strain was harvested (total dry weight was 74 mg.) and both filtrate and mycelium were tested for inositol activity. No activity was present in the filtrate, but the following values were obtained in micrograms of inositol per mg. of dried mycelium in duplicate sample tests: with the Neurospora assay, 0.19 and 0.13; with the Ashbya assay, 0.17 and 0.24. The value obtained for wild type mycelium in com-

TABLE 2

TESTS FOR SUPPRESSION OF OTHER INOSITOLLESS MUTANTS BY A SUPPRESSOR OF INOSITOLLESS 37401 (37401+Su.)

CROSSES TO INOSITOLLESS MUTANTS	NUMBER OF COMPLETE ASCI WITH SEGREGATION TYPES INOSITOL-INDEPENDENT:INOSITOLLESS 4:4	6:2	8:0
(*A*) Wild type + suppressor, culture No. 1, crossed with:			
37102	3	0	0
46316	18	0	0
46802[a]	5	0	0
64001	19	0	0
89601	21	0	0
37401	2	15	2
J.H. 2626	7	0	0
J.H. 5202	2	0	0
(*B*) Wild type + suppressor, culture No. 2, crossed with:			
89601	9	0	0
37401	1	10	0

[a] 8-Spored asci only.

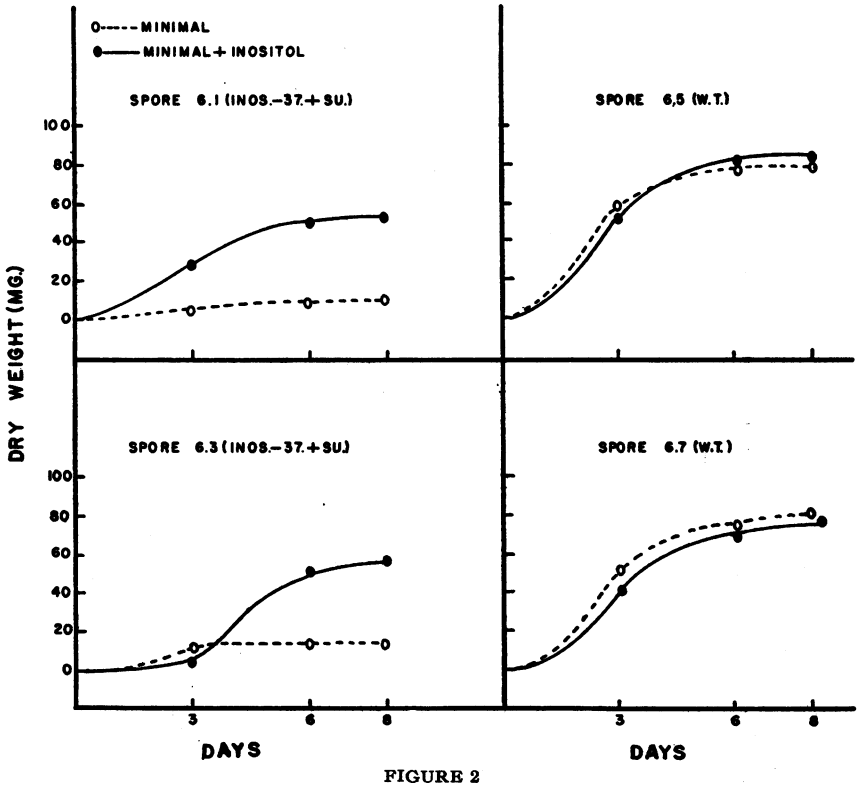

FIGURE 2

parable assays is approximately 2.0, indicating that the suppressor has only about one-tenth the mycelial concentration of inositol present in the wild type.

Genetic Tests of the Suppressor in Combination with Other Inositolless Mutants.—Following the demonstration that the reversion in 37401 was actually the result of a suppressor mutation at an independent locus, it became of interest to determine whether this suppressor would also suppress other inositolless mutants. Consequently, simultaneous crosses were made between the suppressor combined with wild type (W.T. +Su.)

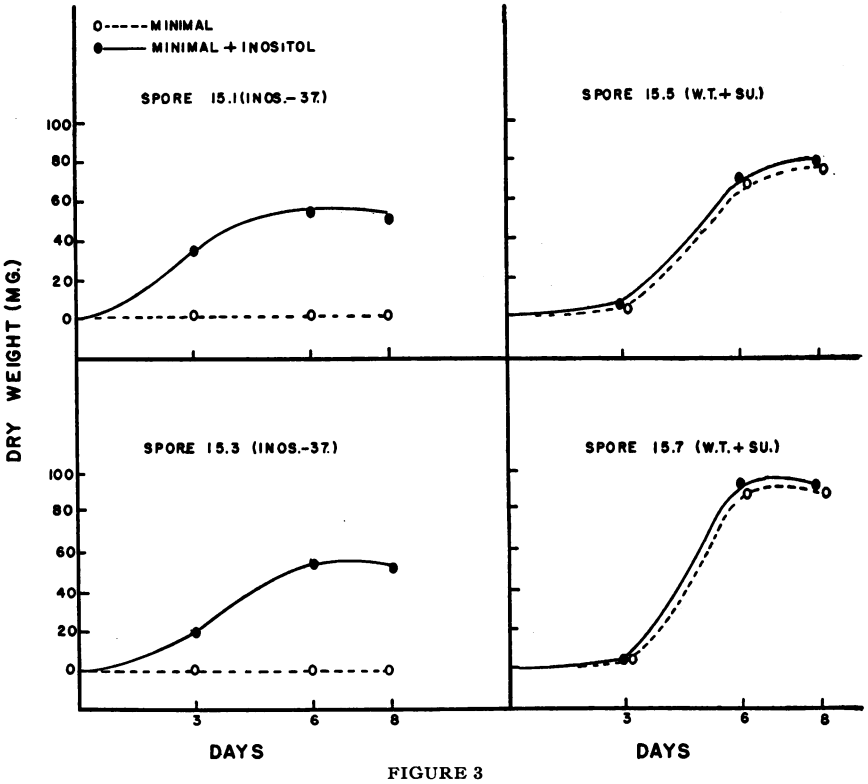

FIGURE 3

and several different inositolless mutants. Serial ascospore isolations were then performed to test for the presence of 6:2 and 8:0 inositol-independent: inositolless segregations, since more than four inositol-independent cultures in a single ascus would indicate a suppression of the particular inositolless mutant being tested by the suppressor of mutant 37401. Some of these crosses were also segregating for albino (al_2-15300) which facilitates the visual detection of any segregation irregularities.

The results of these crosses, shown in table 2, indicate that only 4:4 segregations were obtained with seven different inositolless mutants

(although relatively few complete asci were obtained with mutants 5202 and 37102), whereas mutant 37401 again showed the expected three types. Typical growth curves obtained from the four cultures in one ascus (No. 3) from a cross with mutant 89601 are shown in figure 4.

In order to be certain that the suppressor was actually present, even though inactive, with the various inositolless mutants tested, further

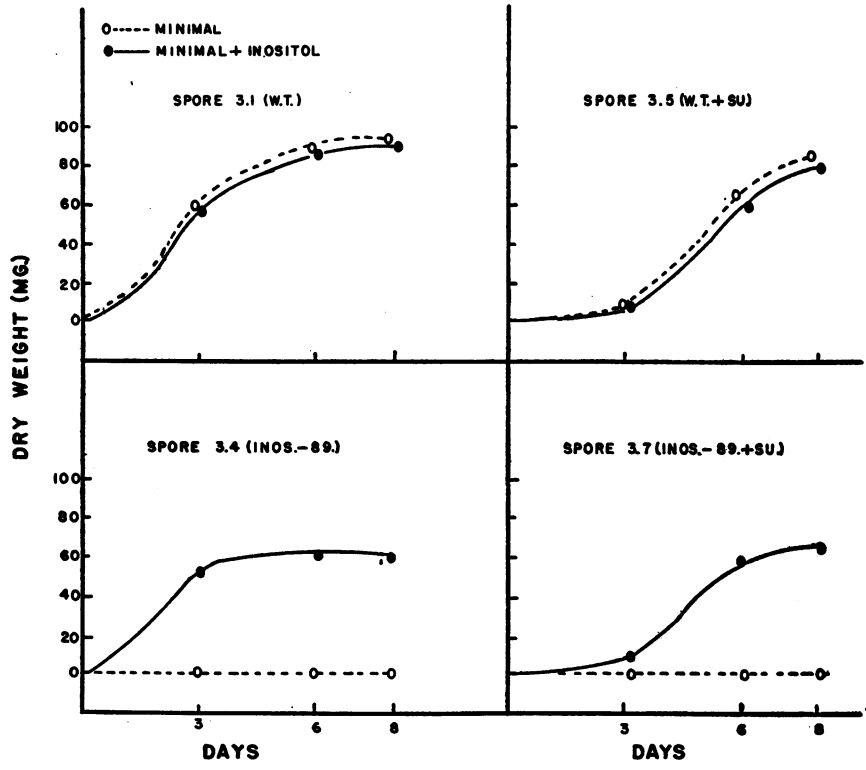

FIGURE 4

Comparative growth curves obtained by methods indicated in figures 1–3, but from a cross of the suppressor of 37401 combined with wild type (W.T. + Su.) with inositolless 89601 (inos. − 89). Cf. table 3.

crosses to 37401 were made utilizing the four cultures from ascus 3 in the cross with 89601. The results of these crosses are shown in table 3. It can be seen from the ascus segregation patterns that the suppressor can be re-extracted from mutant 89601 and is again able to suppress mutant 37401, although it did not effect growth in the absence of inositol when combined with mutant 89601.

Discussion.—The particular reversion selected for study in the present

investigation was obtained as a phenotypically distinctive, slow-growing mutant from inositolless microconidia (of mutant strain 37401) exposed to ultraviolet irradiation and plated on minimal medium. Extensive genetic analyses of phenotypically "normal" reversion types—those exhibiting a morphology and growth rate on minimal medium similar to inositol-independent, "wild type" strains[2]—have demonstrated that the vast majority of these behave in crosses as instances of reverse mutation. Preliminary evidence that certain of the slow-growing types represent instances of suppressor mutation rather than of reverse mutation was obtained when crosses of certain of these mutants to wild type yielded inositolless cultures from random ascospore isolations.[2] The present investigation gives detailed genetic evidence from serial ascospore isolations for one such mutant and demonstrates conclusively that reversions may be obtained which are able to grow in the absence of inositol as a result of suppressor mutation at a locus completely independent of the original inositolless locus.

TABLE 3

TESTS TO DEMONSTRATE THE PRESENCE OF THE SUPPRESSOR OF 37401 IN COMBINATION WITH INOSITOLLESS MUTANT 89601. CROSSES OF ONE CULTURE FROM EACH SPORE PAIR IN A COMPLETE ASCUS ISOLATED FROM A CROSS OF WILD TYPE PLUS SUPPRESSOR WITH 89601-al_2 (CF. FIG. 4)

SPORE NO.	CROSSED WITH	NO. OF COMPLETE ASCI OF SEGREGATION TYPES INOSITOL-INDEPENDENT:INOSITOLLESS 4:4	0:8	2:6	6:2	AL_2 SEGREGATION	INDICATED GENOTYPE OF SPORE
3.1*a* al_2^+	37401-al_2	15	0	0	0	1:1	W.T.
3.4*a* al_2^+	37401-al_2	0	17	0	0	1:1	inos. − 89.
3.5*A* al_2	37401-al_2^+	4	0	0	11	1:1	W.T. + Su.
3.7*A* al_2	37401-al_2^+	2	4	10	0	1:1	inos. − 89. + Su

Growth tests of the present suppressor indicate that this mutation only partially suppresses the effect of the original mutation giving an inositolless phenotype. Although growth occurs in the absence of this substance, it is much less rapid than in inositol-independent strains (either wild type or typical reverse mutation types), and growth is markedly stimulated by the addition of inositol to the medium. The possibility that some change has occurred as a result of the suppressor mutation such that inositol is no longer required by the reversion, or is being replaced by some other substance, appears to be eliminated by the demonstration by microbiological assay that *meso*-inositol is present in the mycelium of the suppressor strain, although in a reduced amount compared with typical inositol-independent types. This latter condition presumably explains the relatively restricted growth of the suppressor on minimal and its stimulation by added inositol.

Perhaps the most notable finding in the present study is the apparent marked specificity of this suppressor strain. Crosses of the suppressor in combination with the wild type allele at the inositolless locus were made to seven allelic inositolless mutants (all of independent origin). A simultaneous cross was made to mutant 37401, in which the suppressor was obtained, as a control. The expected 6:2 and 8:0 inositol-independent: inositolless segregations were recovered in serial ascospore isolations from the control cross, but in all the other crosses only 4:4 segregations were observed. Appropriate additional genetic tests with one of the mutants (89601), involving re-extraction of the suppressor, demonstrated that the suppressor was definitely present, but that no growth on minimal occurred even in protracted growth tests in tubes and in flasks.

Previous studies of the same series of inositolless mutants used in the present tests for suppressor activity have demonstrated that these mutants are all allelic (or in some instances possibly pseudoallelic), but that several differ markedly in at least one respect—the frequency with which they undergo reverse mutation spontaneously or following ultraviolet radiation.[2] With respect to its frequency of reverse mutation induced by ultraviolet, mutant 37401 appears to be distinct from the other seven inositolless types, and the present evidence for the specificity of its suppressor adds another criterion of distinction. The evidence obtained from this suppressor does not, however, aid in elucidating the problem of possible pseudoallelism at this locus. The best evidence to date for recombination is that between mutants 37401 and 64001, from crosses of which inositol-independent cultures (which have been shown not to be pseudo-wild types[6]) are regularly recovered.[2] On the other hand, no evidence of recombination has been obtained from crosses of 37401 and 37102, involving tests of comparable numbers of ascospores.[2] With both mutants, however, there is no effect of the suppressor of 37401.

A similar specificity of suppressor effect in *Neurospora crassa* for one of two apparently allelic mutants of independent origin has been reported by Yanofsky.[7] In this instance it was shown that the particular enzyme, tryptophan desmolase, which was absent from the original mutants, was present in the suppressed mutant, but could not be detected in the mutant which did not grow without tryptophan when carrying the suppressor. An additional noteworthy similarity exists between the results reported for the tryptophanless mutant and those obtained in the present study. When the suppressor mutation is combined with the wild type allele at the inositolless locus, a marked interaction occurs such that the growth of the resulting strain is greatly retarded compared with the normal wild type. Further, this effect cannot be overcome by added inositol. The nature of this interaction is not clear. At present it does not appear particularly profitable to indulge in speculations as to the biochemical mechanism by

which the present suppressor is able to overcome, at least partially, the effect of mutation at the original inositolless locus. Certain aspects of this general problem have been considered for the tryptophanless suppressor,[7] a situation in which the biochemical evidence is much more extensive than in the present case.

The present instance of a suppressor which is effective in combination with only one of a series of allelic (or in some instances possibly pseudoallelic) mutants, provides additional evidence that similarity of response by two or more mutants in Neurospora to a particular suppressor cannot be used as a criterion of allelism. Although the original investigation of suppressors in Neurospora[4] suggested that such mutants might be useful in tests for allelism, it is now apparent that the effects of suppressor mutation may be more complex than was previously anticipated.[8] In addition to instances such as the present case, in which allelic mutants are not all affected by the same suppressor, instances are also known in Neurospora in which clearly non-allelic mutants are suppressed by the same suppressor.[2, 8, 9]

Other instances of reversions are known in Neurospora inositolless mutants which behave in preliminary tests like suppressors, and at least some of those in strain 37401 appear to be different from the present mutant. Additional investigations are contemplated to determine if genetically distinct suppressors do occur. Such tests may make it possible to establish other criteria for distinguishing among the apparent isoalleles at the inositolless locus, much as has been done recently at the vermillion locus in *Drosophila melanogaster*.[10]

Summary.—A genetic analysis has been made of a slow-growing reversion obtained from platings on minimal medium of ultraviolet-treated microconidia of inositolless mutant 37401 in *Neurospora crassa*. These tests indicate that the reversion arose as a result of an independent suppressor mutation rather than by the more usual reverse mutation. The resulting suppression of the inositolless phenotype is only partial, however, since the suppressor grows much less in the absence of inositol than do typical inositol-independent strains and is markedly stimulated by the addition of inositol. Microbiological assays indicate that inositol is present in the mycelium of the suppressor but in reduced amounts compared with wild type strains. Genetic tests have been made to determine the effect of the suppressor of mutant 37401 on seven other allelic (or possibly pseudoallelic) inositolless mutants of independent origin. The results indicate that the suppressor is markedly specific for mutant 37401, since growth does not occur in the absence of inositol when the suppressor is combined with any of the other mutants.

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THE EFFECT OF PRETREATMENT WITH INFRA-RED RADIATION ON THE X-RAY INDUCED SEX-LINKED RECESSIVE LETHAL AND VISIBLE MUTATION RATE IN DROSOPHILA MELANOGASTER

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Pretreatment with infra-red radiation has been shown to cause an increase in the number of gross chromosomal aberrations induced by x-radiation in Drosophila;[1] however, no increase in the number of recessive lethals has been found with similar treatment.[2] These findings are of interest, since recessive lethals have been thought to arise, in large part, as a direct consequence of chromosome breakage.[3–5] Kaufmann and Gay[2] have explained these results by proposing that rearrangements do not give rise to any large portion of recessive lethals, and that the infra-red acts to sensitize the breakage ends of the chromosome so that recombination results rather than restitution. Thus there is an increase in the number of gross chromosomal rearrangements without an increase in the total number of primary breaks, and, consequently, there is no increase in the number of recessive lethals. The data of Kaufmann and Gay are in general agreement with the hypothesis of Lea and Catcheside.[4]

Difficulties of interpretation arise when the foregoing arguments are