THE INFLUENCE OF X-RAYS AND NEAR INFRA-RED RAYS ON RECESSIVE LETHALS IN DROSOPHILA MELANOGASTER*

BY BERWIND P. KAUFMANN AND HELEN GAY

DEPARTMENT OF GENETICS, CARNEGIE INSTITUTION OF WASHINGTON, COLD SPRING HARBOR, NEW YORK

Communicated October 1, 1947

The study here reported concerns the effect of supplementary treatment with near infra-red radiation on the frequency with which x-ray-induced recessive lethal mutations are produced in *Drosophila melanogaster*. Earlier work had shown that when near infra-red rays (λ ca. 10,000 A) are used prior to x-rays in the treatment of the spermatozoa of *D. melanogaster*, a marked increase occurs in the frequency of detectable chromosomal rearrangements over that of controls receiving only the dose of x-rays. On the other hand, the percentage of dominant lethals is not significantly higher when near infra-red treatment precedes the x-rays than when x-rays alone are used.[1] We have now collected additional data which indicate that such supplementary treatment has no significant effect in this species on the frequency of production by x-rays of sex-linked recessive lethals.[2]

Experimental procedures involved the use of the *ClB* method for detection and verification of X-chromosome lethals. Irradiated males were mated with females of the constitution *ClB v/ec ct*^{6} *v g*^{3}; *Cy/Pm*. The maintenance of *Pm* in the stock, and the selection of F_1 females carrying this marker, readily permitted the detection of the non-disjunctional *XXY* exceptions, because of the suppressing effect of the Y-chromosome on eye-color variegation, and facilitated thereby the diagnosis and scoring of the F_2 cultures.

Males of the Swedish-*b*^{6} stock were used; they were obtained from cultures derived from a single pair of flies that had been tested cytologically to insure freedom from chromosomal aberrations. The males were selected three to five days after their emergence and divided into two groups of approximately equal numbers. One of these groups was exposed to near infra-red radiation (as described by Kaufmann, Hollaender and Gay, 1946)[1] for a period of 48 hours either preceding (pretreatment) or following (post-treatment) exposure to x-rays. During this two-day period the group of males that represented the "controls" was kept at a temperature of 18°C. Both groups were exposed simultaneously to a 3000-roentgen dose of x-rays in capsules lying side by side. Following the combined treatment, the males were mated with *ClB* females and allowed to remain in culture bottles for three days before being discarded. This relatively short mating period was chosen as a standard in order to permit determi-

nation of the effect of post-treatment, as well as that of pretreatment, since in the span of three days the accelerating action of near infra-red radiation on the progress of spermatogenesis (detected in an earlier study)[1] is not sufficient to make sperm that was immature at the time of x-ray treatment available for transfer in copulation.

Results.—The lethal-mutation rates determined by these tests are indicated in table 1. Males exposed to near infra-red radiation alone provided only 4 lethals among 2316 spermatozoa tested. The percentage of mutations (0.17 ± 0.08) is not significantly different from that occurring

TABLE 1

LETHAL MUTATION RATE (*ClB* TESTS) AMONG SPERMATOZOA OF MALES EXPOSED TO X-RAYS OR TO X-RAYS PLUS NEAR INFRA-RED RAYS

TYPE OF TREATMENT: X-RAY IN ROENTGENS, NEAR INFRA-RED IN HOURS	NUMBER		PER CENT MUTATIONS
	SPERMS TESTED	LETHAL MUTATIONS	
NIR 48 hrs.	2316	4	0.17 ± 0.08
X-ray 3000 r	3393	253	7.46 ± 0.45
3000 r + 48 hrs.	1989	145	7.29 ± 0.58
48 hrs. + 3000 r	1770	124	7.01 ± 0.61

among spermatozoa of one- to two-day-old males of the *Sw-b* stock at 22°C.[3] Near infra-red radiation in itself, therefore, does not appear to be effective in inducing the types of change that are represented among the group of recessive lethals. Nor does this type of radiation when used prior to or subsequent to a 3000-r dose of x-rays modify to an appreciable extent the frequency of induced lethal mutations, since, as table 1 indicates, samples of the pretreatment and post-treatment series and the controls all yielded about 7 to 7.5% of lethals. (The difference between this frequency and the 8 to 8.7% reported for the 3000-r dosage level by other workers may be attributable in part to differences in the stocks used, or to differences in dosimetry.)

In order to appraise these findings, an effort was made to determine the frequency of occurrence of chromosomal rearrangements among the group of recessive lethals. Previous studies, utilizing salivary-gland-chromosome preparations, had revealed that in some cases there is no detectable chromosomal alteration at the locus of the induced lethal, whereas in others there is a deficiency of one or more bands, or involvement in a gross rearrangement, often without any visible deletion.[4-7] The proportion of the lethals associated with chromosomal rearrangements has been determined in a series of experiments;[4, 8-11] it appears, on the basis of the limited data available, to vary wth the x-ray dose, but at the 3000-r level is of the order of magnitude of 35% (summarized data are given by Lea and Catcheside).[6] Comparable values were obtained for 100 of the 526 lethal mutations detected in our experiments. The 100,

selected at random in equal numbers from the combination-treatment series and the controls, were analyzed by the salivary-gland-chromosome method. Among 50 derived from the combination treatment, 18, or 36%, showed gross rearrangements involving the X-chromosome; 11 were found among 25 lethals examined in the pretreatment series, and 7 among 25 in the post-treatment series. In the control group, 14 out of 50, or 28%, revealed X-chromosome rearrangements. The aberrations were of the types that are customarily detected by salivary-gland-chromosome analysis following treatment of males with a 3000-r dose of x-rays, and included large deficiencies, transpositions, inversions, reciprocal translocations, and complex rearrangements involving two or more chromosomes. We have not carried out the extensive series of genetic tests that would be required to determine the precise location of the lethal mutation with respect to the points of breakage involved in each rearrangement; but, in the light of Demerec's finding[4] that a breakage point coincided with the locus of the lethal in 24 of 26 cases studied (92.3%), it appears that a similar correlation may obtain in our material.

Discussion.—The data and the considerations here presented indicate, therefore, that a considerable fraction of the lethal mutations induced in our experiments is associated with gross structural changes in the chromosomes. Rearrangements of the types represented had been found in an earlier experiment to increase in frequency about 50% when treatment of the spermatozoa with near infra-red radiation preceded a 4000-r dose of x-rays.[1] However, we have not found a corresponding rise in the frequency of recessive lethals when such combination treatment is applied. This suggests that the lethals associated with gross chromosomal alterations are not dependent for their expression on the production of rearrangements. If they were, pretreatment with near infra-red radiation should effect an increase of about 1 to 1.5% in the frequency of recessive lethals over that in the x-ray controls.

Analysis of dose-frequency relations determined experimentally in studies of lethals and chromosomal aberrations had previously led Lea and Catcheside[6] to formulate a detailed theory based on the alternative assumption that radiation-induced recessive lethals and chromosomal rearrangements in *Drosophila* result independently from a single type of primary effect. This interpretation has also been formulated by Herskowitz.[11] Fano,[13] however, in a recent note, has pointed out that the consequences of this alternative assumption also are at variance with the total experimental evidence. The evidence now available from the near infra-red experiments makes it seem reasonably certain that the negative portion of the theory advanced by Lea and Catcheside concerning the origin of recessive lethals is essentially correct—namely, that the lethals associated with chromosomal rearrangements in *Drosophila* do not repre-

sent a special class caused by position effect and requiring two ionizing particles for their production.

In the absence of any acceptable comprehensive theory of the mechanism of induction of lethals and of viable rearrangements, it may still be useful to compare the values obtained experimentally following the combination treatment with theoretical estimates derived from alternative assumptions. These estimates, supplied by Dr. U. Fano, were based on the methods developed in his previous note;[13] the pertinent calculations will be published in Year Book No. 46 of the Carnegie Institution of Washington. In making the estimates it has been assumed tentatively (in agreement with the data obtained at 4000 r) that the frequency of cytologically detectable X-chromosome breaks induced by an x-ray dose within the range from 2000 r to 4000 r will be increased by 50% under the influence of infra-red treatment. The infra-red treatment should then have the following effects:

1. The frequency of sex-linked recessive lethals at 3000 r should increase by about 17% if $^1/_3$ of those lethals were due to position effect, and *decrease* by about 5% or more according to the Lea-Catcheside hypothesis. Our experimental results show a decrease of this order of magnitude (table 1, line 4 compared with line 2), although the difference is not statistically significant.

2. The fraction of sex-linked recessive lethals associated with rearrangements should increase from approximately $^1/_3$ to $^3/_7$ according to the position-effect hypothesis, and to over $^1/_2$ according to the Lea-Catcheside hypothesis. Our experimental data show an over-all increase from 28 to 36%, which is more nearly comparable in magnitude with that expected on the former hypothesis than on the latter. It should be pointed out, however, that in these experiments the number of cases analyzed is small, and the errors correspondingly large (28 ± 6%, and 36 ± 7%). Moreover, among the group of lethals induced by x-rays following pretreatment with near infra-red radiation—the type of experiment in which the 50% increase in frequency of viable chromosomal rearrangements was effected—there were 11 lethals associated with chromosomal rearrangements out of 25 examined, a frequency of 44 ± 10%; and this value, although not statistically significant, might be reconciled with the Lea-Catcheside hypothesis.

3. The fraction of eggs hatching when the spermatozoa used in their fertilization had been exposed to 2000 roentgens of x-rays should be reduced by not less than about 10% (of the fraction itself), according to either hypothesis. Our actual counts[1] correspond closely to this expected value, since 51.4% of the eggs hatched when sperms were treated with 2000 roentgens of x-rays, and only 46.7% when treatment with near infra-red radiation preceded the x-rays.

It is obvious, therefore, that the application of this type of analysis to the formulation of a general theory of the origin of lethals and chromosome rearrangements will require more extensive data than have been provided in the present paper. Further pertinent data might also be obtained from a study of frequency of the various types of change induced by x-rays at different dosage levels following pretreatment with near infra-red radiation.

The experimental data here presented, in conjunction with those obtained in earlier experiments,[1, 14] permit a more comprehensive view than was previously possible of the action of near infra-red radiation in modifying the frequency of x-ray-induced chromosomal rearrangements in *Drosophila*. This "sensitizing" action now appears to apply to the production of viable chromosomal rearrangements, which are detected by salivary-gland-chromosome analysis, and presumably also to their inviable counterparts, the multiple-break type of lethals that lead to death in embryonic stages of the individuals carrying them. Supplementary treatment with near infra-red radiation did not effect any significant increase in the frequency of the single-break type of dominant lethal, or of sex-linked recessive lethals. These findings, together with our observations that near infra-red radiation in itself is ineffective in inducing either lethal mutations or chromosomal rearrangements, suggest that this agent is not responsible for initiating or producing breakage of chromosomes. As was pointed out in our earlier publication,[1] near infra-red radiation of wave-length 10,000 A provides only about 1.2 electron volts wherever a quantum is absorbed, and this amount is not as a rule sufficient to break chemical bonds. Under these conditions the production of primary breaks would be a function of the x-ray dose alone, even in treatments that combine near infra-red and x-rays. In appraising this interpretation, consideration must be given to the finding of Swanson and Hollaender[12] that treatment of microspores of *Tradescantia* with near infra-red rays following their exposure to x-rays produces a significant increase in chromosome breakage beyond that found in the controls. In order to relate to a single mechanism the modifying action of near infra-red radiation when used prior to or subsequent to x-rays, these authors have suggested that the chromosome structure may be weakened by either type of radiation and that the supplementary action of the other may then be effective in producing a thoroughgoing break. This interpretation attributes the increased frequency in detectable chromatid breaks to an increase in the number of primary breaks. Since we have not found evidence in our experiments with *Drosophila* to support this assumption, we are inclined to the point of view indicated in our earlier publication[1]—that near infra-red radiation acts as a "sensitizing" agent by increasing the number of breaks available for participation in the production of new chromosomal rearrangements. This increase could be realized if recombination were

facilitated at the expense of restitution. Such a mechanism would account for the fact that in *Tradescantia* the frequency of double deletions, which presumably are realized immediately following the production of the lesion by x-rays, is increased by pretreatment but not by post-treatment with near infra-red, whereas single deletions and interchromosomal exchanges are increased in frequency both by pretreatment and by post-treatment. We have not completed a detailed analysis of the effect on the chromosomes of the x-rayed spermatozoan of *Drosophila* of exposure to a dose of near infra-red radiation equal to that used in the pretreatment experiments; but exposure for shorter intervals of eggs fertilized by x-rayed sperm has elicited chromosomal rearrangements with a frequency in excess of that obtained in controls kept at 18° to 28°C.[14] Facilitation of chromosome movement may have been an important factor in promoting recombination in these cases, since the eggs were exposed during the period of syngamy and early cleavage, when the potential breaks first become available for participation in the formation of rearrangements. On the other hand, the pronounced effect of near infra-red radiation in the pretreatment experiments emphasizes the possibility that this agent may produce such changes in the materials of the chromosomes that the process by which restitution is normally effected is either inhibited or delayed, thereby making additional breaks available for the formation of new combinations.

The more precise definition of the mode of action of near infra-red radiation that is now possible opens the way for an attack on questions relating to its effect on specific cellular components; it also emphasizes the potentialities of this agent as a tool in experiments designed to modify the recombination phase of the process of induced structural change.

Summary.—Supplementary treatment of the spermatozoa of *D. melanogaster* with near infra-red radiation does not effect any significant increase in the frequency of production by x-rays of recessive, sex-linked lethal mutations. Analysis of a sample of 100 of these lethals by the salivary-gland-chromosome method revealed that 32% were associated with gross chromosomal alterations. A consideration of these data and those obtained in previous experiments suggests that radiation-induced recessive lethals are not attributable primarily to a position effect dependent on the establishment of new associations by the gene concerned. Consideration of the combined data also suggests that near infra-red radiation, when used as a supplementary treatment, is effective in increasing the frequency of chromosomal rearrangements by facilitating recombination, presumably at the expense of restitution, among the ends of chromosomes broken by the ionizing radiation.

Acknowledgments.—The authors wish to express their appreciation to Dr. U. Fano, of the x-ray section of the National Bureau of Standards, for

his contributions to the discussion of the problem and to the analysis of the data, and to Miss Katherine Wilson for her assistance with the preparation and analysis of the salivary-gland-chromosome slides.

* Progress of this study was facilitated by a grant-in-aid from the United States Public Health Service.

[1] Kaufmann, B. P., Hollaender, A., and Gay, H., *Genetics*, **31**, 349–367 (1946).
[2] Kaufmann, B. P., and Gay, H., *Anat. Rec.*, **96**, 34–35 (1946).
[3] Kaufmann, B. P., *Am. Nat.*, **81**, 77–80 (1947).
[4] Demerec, M., *Cytologia*, Fujii Jubilee Volume, 1937, pp. 1125–1132.
[5] Fano, U., *Cold Spring Harbor Symp. Quant. Biol.*, **9**, 113–120 (1941).
[6] Lea, D. E., and Catcheside, D. G., *J. Genetics*, **47**, 10–24 (1946).
[7] Lea, D. E., *Actions of Radiations on Living Cells*, Cambridge University Press, 1946.
[8] Oliver, C. P., *Zeit. f. indukt. Abstam. Vererb.*, **61**, 447–488 (1932).
[9] Demerec, M., and Fano, U., *Proc. Nat. Acad. Sci.*, **27**, 24–31 (1941).
[10] Fano, U., *Genetics*, **29**, 361–369 (1944).
[11] Herskowitz, I. H., *Am. Nat.*, **80**, 588–592 (1946).
[12] Swanson, C. P., and Hollaender, A., *Proc. Nat. Acad. Sci.*, **32**, 295–302 (1946).
[13] Fano, U., *Science*, **106**, 87–88 (1947).
[14] Kaufmann, B. P., *Genetics*, **31**, 449–453 (1946).

---

*ON INFINITE COMPLEXES WITH AUTOMORPHISMS**

BY BENO ECKMANN

INSTITUTE FOR ADVANCED STUDY, PRINCETON, N. J., AND UNIVERSITE DE LAUSANNE SWITZERLAND

Communicated July 28, 1947

When in an infinite complex K a group G of automorphisms operates without fixed cells and with finite fundamental domain, then there exist purely algebraic relations between certain homology properties of K and the abstract structure of G. This is established in the present note as an application of a previous general result,[1] and geometric examples are investigated, concerning, in particular, coverings of closed manifolds and their homotopy groups.

1. Let K be a closure finite complex in the sense of combinatorial topology; its finite integer chain groups will be denoted by C_n, the boundary homomorphism of C_n into C_{n-1} by ∂, $n \geqq 0$ (C_{-1} is the additive group of integers, and the boundary of a 0-cell c_0 is defined by $\partial c_0 = 1$), and the homology groups based upon finite integer chains by H_n. If $H_n = 0$, K is said to be *acyclic* in the dimension n; when K is a geometric complex, $H_0 = 0$ means that it is connected.

For a given Abelian coefficient group J, an n-cochain f^n in K, $n \geqq 0$, is a J-valued function of the n-cells c_n of K, or a homomorphism of C_n into J; its coboundary δf^n is the $(n + 1)$-cochain defined by $\delta f^n(a_{n+1}) =$