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*THE INDUCTION OF ACTIVATED, STABLE STATES IN THE CHROMOSOMES OF TRADESCANTIA BY INFRA-RED AND X-RAYS**

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Infra-red by itself is ineffective in inducing chromosomal aberrations. When employed in combination with x-rays, however, it is an effective potentiating agent, and significant increases in the frequency of aberrations are readily obtained. This phenomenon was first demonstrated in Drosophila by Kaufmann, Hollaender, and Gay.[1] They succeeded in showing that an exposure of Drosophila males to infra-red prior to x-radiation increased the frequency of aberrations for any given dose of x-rays, but that a post-treatment was generally ineffective except under certain circumstances.[2] Subsequent studies in Tradescantia have revealed that

infra-red is an effective potentiating agent when used either as a pre- or as a post-treatment, although not equally so for all types of aberrations.[3-4] In addition, the duration of time between exposure of the inflorescences to infra-red and to x-rays was shown to be unimportant; the effect, once induced, was relatively permanent and did not decay with time to any appreciable degree or in any consistent manner.

Two hypotheses have been advanced to account for the action of infra-red in these combined radiation experiments. The Drosophila workers have suggested that infra-red acts principally to favor the process of recombination of broken ends over that of restitution.[1, 6] According to this hypothesis, x-rays alone can induce breaks; infra-red simply influences their subsequent behavior. We have assumed, on the other hand, that the primary action of infra-red is such as to increase the frequency of primary breaks which may or may not become involved in the process of recombination.[3-5] As a corollary hypothesis, we are now proposing that both infra-red and x-rays can induce activated states in the chromosome which, under the influence of the other type of radiation, are more readily moved along the reaction pathway which leads to complete breakage of the chromosomes. Following the ideas of Eyring and Stearn[7] (see also McElroy and Swanson[8]), it is considered that the activated states occupy a stable position in an energy depression somewhere along the reaction pathway, and that their subsequent behavior will depend upon the environmental factors which impinge upon them. The activated states are different for each type of radiation, and our studies indicate that each is acted upon by the other type of radiation, i.e., the radiations must be in combination for an increase in breaks to be achieved. Temperature shocks were therefore employed to test for the presence of these activated states since many studies of both a biological and a physical nature have indicated that such states are sensitive to such environmental changes.

Experimental Procedures and Results.—Methods for the exposure of inflorescences of Tradescantia to infra-red radiation have been described previously.[3, 5] The infra-red source used in these experiments was a 250-watt drying lamp, the exposure time was three hours, and the radiation was passed through a Corning No. 2540 glass filter. All x-ray exposures were made at 80 kv. and 10 ma., with the total dose in each case being 67 *r* units delivered in one minute. All temperature shocks consisted of immersion of the inflorescences in water at 48°C. for 30 seconds. This temperature was found to be effective in bringing about a change in the state of sensitivity of the chromosomes, and was therefore adhered to in all of the experiments herein reported. The chromosomes were smeared 22–24 hours after x-radiation regardless of what other treatments were given. Only metaphase figures were scored.

Table 1 summarizes the data obtained from two experiments in which

a temperature shock was interposed between the infra-red and x-ray exposures. The *x-ray* and *temp.-x-ray* series serve as controls against which the *infra-red-temp.-x-ray* and the *infra-red-x-ray* series can be evaluated. Whenever a temperature shock was employed it preceded the x-ray exposure by one hour to avoid any effects which temperature might have on the x-ray effects *per se*. A comparison of the *x-ray* and the *temp.-x-ray* series indicates that there is no such effect. All categories of aberrations in the *infra-red-x-ray* series are significantly increased over those in the *x-ray* and *temp.-x-ray* series, while the data in the *infra-red-temp.-x-ray* show no significant increases. A temperature shock of the magnitude used clearly removes the effect induced by the infra-red, and the chromosomes thus treated react to x-rays as if no prior infra-red exposure had been made. The action of infra-red on chromosomes therefore appears to be such as to raise them to a state of higher reactivity to subsequent doses of x-rays, a state from which they can be returned to one of normal reactivity by the temperature shock.

TABLE 1

EFFECT OF A TEMPERATURE SHOCK WHEN GIVEN BETWEEN EXPOSURES OF INFRA-RED AND X-RAYS. ALL *P* VALUES DERIVED FROM χ^2 CALCULATIONS, AND IN TERMS OF THE *X-Ray Control* SERIES. DATA IN PER CENT OF ABERRATIONS ON A CHROMOSOME BASIS

TREATMENT	NUMBER OF CHROMOSOMES	SINGLE DELETIONS	ISOCHROMATID DELETIONS	EXCHANGES
X-ray control	1800	4.72	6.34	1.39
Temp.-x-rays	2400	4.55	6.43	1.29
		P = 0.70–0.80	0.80–0.90	0.70–0.80
Infra-red-temp.-x-rays	2580	4.31	7.06	1.32
		P = 0.50–0.70	0.20–0.30	0.80–0.90
Infra-red-x-rays	1800	8.55	9.62	3.22
		P = <0.001	<0.001	<0.001

It was then decided to test the effect of a similar temperature shock when applied between x-rays and infra-red, the latter being given as a post-treatment. It was first necessary, however, to make certain that a temperature shock of this magnitude would not interfere with the capacity of the chromosome to respond to the influence of infra-red. The data in table 2 were obtained with this in mind, and it is evident that the *infra-red-x-ray* and the *temp.-infra-red-x-ray* series are similar for all categories of chromatid aberrations.

Having established this fact, the data in table 3 can be more properly evaluated. The temperature shock in this experiment was delayed for an hour after the end of the x-ray exposure to avoid any possible interference with the processes leading to recombination. In the *x-ray-infra-red* series, the lack of increase in the isochromatid deletions agrees with the data from earlier experiments[3] (Swanson's[4] later conclusions bearing on this subject are incorrect, and are to be disregarded). The chromatid deletions

and the exchange aberrations are increased as expected. When the temperature shock is interposed between the two radiations, the isochromatid deletions remain unaffected, the chromatid deletions are significantly decreased, and the exchanges are returned to a level comparable to that induced by x-rays alone. It is reasonable to conclude, as a consequence, that the temperature shock acts not to decrease the capacity of the chromosome to react to infra-red, but rather to "desensitize" the chromosomes by eliminating the x-ray induced activated states which ordinarily respond to the influence of infra-red.

Discussion.—The experiments just cited indicate that both x-rays and infra-red can induce altered states in the chromosome which have no morphological expression, but which respond in combined radiation experiments in such a manner as to increase significantly the frequency of aberrations. These states can persist unchanged for long periods of time

TABLE 2

EFFECT OF A TEMPERATURE SHOCK WHEN GIVEN PRIOR TO INFRA-RED, WITH INFRA-RED BEING USED AS A PRETREATMENT. *P* VALUES ARE DERIVED FROM χ^2 CALCULATIONS WITH P^x BEING COMPUTED IN TERMS OF THE *X-Ray Control* SERIES, AND P^i IN TERMS OF THE *Infra-Red-X-Ray* SERIES. DATA IN PER CENT OF ABERRATIONS ON A CHROMOSOME BASIS

TREATMENT	NUMBER OF CHROMOSOMES	SINGLE DELETIONS	ISOCHROMATID DELETIONS	EXCHANGES
X-ray control	3616	5.25	6.75	1.41
Temp.-x-ray	3600	5.75	7.25	1.42
		P^x = 0.70–0.80	0.30–0.50	0.90–0.95
Infra-red-x-rays	3600	8.84	8.35	3.00
		P^x = <0.001	0.01–0.02	<0.001
Temp.-infra-red-x-ray	3864	8.41	8.87	2.82
		P^x = <0.001	0.01–0.001	<0.001
		P^i = 0.50–0.70	0.50–0.70	0.50–0.70

at room temperatures without an appreciable decay in the induced effect; they can, on the other hand, be removed from participation in the breakage-recombination processes by a temperature shock. Additional, but incomplete, data also show that an infra-red effect cannot be induced if the inflorescences are irradiated at temperatures of 23°C. and above (this, despite the fact that if the infra-red is given at a lower temperature the states are nevertheless stable when the inflorescences return slowly to room temperature), but that at 18°C. and lower the infra-red effect is quite pronounced. There is also the suggestion in these data that temperatures lower than 18°C. during infra-red exposure—e.g., 12° and 4°C.—led to progressively greater increases. The exact temperature relationships remain to be worked out in detail, but the available data are in agreement with expectations if it is assumed, as is likely, that the lower the temperature the greater would be the number of activated states retained, and the longer would be their half-life.

These considerations suggest that we are dealing with induced states of a higher energy content than that characteristic of the unirradiated molecular structure of the chromosome, and that in this energized condition the chromosomes react with greater fragility to subsequent radiation. If this be the case, then the further assumption must be made that the activated states induced by infra-red and by x-rays are dissimilar either in the energy levels attained or in the reaction pathways subsequently followed. This assumption is made necessary by the fact that while infra-red, when employed in post-treatment exposures, can raise the x-ray induced states to the level of primary breaks and thus increase the frequency of aberrations, additional infra-red radiation cannot do likewise with those states previously induced by itself. That is, infra-red cannot by itself induce chromosomal aberrations regardless of the duration of exposure or, so far as our experiments show, of the conditions of exposure.

TABLE 3

EFFECT OF A TEMPERATURE SHOCK WHEN GIVEN BETWEEN EXPOSURES TO X-RAYS AND INFRA-RED. P VALUES ARE DERIVED FROM χ^2 CALCULATIONS WITH P^x BEING COMPUTED IN TERMS OF THE *X-Ray Control* SERIES, AND P^i IN TERMS OF THE *X-Ray-Temp.-Infra-Red* SERIES. DATA IN PER CENT OF ABERRATIONS ON A CHROMOSOME BASIS

TREATMENT	NUMBER OF CHROMOSOMES	SINGLE DELETIONS	ISOCHROMATID DELETIONS	EXCHANGES
X-ray control	6300	4.27	4.49	1.43
X-ray-temp.	3900	4.60	4.00	1.46
		P^x = 0.30–0.50	0.20–0.30	0.80–0.90
X-ray-temp.-infra-red	4800	4.79	4.90	1.37
		P^x = 0.10–0.20	0.20–0.30	0.80–0.90
X-ray-infra-red	6000	5.35	4.08	3.72
		P^x = 0.01–0.001	0.20–0.30	<0.001
		P^i = 0.10–0.20	0.05–0.02	<0.001

If we have correctly interpreted the present data, they provide the first concrete evidence that radiations can induce activated, stable states in the genetic material of the cell. The nature and frequency of these states is not known, and it is unlikely that the present technique, which depends upon the determination of an end-product rather than an exact and quantitative description of the states themselves, will provide other than preliminary data relating to their presence or absence, but an understanding of them is essential to an understanding of the actions of radiation as they affect the heriditary potential of the cell. Examples from other fields, however, can provide us with possible clues and avenues of approach to a further study of these effects. Phosphorescent materials, for example, exhibit a strikingly parallel behavior to the conditions herein described. Metastable states can be induced photochemically, they can persist for considerable intervals of time, and they can be returned to a ground state by high temperatures.[9] An analogous situation also holds for certain

of the high polymers of a synthetic nature.[10] Both of these examples may be explained by assuming that energy in the form of photoelectrons is trapped within the irregularities of a lattice network; in the case of phosphorescent materials, impurities in the crystal structure are involved in creating distortions which facilitate the trapping process. The situation found in high polymers, however, is likely to be more closely analogous to that described here not only because of the polymeric nature of the chromosome, but because of the roles in polymerization processes played by peroxides and oxygen. The importance of both in the interpretation of radiation effects on genes and chromosomes has been discussed by many authors, and need not be considered here.

Final mention should be made of the fact that mustard, which in many respects acts in a manner similar to x-rays, can apparently induce activated genic states which are temperature sensitive.[11] It has also been shown that the mustard effects on chromosome breakage can be considerably enhanced by infra-red radiation.[12] It is possible, in the light of these studies, that a reinterpretation of radiation effects on genetic material in terms of activated states may assist in an elucidation of the role played by such environmental agents as oxygen tension and temperature. In a theoretical way, this topic has been considered by McElroy and Swanson,[8] but further experimentation is clearly needed.

Summary.—A temperature shock of 48°C. for 30 seconds, given between exposures of inflorescences of *Tradescantia* to infra-red and x-rays, and to x-rays and infra-red, effectively prevents the potentiating action of infra-red from being expressed in the form of an increase in the frequency of chromosomal aberrations. It is proposed that both infra-red and x-rays induce activated, stable states in the chromosomes, and that in combined radiation experiments these states are raised to the level of primary breaks which may remain open as deletions or enter into recombination with other similarly broken ends to give gross aberrations. These states are removed by high temperatures when once induced, and have a transitory, and hence undetectable, existence if induced at temperatures of 23°C. and above.

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THE INTERACTION OF A MINUTE MUTATION WITH A SEX-LINKED LETHAL OF DROSOPHILA MELANOGASTER

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Despite the fact that the analyses of lethal mutations of Drosophila present a promising approach to the study of gene action, phase specificity has been determined for only a few and the possibility of interaction between lethals and other mutants has received little attention.[1] An experiment was designed, therefore, to determine the effect of a minute mutation, *M*(3)*w*, upon the lethal, *l*(1)7, and the average time of death and the distribution of deaths among the *l*(1)7 larvae.

Method.—The recessive sex-linked lethal *l*(1)7, characterized by the appearance of melanomas, was most recently investigated by Russell[2] who estimated that death occurs in the male larvae about 100 hrs. after hatching. The minute *M*(3)*w*, is lethal when homozygous, but Dunn and Mossige[3] found that the heterozygous mutation causes a pronounced retardation in the rate of larval development. To test the effect of this retardation upon the activity of *l*(1)7 it was necessary to obtain flies which could be crossed to produce male larvae containing *l*(1)7 only and other males with both *M*(3)*w* and *l*(1)7. Accordingly, two stocks were prepared one of which contained $\frac{e}{e}\frac{l(1)7}{\text{In}(1)dl\text{-}49,}$ females and the other $\frac{M(3)w}{\text{In}(3R)C}\frac{+}{e}$ males. In(1)*dl*-49, in addition to carrying the marker gene yellow, served as a crossover suppressor in the *l*(1)7 region of the sex chromosome while In(3R)C performed a similar function in the *M*(3)*w* and ebony region of the third chromosome. Crossing these two types produces an F_1 generation containing larvae of eight different genotypes among which are $\frac{M(3)w}{e}\frac{+}{}$ *l*(1)7 males and $\frac{\text{In}(3R)C}{e}\frac{e}{}$ *l*(1)7 males. Several hundred eggs from such a cross were collected and the hatching larvae removed at hourly intervals and placed in Stender dishes containing yeasted Cream-of-Wheat medium. Between 40 and 50 hrs. after hatching,