in these experiments from those reported by others who have not observed high incidences of the tumor (*2, 4*). Experiments are planned to determine which, if any, of the factors named can influence the tumor incidence.

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Interaction of Chromatid Breaks Produced by X-rays and Radiomimetic Compounds

Abstract. The results of combination treatments of the roots of *Vicia faba* with certain radiomimetic compounds (8-ethoxycaffeine, maleic hydrazide, β-propiolactone, potassium cyanide) and x-ray as well as combination treatments of certain radiomimetic compounds with one another were observed to determine whether interaction will occur between chromosomal breaks induced by different agents. Interaction was observed between breaks induced by x-rays and all of the breaks induced by chemicals but not between breaks induced by any two chemicals. The results are discussed in terms of possible breakage bond differences and the effects of temporal and spatial differences in breaks induced by different agents.

It has been known for some time that treatment with certain chemicals classed as radiomimetic results in chromosomal aberrations that are indistinguishable, when observed at metaphase, from radiation-induced changes. Whether or not the breaks induced by these compounds are qualitatively similar to one another and to radiation-induced breaks is still a moot point. Evidence at this time indicates that regardless of the similarity or dissimilarity of the breaks, the areas in which the breaks induced by a given compound occur are often quite specific. For example, in *Vicia faba*, the chromosomal breaks induced by 8-ethoxycaffeine are most frequently located in the nucleolar organizer region of the satellited chromosomes. Breaks induced by maleic hydrazide are most frequently observed in the two segments of heterochromatin located on either side of the centromere of the satellited chromosomes. Breaks induced by radiation seem to be more at random. They are observed in euchromatic as well as heterochromatic regions, and the frequency with which breaks occur in the long as against the short chromosomes approaches randomness.

Wolff and Luippold (*1*), in their studies of the rejoining system, and Cohn (*2*), in his study of the interaction of x-ray-induced breaks, have demonstrated that whatever the nature of the bonds broken during treatment with x-rays there is sufficient similarity between enough of these ruptured bonds to result in the interaction of broken ends. That all or most x-ray-induced breaks are similar enough to interact is indicated by the fact that Cohn observed a frequency of interaction as great as that which he calculated for complete interaction (*2*). It is feasible, in the light of existing evidence, to consider that all breaks, no matter what their origin or chemical nature, interact through the mediation of a common repair system that does not distinguish between one broken bond and another so that the difference between an interchange and a noninterchange situation is a function only of availability of the breaks in terms of time and space and not of chemical differences between the bonds that are broken.

It is of some interest to test the hypothesis that all breaks, no matter what their nature, can interact with one another. One method would be to confront the "rejoining system" with breaks induced simultaneously by different agents and observe the amount of interaction taking place. These breaks would be randomly dispersed or concentrated in specific localities in the chromosomes, depending on the agent or agents employed.

Specificity in terms of localized breakage can be explained in two ways. First, the compound may be incorporated at specific sites and is therefore restricted in its action to a few localities. The only evidence bearing on this point makes this an unlikely hypothesis since autoradiographic studies of tritiated 8-ethoxycaffeine indicate that at least this compound is not incorporated at the site where most of the damage is observed (*3*). Second, specificity can be explained by assuming that an agent inducing breaks in specific localities in few or all chromosomes is capable of producing breaks in one or few kinds of bonds which occur rarely along the length of the chromosomes. Conversely, an agent inducing breaks which are randomly located along the length of all chromosomes can be considered capable of producing breaks in several kinds of bonds, or, assuming some specificity of action, of breaking a particular bond that is exceedingly common along the length of the chromosomes. Regardless of the explanations for observed localized breakage, it is of interest to test whether or not breaks induced at specific sites by particular agents interact with other breaks, randomly or specifically located.

The experimental material used in the following experiments (*4*) consisted of lateral root tips of the broad bean, *Vicia faba*. The variety used, Seville Long Pod, was obtained from Carter's Tested Seeds Limited, London, England. The roots were grown in shell vials in the dark at 25°C for 6 days following an initial 24-hour period of soaking. The tap water was changed twice daily during growth and recovery (the latter being the time interval between treatment and fixation). Twenty-four hours before treatment the roots were placed in an incubator at 17°C. Treatments were performed at 20°C, and recovery took place at 17°C, in the dark. The root tips were treated with 0.05 percent colchicine for 3 to 4 hours before fixation in a cold mixture of alcohol and acetic acid (3:1), and slides were prepared as Feulgen squashes. The treatments were carried out in shell vials and were performed in three different ways.

The two agents that induce breaks whose ability to interact was being tested were introduced simultaneously, and each was used as pretreatment for the other. The agents used to induce specific and randomized breaks are as follows: β-propiolactone, potassium cyanide, 8-ethoxycaffeine, maleic hydrazide, and x-rays. Dinitrophenol was also used in an occasional experiment to keep the breaks open between treatments (*1*) to determine whether or not it had any effect on the exchange frequency (interaction) (*2*). The following molar concentrations of the various agents were use: β-propiolactone, 7×10^{-3}; KCN, 1×10^{-3}; 8-ethoxycaffeine, 1×10^{-2}; maleic hydrazide, 2×10^{-4}; dinitrophenol, 1×10^{-4}. The x-ray dose, when used, was 100 r.

Results of the different combination treatments are summarized in Table 1. The experiments listed here are based on the assumption that x-ray-induced breaks, being more or less randomly distributed, are likely to include amongst them enough broken bonds similar to those induced by radiomimetic compounds that interaction will result from combination treatments including radiation as one of the agents. Conversely, combination treatments with two agents that induce breakage in relatively localized areas are likely to break few, if any, similar bonds, and therefore

interaction will not result from these treatments.

The results of these treatments seem to bear out this assumption. All the combination treatments that include x-rays and a radiomimetic compound result in interaction. Combination treatments with agents that induce breaks in rather specific chromosomal areas do not appear to result in interaction of these breaks. In the case of 8-ethoxycaffeine and maleic hydrazide, where the breaks are localized in the satellited chromosomes, interaction, if it occurs, can be observed directly. If it is assumed that interaction takes place, exchanges would be expected between the nucleolar organizer region (induced by 8-ethoxycaffeine) and the heterochromatic regions proximal to the centromere of the long chromosomes (induced by maleic hydrazide). Instead, all exchanges in the satellited chromosomes involving one or the other of these two break points always involve identical break points in both chromosomes. None of the combination treatments resulting in interaction showed any change in exchange frequency in the presence of dinitrophenol, nor did the frequency differ when the order in which the agents were presented was shifted. This, of course, was to be expected when KCN was used, for it has the same depressant effect on the rejoining system as dinitrophenol. No experiments in which KCN was combined with either 8-ethoxycaffeine or maleic hydrazide could be carried out, nor could dinitrophenol be used with the 8-ethoxycaffeine–maleic hydrazide combination treatments to inhibit rejoining, because the resulting decrease in exchange frequency produced by these two compounds in the presence of KCN or dinitrophenol is too great to permit the determination of any degree of interaction.

The results obtained, although supporting the assumption made previously, can be variously interpreted. It can be argued, as Auerbach (*5*) has (after having demonstrated interaction between breaks induced by nitrogen mustard and by x-rays), that failure to observe interaction can be misleading in that breaks induced by two agents are so spaced temporally that restitution or rejoining of one set occurs before the other set of breaks has been induced. Since the breaks induced by one agent may be concentrated in a completely different mitotic stage than breaks induced by the second agent, breaks induced by both agents during the relatively short treatment may only rarely occur at the same time in the same cell. Interaction obviously would not occur under these circumstances even though the breaks may be qualitatively similar and are otherwise capable of interaction.

This argument adequately explains some of the results listed in Table 1 if removed from the context of other results listed therein. For example, the combination treatment with x-rays and 8-ethoxycaffeine produced an exchange frequency to be expected if interaction occurred. These are agents that are dissimilar in localization pattern (*6*), but the breaks induced by both of them are so placed temporally that they are hard to distinguish. Interaction therefore is to be expected if the previous argument is assumed to be correct even though these agents (x-rays and 8-ethoxycaffeine) are quite dissimilar in many ways. Auerbach's hypothesis would also explain the results observed after combination treatments with 8-ethoxycaffeine and maleic hydrazide, which are temporally quite different (*7*) and which do not seem to induce breaks that interact, and after treatments with KCN and β-propiolactone (*8*, *9*) that also do not result in interaction. In the latter case, however, one might expect a change in the temporal differences between the two compounds since the presence of KCN results in an inhibition of the rejoining system, thus increasing the probability of interaction (*1*). Interaction, however, is not indicated by the exchange frequency resulting from this combination treatment.

The argument that noninteraction results from temporal differences between breaks induced by different agents fails when the results of the combination treatments are viewed as a whole. The highest frequency of breaks induced by x-rays seems to be produced at a different time in the mitotic cycle than breaks induced by KCN (*8*), maleic hydrazide (*7*), and β-propiolactone (*9*). It would therefore be expected that the breaks induced by the radiomimetic compounds would have rejoined before the x-ray-induced breaks occur and would not be available for interaction with succeeding breaks. This is evidently not the case, however, since the combination treatments produce exchange frequencies indicating that interaction has occurred. It would clearly be a most complex situation if one must state that when interaction does not occur it is the result of temporal differences but when interaction does occur temporal differences are of no importance. The data herein presented are certainly equivocal in the sense that mitotic inhibition may well distort the results so that simple comparisons between single treatments and combination treatments are difficult. This may well be the case in the 8-ethoxycaffeine–maleic hydrazide combination, where the exchange frequency as well as the isochromatid frequency obtained is well below what is to be expected on and additive basis. It is not the case in the KCN–β-propiolactone combination, where the exchange frequency does not indicate that interaction has occurred although it is not only as high but slightly higher than additive.

It seems reasonable at the moment to conclude that interaction may not *only* be a function of time and space but also that it may occur only between the same or very similar kinds of breaks, and that those breaks involving very different kinds of bonds do not

Table 1. Comparison of the interaction of chromatid breaks induced by x-rays and radiomimetic compounds in different combination treatments. BPL, β-propiolactone; EOC, 8-ethoxycaffeine; KCN, potassium cyanide; MH, maleic hydrazide.

Treatment	Cells scored (No.)	Normal (%)	Abnormal (%)	Isochromatids (%)	Exchanges (%)		
					Observed	If additive	Calculated for interaction
X-rays	750	73	27	24	5		
KCN	650	71	29	25	7		
KCN + x-rays	1300	46	54	37	23	12	24
X-rays	750	73	27	24	5		
BPL	500	44	56	54	22		
BPL + x-rays	200	26	74	68	54	27	49
X-rays	750	73	27	24	5		
EOC	200	87	13	14	1		
EOC + x-rays	200	63	37	24	12	6	11
X-rays	750	73	27	24	5		
MH	200	35	65	71	20		
MH + x-rays	200	25	75	64	45	25	46
EOC	200	61	39	42	6		
MH	200	40	60	62	15		
EOC + MH	200	53	47	56	8	21	40
KCN	300	85	15	14	5		
BPL	500	44	56	54	22		
BPL + KCN	200	30	70	74	34	27	49

interact to any appreciable extent, if indeed they interact at all. What this means in terms of chromosome structure is not yet clear. It is clear, however, that it cannot be assumed on the basis of the available evidence that interaction occurs through a common repair system that is unable to distinguish one broken bond from any other. It is possible that the repair system cannot join two unlike bonds or that there is more than one system operating in the process we call rejoining.

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Fluorescence of Photosynthetic Organisms at Room and Liquid Nitrogen Temperatures

Abstract. Fluorescence spectra of algae and higher plants show two bands, ascribable to monomer and aggregate forms of chlorophyll. At low temperature, the long-wavelength emission is greatly enhanced and often appears as a new band. Photosynthetic bacteria, on the other hand, show no new bands at low temperature, within the spectral coverage and sensitivity of these measurements. A green fluorescence is also found in algae, which we attribute to carotenoids.

Fluorescence and absorption studies indicate that chlorophyll may exist in vivo in both monomeric and aggregated forms (*1–4*). Particularly strong evidence supporting this view has been given by S. Brody, who attributed a long-wave fluorescence appearing at low temperature to such an aggregate, on the basis of analogous behavior of concentrated chlorophyll solutions. It has been further suggested (*3–5*) that the decline of photosynthetic efficiency at long wavelengths, first observed by Emerson and Lewis (*6*), is due to preferential light absorption by this aggregated species. Other explanations have also been discussed for this long-wavelength decline (*7*, *8*). In this report, we present a summary of new experiments on the fluorescence of various photosynthetic organisms which further extend the considerations mentioned above.

Fluorescence spectra were determined at room and liquid nitrogen temperatures (*9*) for various algae (*Ochromonas danica, Chlorella pyrenoidosa, Euglena gracilis, Porphyridium cruentum*), photosynthetic bacteria (*Rhodospirillum rubrum, Rhodopseudomonas palustris, Chromatium d*), and leaves of higher plants (*Prunus virginiana, Betula papyrifera*). These forms were selected as representative of various plant pigment systems.

Figure 1 shows typical fluorescence spectra of *Ochromonas*. The room temperature chlorophyll spectrum consists of a single broad band, with maximum at 685 mμ. At low temperature, two bands are found, shifted from the room temperature peak toward longer wavelengths by about 3 mμ and 20 mμ respectively. The effects are reversible. The low-temperature, long-wave emission is again attributed to aggregated chlorophyll, while the band at shorter wavelength is assigned to the monomer. The slight shift in wavelength of this latter band with temperature is observed also in chlorophyll solutions in vitro (*3*), and is probably due to Franck-Condon effects.

In general, at low temperature (with, typically, 436 mμ excitation), the intensity of fluorescence from the chlorophyll aggregate relative to that from the monomer increases with increasing age of the algal culture or leaf, until a stable ratio is reached. In the case of *Ochromonas, Chlorella,* and the higher plants, the final relative (uncorrected) intensities of the two low-temperature bands are roughly equal (Fig. 1).

In another group of algae, the low-temperature aggregate emission in mature cultures far exceeds the monomer emission. This is the case, for example, in *Euglena* (Fig. 2) and *Porphyridium*. Direct measurements of absorption spectra of *Euglena* at room and liquid nitrogen temperatures show surprisingly little difference and rule out the possibility that the observed fluorescence changes are artifacts, due to selective reabsorption of fluorescent light. In these algae, we have confirmed the observations of French that, indeed, even the room-temperature fluorescence spectrum shows marked broadening, shifts in peak location, and appearance of shoulders, as the culture ages (*10*). Such effects, found to a lesser degree also in the other green

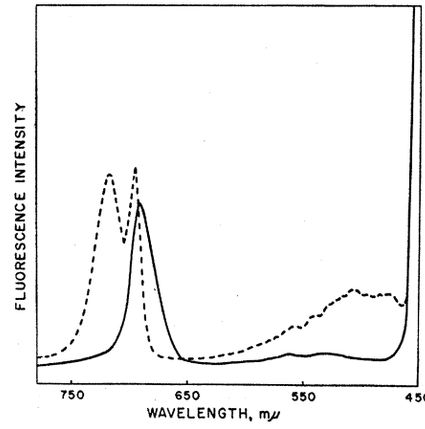

Fig. 1. Fluorescence spectra of 2-week culture of *Ochromonas danica*, at room temperature (solid line) and liquid nitrogen temperature (broken line). Excitation, 436 mμ. Spectra in Figs. 1, 2, and 3 are uncorrected for variation in photomultiplier sensitivity (Du Mont, 6911). Increased scattering, resulting from freezing of the algal suspension, makes the fluorescence yield at the lower temperature appear smaller than it actually is.

plants, may arise from a sufficiently strong aggregate emission in mature cultures to be observable even at room temperature. It is noteworthy that in *Porphyridium*, in which such large amounts of aggregated chlorophyll are found, the long-wavelength decrease in photosynthetic efficiency begins at about 650 mμ (*7*), whereas in algae of the first group, as typified by *Chlorella*, the decline sets in at about 685 mμ (*6*).

Several explanations are possible for the enhancement of long-wave fluorescence at low temperature. As pointed out earlier (*3*), cooling may cause an

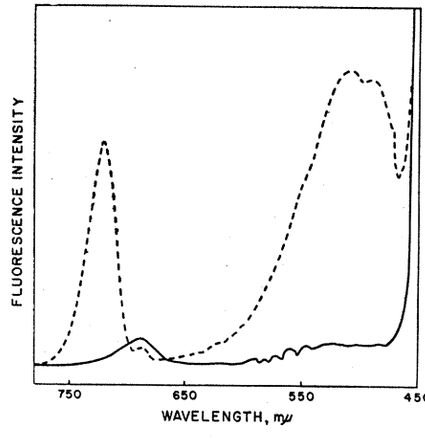

Fig. 2. Fluorescence spectra of 48-hour culture of *Euglena gracilis*, at room temperature (solid line) and liquid nitrogen temperature (broken line). Excitation, 436 mμ. In older cells the monomer band is difficult to detect because of the great height and breadth of the aggregate band.