

under similar conditions. This was further justified by the similarity of the two sets of data. The spectral response of *Drosophila* was determined at four intensities of each of a series of monochromatic lights. These relative intensities were 1, 1/10, 1/100, and 1/1000. The intensity of the light was altered through the use of sector disks rotated by an electric motor. The speed of rotation was well above the flicker fusion frequency for the human eye; this factor would not be expected to influence the character of the results, inasmuch as a constant rate of rotation was employed.

It is obvious from Fig. 1 that when the percentage response of *Drosophila* at each of the four intensities is plotted against the wavelength of monochromatic light, the wavelength of maximal response and the over-all spectral sensitivity of the eye shift toward the shorter wavelengths, just as in the Purkinje shift of vertebrates. In response to monochromatic light of the relative intensities of 1 and 1/10, the maximal response is recorded in the region of 520–540 m μ , whereas, with the relative intensities of 1/100 and 1/1000, the maximal response is observed in the region of 480–500 m μ . It is readily seen from Fig. 1 that the shift is a gradual one. The shift in maximum response of approximately 40 m μ is nearly as large as the shift occurring in vertebrates.

An equal energy spectrum was not used in the present investigation because such a spectrum is unnecessary for the demonstration of a Purkinje shift. Himstedt and Nagel (4) demonstrated the Purkinje shift in the retina of the frog using light of unequal energy content.

Furthermore, the results of Fingerman (3) have demonstrated the questionable desirability at this time of reducing the data of *Drosophila* involving responses to monochromatic light to energies yielding equal response. However, if this were done, it would not be expected to alter the relative difference obtained between the wavelengths of maximum response at high and low intensities.

A shift of this sort can only be explained by a Duplicity Theory, the presence of two distinct photoreceptive mechanisms with photoreceptive pigments. Such a shift is not entirely unexpected because of the presence of two types of photoreceptor cells in the compound eye of *Drosophila*.

Drosophila has an apposition eye, which is better adapted for bright-light than for dim-light vision. This shift toward the shorter wavelengths with decrease in the intensity of the light stimulus is undoubtedly due to a visual pigment, different from that predominating in bright light, that can function in dim light. Such a shift of the spectral sensitivity of the eye toward the shorter wavelengths would also tend to increase the relative efficiency of the eye in dim light as compared with the efficiency in bright light, because it is at these shorter wavelengths that the eye pigments absorb most strongly light that has entered an ommatidium obliquely, resulting in an increased localization on the retina of the image of the orienting beam (3). This is the first description

of a Purkinje shift occurring in the compound eye.

Experiments are now in progress to determine whether *Drosophila* has a true color vision and whether this is associated with one or both of the two types of photoreceptive cells.

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The Protective Effect of Glutathione against Radiation-induced Chromosome Aberrations¹

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It has been known for some time that the sensitivity of chromosomes to breakage by ionizing radiation can be modified by the amount of oxygen present. Thoday and Read (1) showed that a decrease in the frequency of chromosome aberrations occurred when root tips of *Vicia faba* were exposed to x-rays in an atmosphere of nitrogen as compared to root tips exposed in the presence of oxygen. Hayden and Smith (2) demonstrated that barley seeds x-rayed in a vacuum gave a lower frequency of chromosomal aberrations than seeds x-rayed in air. Similar results were obtained by Giles and Riley (3, 4) in *Tradescantia* microspores, where a marked decrease in chromosome aberrations was obtained when the oxygen tension was decreased to 2% or lower.

Using survival as the criterion, Hollaender *et al.* (5) were able to decrease the susceptibility of *Escherichia coli* suspensions to x-rays by lowering the oxygen tension. Burnett *et al.* (6) showed that the sensitivity of *E. coli* to x-rays also could be reduced by the presence of chemical agents such as certain sulfhydryl compounds, glycols, and alcohols. These results suggest that chemicals also might be effective in reducing chromosome aberrations caused by irradiations. Baron *et al.* (7, 8) demonstrated an inhibition of the action of certain sulfhydryl-containing enzymes after exposure to x-rays and α -particles and showed that reduced glutathione had the ability to reactivate partially the irradiated enzymes. Glutathione has been used for the treatment of radiation sickness (9) and x-rays burns (10) and has been shown to increase survival and to limit loss of weight in irradiated mice (11) and other animals (12).

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In the present study *Tradescantia paludosa* cuttings were used as experimental material to determine the effect of glutathione on the frequency of radiation-induced chromosomal aberrations. Several methods were tried to initiate root development and growth. The best method involved placing the cuttings in tap water with continual aeration at ordinary greenhouse temperature (65°–70° F). When the cuttings showed good development of primary roots, they were transferred to especially designed Lucite containers containing Hoagland and Snyder's nutrient solution (13). The containers were 30 × 2 × 12 cm, and the side of the container facing the source was 3 mm thick. After 24 hr in the nutrient solution, the cuttings were transferred to fresh nutrient solutions containing the various concentrations of reduced glutathione. After 1 hr preabsorption of glutathione, the plants, still in the glutathione solution, were exposed to continuous γ -irradiation from a 3 curie Co⁶⁰ source for 48 hr. The Co⁶⁰ source was located in a cylindrical stainless steel container with a wall thickness of 0.8 mm on the front side. The side walls were 1 mm thick. This source holder was placed in the center of a spherical lead pig with a cone-shaped opening for the beam. Dosages, as measured by the film badge method (14), indicated that the plants received approximately 25 r/day, and hence a total dose of 50 r.

The effects of the irradiation and treatment with glutathione were determined by counting the number of chromosome fragments and bridges in root tip anaphases. The root tips were fixed immediately after the radiation exposure in Carnoy's fixative (3:1), and slides were prepared using the acetocarmine squash technique. The experiments included concentrations of glutathione ranging from 1×10^{-5} to 1.5×10^{-3} M. None of these concentrations seemed to cause any serious disturbances in the roots not subjected to radiation. At a higher concentration, 3×10^{-3} M, which also was tried, the roots became soft and were obviously injured, and the meristematic tissue showed no mitotic activity. These roots were unsuitable for study.

The frequencies of fragments and bridges were calculated as the number of aberrations/100 anaphase cells (Table 1).

TABLE 1
THE EFFECT OF DIFFERENT CONCENTRATIONS OF
GLUTATHIONE ON FREQUENCIES OF CHROMO-
SOME FRAGMENTS AND BRIDGES AFTER
CHRONIC γ -IRRADIATION

Conc of GSH added	Total No. ana- phases	No. frag- ments	Cor- rected No. frag- ments/ 100 cells	No. bridges	Cor- rected No. bridges/ 100 cells
None	661	115	17.40	20	3.03
1×10^{-5} M	250	42	15.20	2	.63
1×10^{-4} M	235	29	11.17	2	.67
3×10^{-4} M	474	40	8.63	5	1.03
1.5×10^{-3} M	298	25	8.13	5	1.74

In Table 1 results from three different experiments are pooled. One control series was included in each experiment, but the complete range of concentrations of glutathione used could not be included in all experiments. There was a small variation in the frequencies of fragments and bridges in the several control series. In Table 1 the relative frequencies of fragments and bridges in each glutathione series, when compared with its own control series, have been adjusted to the same relative frequencies of the combined control series.

Chronic γ -radiation (without glutathione) gave 17.40% fragments (Table 1). With 1×10^{-5} M glutathione there was a slight but not significant decrease in the frequency of fragments to 15.20%. A more pronounced and significant effect was obtained with 1×10^{-4} M, which decreased the fragment frequency by about one third, to 11.17%. At a concentration of 3×10^{-4} M the number of fragments decreased to 8.63%, which is a reduction of more than half. The maximum decrease was apparently reached around this value, since at 1.5×10^{-3} M, a fivefold increase in concentration, there were 8.13% fragments, a frequency that is only slightly less and not significantly different from that of the next highest concentration used.

Reunions resulting in bridge formations at anaphase were infrequent (Table 1). Following chronic γ -irradiation in the absence of glutathione, they occurred with a frequency of only 3.03%. The two strongest concentrations of glutathione applied (3×10^{-4} M and 1.5×10^{-3} M) gave bridge frequencies of 1.03 and 1.74%, respectively. This is apparently a decrease of approximately the same degree as for fragmentation. The values for the two lowest concentrations, 1×10^{-5} M and 1×10^{-4} M, were .63 and .67% of cells with bridges, apparently a much more pronounced decrease than for the two strongest concentrations. No definite conclusions, however, can be reached on the basis of these data, since the frequency of bridges is so low.

The data show conclusively that the frequency of fragments induced by chronic γ -radiation at 25 r/day is decreased when irradiation is given in the presence of glutathione at a concentration of 1×10^{-4} M or higher. There are two possibilities for explaining the mechanism of the glutathione effect: (a) glutathione may either protect the chromosomes from breakage, or (b) may have a stimulating effect on restitution. The first possibility seems more likely. Barron and Flood (15) have shown that glutathione is readily oxidized by ionizing radiation in aqueous solution. The oxidation takes place at the —SH groups and proceeds efficiently, although not to any great extent with small doses of γ -radiation. Weiss (16) and others have stated that the interaction of ionizing radiation with water leads to the formation of OH and O₂H radicals, H₂O₂, and atomic oxygen. It seems likely, therefore, that glutathione is oxidized by these oxidizing agents. Since water is present in all organisms, the same kind of ionization products should be formed within cells.

The protective ability of glutathione may therefore be due to its reactions with the oxidizing agents produced by irradiation. Such reactions would decrease the amount of reactive ionization products available for reactions with the chromosomes and result in fewer breakages.

Apparently, a maximum protective effect was obtained at the two highest concentrations, but it is not known whether the maximum occurred because the glutathione reacted with all the oxidizing agents produced by the radiation or whether the maximum possible absorption of glutathione in the cell nuclei was obtained at the second highest concentration used ($3 \times 10^{-4} M$). In the absence of oxygen the aberration frequency was reduced to one third in some experiments (4). This may indicate that the optimal effect of oxidizable chemicals on chromosome aberrations is not obtained with glutathione, which gave a reduction of about one half in these experiments. If these arguments are correct, however, the results indicate the importance of the indirect effect of the oxidizing reactants produced in water by radiation in the process of chromosome breakage. The data presented are preliminary, and further work is being conducted on various aspects of the problem.

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Absorption Spectra and Isomerization of the Chlorophylls

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In the search for clues to the mechanism of photosynthesis, several isomerization reactions and numerous variations of the absorption spectra of the chlorophylls have been observed. These isomerizations and spectral changes take place in killed plant tissue and in extracts of plants. They have not been observed during the photosynthetic utilization of sunlight by living plants (1, 2).

The wavelength of the absorption maxima of the

chlorophylls depends upon the physical state or condition of these pigments. In living organisms, in many colloidal suspensions, and in the solid state, chlorophyll *a*, the principal photosynthetic pigment, exhibits a spectral absorption maximum near 680 mμ (2). But in solution in organic solvents, this chlorophyll exhibits a spectral absorption maximum at shorter wavelengths (about 660-673 mμ, depending upon the solvent). The similarity between the spectral properties of chlorophyll *a* in living plants and in colloidal dispersions indicates that this green pigment may occur naturally in colloidal form (2). In colloidal suspensions prepared by grinding plant tissues, however, the chlorophyll is always associated with carotenoid pigments and with various colorless substances such as proteins and fats. Photosynthetic activity has been observed only in living plants in which the chlorophyll occurs in this special labile association (2, 3).

Reversible isomerization reactions of the chlorophylls take place spontaneously in solutions of the pigments (4). These isomerizations are not accompanied by significant spectral changes. Chlorophyll *a* yields the similar chlorophyll *a'*. Chlorophyll *b*, the minor green pigment of green algae and of higher plants, also yields a similar interconvertible isomer, chlorophyll *b'*. These isomeric chlorophylls are usually detected and isolated by chromatographic adsorption in columns of powdered sugar.

Spectral changes of the chlorophylls occur when the solutions in hydrocarbon solvents are cooled (5). With decreasing temperature, the maxima are shifted gradually to longer wavelengths, from about 663 mμ at 293° K to 675 mμ at 75° K for chlorophyll *a*, and from about 643 mμ at 293° K to 660 mμ at 75° K for chlorophyll *b*. These shifts of the spectral absorption maxima have been ascribed to a reversible isomerization of the chlorophylls, the nature of the isomers being unknown.

Pronounced shifts of the spectral absorption maxima of the chlorophylls dissolved in petroleum ether plus methanol have now been observed when the alcohol is removed from the solutions. These spectral shifts, which are influenced greatly by the presence of colorless impurities and by the solvent itself, may be greater than those observed when the usual preparations of chlorophyll are dispersed in water, or when solutions of the chlorophylls in hydrocarbons are cooled. The shifts produced by the removal of alcohol from petroleum ether solutions of the highly purified chlorophylls are due to variation of the physical state of the pigments, not to the formation of isomeric substances.

For observation of these spectral shifts, the chlorophylls and their isomers were extracted from heated leaves and were separated by chromatographic adsorption with powdered sugar as adsorbent and with freshly washed and distilled (bp, 35°-40°) petroleum ether plus 0.5% propanol as solvent. The chlorophylls, separated in the column as four green zones, were eluted from the respective portions of the sugar with the low-boiling petroleum ether containing about