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PARTIAL DOCUMENT

# INDIRECT EFFECTS OF RADIATION ON GENETIC MATERIAL*

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The pioneer work following Muller's discovery (1) that x-rays would produce mutations seemed to point to a simple direct relation between ionizing radiation and genetic changes (2). It was soon found that mutations and chromosomal abnormalities showed differences. Further, the work with ultraviolet early demonstrated that different wave lengths differed in their effectiveness in producing mutations because of differential penetration and differential absorption by genetic material (3-7). The mutation rate was found to be directly proportional to the dose of x-radiation by Oliver (8), Timoféeff-Ressovsky (9), and others. Muller (10) concluded from his evidence that the production of translocations in *Drosophila* was independent of temperature, wave length or intensity of radiations, and interval spacing of treatment. However, Sax (11) had shown that there was a time factor in the production of chromosome aberrations, while Sax and Enzmann (12) and Mickey (13) had demonstrated an effect of temperature on rearrangements in *Tradescantia* and *Drosophila.*

The development of new and improved genetic tools and experimentation, including studies of genetic effects in microorganisms, and the great development in sources of radiations as well as knowledge of their physical and chemical properties have changed our views considerably. We have found that many of the biological systems of the organism are involved in determining the amount and kind of radiation damage, and these factors increase greatly our ability to study modifications of the genetic system.

It was simplest to interpret data as direct damage in terms of a target theory when the only proven mutagens were radiations. Auerbach and Robson (14) and Auerbach (15, 16) demonstrated that mustard gas caused both mutation and chromosome rearrangements in *Drosophila.* Rapoport (17) and Kaplan (18) showed that formaldehyde was mutagenic when fed to *Drosophila* males during development. This was not effective in producing mutations in the females under these conditions (15, 16, 19). Jensen, Kirk, Kølmark, and Westergaard (20) list a number of chemicals that they and other workers have demonstrated to be mutagenic in microorganisms. Oehlkers (21) produced chromosome abnormalities in plants with ethyl urethane, and other such agents have now been found. Levan (22) reported a number of substances with such capacities which he and other workers have discovered.

Lea (23) discussed the indirect effects of radiations, especially the effects in water, emphasizing the pioneer work of Fricke and his associates

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(see Lea's discussion and bibliography). In fact, Fricke and Demerec (24) very early ascribed much of the damage of the genetic systems by radiation to indirect rather than direct effects. In the time that has elapsed since this work, much information has been obtained on the indirect effects of radiations. Much of the evidence has been reviewed in several recent publications, especially in "Radiation Biology" (25, 26). Reference will be made to these very complete review articles and to some of the extensive work of this period, as well as to more recent papers.

Latarjet and Gray (27) describe the chain of events in radiobiologic damage as follows:

| 1 | | 2 | | 3 | | 4 |
|---|---|---|---|---|---|---|
| Absorption of radiant energy | → | Primary radiochemical reactions | → | Chemical reactions chains | → | Observable lesion |

latent period

> In the first step, the distribution of the absorbed energy within the cell is determined by the chemical structure of the cell and the type of radiation to which it is exposed. The absorbed energy will be distributed among the various possible modes of vibration of the molecules within a time of the order of $10^{-13}$ sec, to be followed by the second step which is, in most cases, probably complete in times of the order of $10^{-9}$ sec. At this stage, part of the energy is in the form of very reactive and very short-lived radicals, which in turn give rise to intermediates of longer life, and to reversible and irreversible chemical changes, which we have designated as the third step. Steps 2 and 3 together comprise the latent period, and it is probable that in many cases the course of the changes which take place during steps 2 and 3 will be affected by the chemical composition of the living protoplasm. Those changes which constitute step 3 will also be conditioned by the active metabolism of the cell, and therefore by environmental factors such as temperature and nutrients.

The observable lesion in genetic analyses is a genetic change or lethal effect under certain conditions. The systems have been studied by modifying 1) the type of radiation, 2) the chemical composition of the cell or organism, and 3) the activities of various metabolic systems.

Different types of radiation produce measurably different effects on the genetic system. Stadler and Roman (28, 29) stressed the differences between the results of irradiation with ultraviolet and with x-rays. Both types of radiation produce mutations, but ultraviolet produces chromosome abnormalities very seldom. Fabergé (30) showed that ultraviolet broke maize chromosomes just as do x-rays. In fact, it may shatter chromosomes into small fragments on occasion, as shown by Lovelace (31). Swanson and Stadler (32) reviewed the effects of ultraviolet on the genetic system, particularly the differences between these effects and those of x-rays, as well as wave length differences in penetration and effectiveness. As they noted, a dose of ultraviolet produces many mutations and small deficiencies but few chromosome rearrangements, compared to an equivalent dose of x-rays, based on an equal

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number of mutations, which would produce an appreciable number of gross structural changes in the chromosomes. These data indicate that the sum of the effects of ultraviolet radiation on the chromosome is not the same as that from x-rays.

Neutrons and alpha particles differ from x-rays in their genetic effects (23). The ionization density along the tracks of alpha particles and neutrons, or protons ejected by neutrons, is much greater than that effected by x-rays. For a measured amount of ionization, the effects of the charged particles are much more concentrated in certain regions of the irradiated tissue, and this causes differences in their effectiveness measured in genetic damage.

Muller (2), Kaufmann (33), and Giles (34) reviewed the information on the induction of mutations and chromosome abnormalities in animals and plants. The results with neutrons will be used, since alpha particles do not penetrate into tissue sufficiently for use in all types of studies. More recent work by Giles, de Serres, and Beatty (35) showed that the number of chromosomal abnormalities produced in *Tradescantia* is directly proportional to dosage. The frequency of rearrangements produced by x-rays is reduced in *Tradescantia* by change in intensity or by intermittent treatment, but neutron effects are not modified. Lewis (36) and Muller (2) showed that the frequency of chromosome rearrangements produced by neutrons in *Drosophila melanogaster* is directly proportional to dosage. Stone, Alexander, Clayton, and Dudgeon (37) found the same results with *Drosophila virilis*. Because of its chromosome complex of five pairs of rods and a dot, *virilis* is a much more useful organism for measurement of translocation rate than *melanogaster* which has a rod, two pairs of V-shaped chromosomes, and a dot. Many more of the rearrangements can be scored as translocations in *virilis*. For example, Muller (2) found about 10 percent translocations for 1500 rep, whereas Stone *et al* found over 50 percent at 1600 rep (37).

Figure 1 from the latter paper shows the relation between neutron dosage from two nuclear detonations and translocation frequency -- a

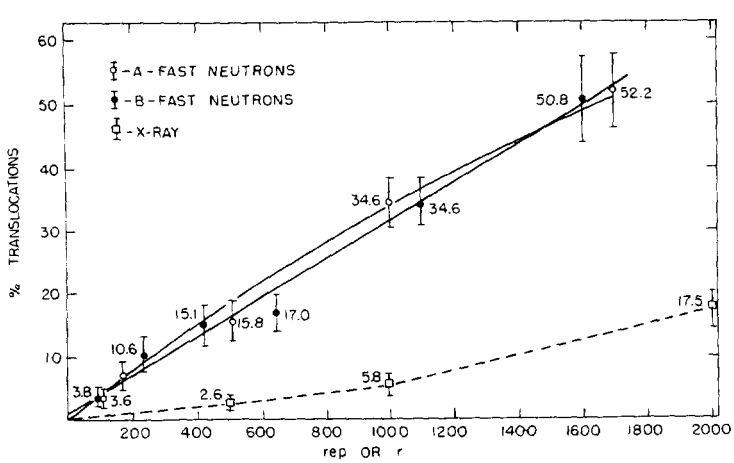


Figure 1. Relation between neutron dosage and translocation frequency.

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direct proportionality to dosage. The straight line is the best fit to the data by least squares. The curve is based on the fact that we cannot distinguish a second hit on a sperm nucleus by the proton produced by the neutron. The curve is calculated from the four low doses, which should represent single hits, to show the additional undamaged nuclei which should be hit by each additional 100 rep. Muller (2) suggested that the overlapping of the chromosomes in the *Drosophila* sperm head would allow several breaks in separate chromosomes for the passage of each proton. The fact that the passage of a single proton can cause so much damage because of the heavy ionization that translocations (which require at least two separate breaks) regularly result from its passage indicates that secondary indirect effects would be difficult to demonstrate. Even so, Gustafsson and his collaborators have demonstrated such effects in a series of papers (38-45).

Ultraviolet and x-rays remain as better agents with which to demonstrate indirect effects from radiation. Many workers using microorganisms have come to the conclusion that much of the effect of ultraviolet on microorganisms is indirect (46-48). Zelle and Hollaender (48) review the literature on bacterial work and give the evidence for this conclusion. Stone, Wyss, and Haas (49) obtained the initial evidence that ultraviolet produced chemical mutagens in bacterial media capable of inducing mutations in unirradiated organisms placed in this media within a short time (less than four hours). Wyss, Clark, Haas, and Stone (50) showed that organic peroxides formed by the action of $H_2O_2$ on broth were mutagenic for bacteria, although $H_2O_2$ was not mutagenic in saline. Wagner, Haddox, Fuerst, and Stone (51) found that irradiated broth and $H_2O_2$ were mutagenic to *Neurospora crassa,* and Dickey, Cleland, and Lotz (52) demonstrated that organic peroxides and $H_2O_2$ were effective mutagens in this organism. Recently Altenburg (53) demonstrated that organic peroxides produce mutations in *Drosophila.* Wyss and his students have been studying the production of peroxide under different conditions; for example, Schaiberger (54) has been testing some unsaturated fatty acids. Irradiation of distilled water with the ultraviolet lamp used produced about 1 ppm $H_2O_2$. Irradiation of water solutions of substances like crotonate (0.1 percent), oleate (0.05 percent), linoleate (0.1 percent), glycerol (1.0 percent), or Tween "80" (1.0 percent) increased the peroxide formed up to 25 to 50 ppm. These solutions increased the incidence of phage and streptomycin-resistant mutations 25 to several hundred times over that of the control unirradiated solution.

Furthermore, Wyss (55) showed that irradiation of a fatty acid ester of a sugar alcohol produced a high concentration of peroxide in the 2600 Å range of ultraviolet. This type of substance is found in the lipid fraction of microorganisms. Therefore, ultraviolet and x-rays, if oxygen is present, will form peroxides and active radicals with various cell constituents which will contribute to cell damage, including mutation. The importance of these peroxides is illustrated by Latarjet (56) from the fact that catalase and peroxidase protect part of the cells from being killed if added to a cell suspension shortly after irradiation. Latarjet shows that these are organic peroxides. He regards the action of catalase and peroxidase as part of a coupled oxidative process with some of

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the cellular substances which divert the radio-lesion process to a more innocuous pathway.

The more important series of investigations of the indirect effects of x-rays stems from the demonstration by Thoday and Read (57) that radiation damage was influenced by the presence of oxygen. These authors showed that the frequency of chromosome aberrations was markedly reduced in *Vicia faba* if air was replaced by nitrogen. They also showed that the amount of oxygen did not influence the effect of alpha particles as much as that of x-rays (58). One of the important points demonstrated by Giles, Riley, and Beatty (59-65) in a series of publications is that the presence and amount of oxygen *during* irradiation, not pre- or post-treatment, determines the amount of radiation damage. Even in a vacuum there are some rearrangements produced by x-rays. Furthermore, there is an oxygen effect with fast neutrons but of considerably smaller magnitude. Baker and Edington (66) showed that oxygen concentration influenced the number of translocations and recessive lethals in *Drosophila* so that the lower the amount of oxygen present, the fewer the abnormalities produced for a given dose of x-rays. Recessive mutations are a mixture of types of change, but in *Drosophila* x-ray mutants resemble spontaneous mutants.

Muller (2) discussed the problems of spontaneous and induced mutations. Kaufmann (33) and Giles (34) discussed radiation-induced rearrangements. Kaufmann discussed pre- and post-treatment with infrared and other agents. He and his co-workers demonstrated that pretreatment with infrared increases the frequency of chromosome rearrangements in *Drosophila*. However, Kaufmann and Gay (67) and Young, Yost, Ives, and Levine (68) did not find a similar increase of mutations due to infrared pretreatment. These results and those of Swanson and Yost (69), Yost (70), and Kaplan (71) indicate that factors other than the direct or indirect action of radiations may contribute to the amount of damage. It is undetermined whether these procedures modify the direct effect of radiation or their agents (the indirect effects of radiation) or modify metastable states of the genes and chromosomes as these last authors suggest.

The effect of $H_2O_2$ and the direct and indirect effects of ultraviolet can be countered in part by the iron-porphyrin enzymes, catalase, and peroxidase (50, 51, 56). In addition, the cytochrome system has been shown to protect from x-ray damage. This was shown for chromosome abnormalities in *Tradescantia* by King, Schneiderman, and Sax (72) and for *Drosophila* by Haas, Dudgeon, Clayton, and Stone (73). The radiation damage is increased if cytochrome oxidase is blocked with carbon monoxide, a light reversible reaction. King *et al* (72) had used 5 atmospheres pressure (4 CO + 1 air, etc.), but Gray (74) found that he had to raise the pressure to between 50 and 60 atmospheres to obtain similar results with *Vicia*. He obtained under optimum conditions an increase in sensitivity by a factor of 1.8, about the same as with *Tradescantia*. No positive pressure is necessary with *Drosophila* (73, 75). Oxygen interfered with the CO effect in the plant material but did not show such a counter action in *Drosophila*. King, Schneiderman, and Sax (72) and Gray (74) agree that the CO inhibits the cytochrome system, resulting in the reduction of

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molecular oxygen by the flavoproteins. Carbon monoxide inhibition may also increase damage by interfering with the energy system from the Krebs' cycle (75). Before going into that evidence, we shall show that cytochrome oxidase protects the organism against radiation damage measured as mutation.

De Busk and I have used a back-mutation test similar to the one described at this conference by Giles (76). Two stocks were used, an *adenineless colonial* and *adenineless colonial poky.* Poky is a cytoplasmic mutant which Mitchell and his co-workers (77-81) have shown lacks cytochrome oxidase and depends on an alternate terminal oxidase system. The effect of CO was tested by holding the two strains in CO for 30 minutes and reversing the CO effect by treating part of the cells in intense white light, then irradiating cells with all types of treatments. The light breaks the CO - cytochrome complex and is not a photoreactivation of ultraviolet damage as it was used first. Figure 2 shows the results of such a series of experiments. The *poky* strain is not affected by CO as would be expected if the CO effect were on the cytochrome oxidase system.

Glass (82) discussed radiation damage in relation to cell cycle. I shall consider some of the new work of Alexander and Stone (118) and our earlier experiments (75) in relation to the indirect effects of x-rays. Patterson, Brewster, and Winchester (83) showed that dominant lethals were more frequent among the eggs laid by x-rayed females during the first 24 hours than on the third day. King (84) confirmed these findings. Whiting (85), using *Habrobracon juglandis* females, and Bozeman and Metz (86), using *Sciara ocelaris,* showed that metaphase or metaphase and anaphase were the most x-radiation-sensitive periods in the meiotic cycle in these *Diptera.* Sparrow (87), in his extensive work on *Trillium erectum,* showed that late prophase through anaphase was the sensitive period in that

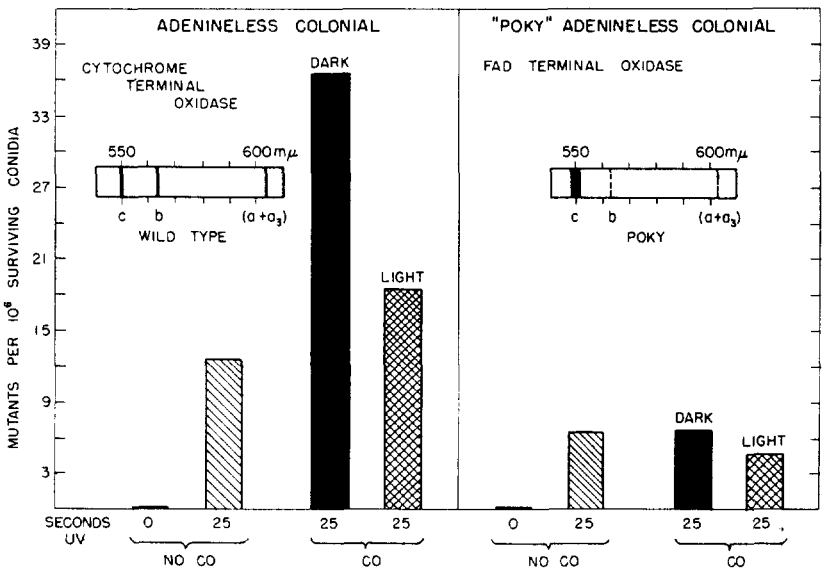


Figure 2. Relation between cytochrome oxidase and radiation damage measured as mutation.

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plant. In a series of papers, Bonnier and Lüning (88-92) showed that there was a difference in x-ray sensitivity at different stages in spermatogenesis with postmeiotic spermeogenesis the most sensitive period. Bonnier (93) and Lüning (94) extended our information by showing that there were more breaks produced when sperm was irradiated in the females' sperm receptacles than when males were irradiated.

Auerbach (95) demonstrated the x-ray susceptibility measured as mutations of the different stages of spermatogenesis. She used crossing over in the male to determine the time of meiosis in *Drosophila melanogaster.* In this species spermatogonia (brood *d*), which produce multiple identical recombinants, are followed by single and double recombinants with excessive sterility (brood *c*), which includes meiosis, followed by single recombinants or mutations (brood *b*). The stages are given in reverse order from the recovery of offspring in successive three-day periods (*a, b, c, d*). The number of lethals was highest in *b*, followed by *a, c*, and *d*, in that order. Sobels (96) confirmed this cycle for lethal mutations. He showed also that cyanide and azide increased the number of x-ray-induced mutations, especially in the sensitive period.

*Drosophila virilis* has a longer life cycle and matures more slowly than *melanogaster.* In our study of the cycle, males 19 to 21 hours of age were irradiated, then mated to three mature females for five days (no offspring are produced during this period), and then mated to three virgin females every two days. The females included one normal and two marker females. After separation, the eggs from the normal female were counted for four days, and the number of pupae to develop indicated the number of dominant lethals. The progeny of the marker females were backcrossed to stock marker females, and the translocations present were scored genetically. This gave the dominant lethals and translocations for each two-day period, A through H. The results are given in Table 1, which includes experiment 12 published earlier (75).

To find out if there was a period when the x-radiation reduced the number of sperm produced, freshly laid eggs were checked for the presence of sperm by means of the procedure devised by Patterson, Stone, and Griffen (97). In stages A through F, over 92 percent of the eggs had been fertilized; in G only 36 percent; and in H only 81 percent. Clearly only the last spermatogonia and meiotic stages failed to produce sperm. When the results of Lüning (92), Auerbach (95), Whiting (85), and Bozeman and Metz (86) are considered along with out data using *virilis,* the susceptibility of the different stages in spermatogenesis is well established.

Our results are given in a series of graphs -- Figures 3 to 9. The figures are given in reverse order from the results to show spermatogonia on the left and mature or nearly mature sperm on the right. Figure 3 shows a test of translocation frequency (the percent of sperm with one or more translocations) and dominant lethals, using 1000 r of x-rays in $O_2$ during one minute at $1^o$ to $3^oC$ (250 kv, 15 mamp, inherent filtration equivalent to 0.3 mm aluminum). At the end of the radiation, the flies of test 13b were transferred to a similar treatment chamber with CO flowing through it and post-treated for half an hour. The other test, 13a, was continued in $O_2$. There is no significant difference in the results of the two tests. Haas, Dudgeon, Clayton, and Stone (73) showed

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Table 1

The effect of dosage and gas post-treatment on the irradiation damage of the developing germ cells of *Drosophila virilis*.*

| Experiment | Periods** | Percent $P_1$ males fertile | Type of translocations $T_2$ | $T_3$ | $T_{2+2}$ | $T_4$ | $T_{2+3}$ | $T_5$ | Sperm with trans-locations | Total sperm tested | Percent sperm with trans-locations ± 2 SD | Dominant lethals Eggs | Pupae | Percent lethals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13a | A | 86 | 21 | 3 | 1 | | | | 25 | 342 | 7.3 ± 2.5 | 3758 | 2233 | 40.6 |
| | B | 100 | | | | | | | | | | 5972 | 3133 | 47.5 |
| 1000 r | C | 100 | 49 | 13 | 2 | 3 | | | 67 | 240 | 27.9 ± 5.9 | 3892 | 818 | 79.0 |
| Gas - $O_2$ | D | 99 | 38 | 12 | 6 | 2 | | | 58 | 218 | 26.6 ± 6.0 | 5399 | 574 | 89.4 |
| Post- | E | 89 | 19 | 15 | 2 | 5 | | | 41 | 79 | 51.9 ± 11.9 | 4781 | 189 | 96.0 |
| treatment | F | 67 | 1 | 1 | | | | | 2 | 63 | 3.2 ± 2.4 | 3171 | 163 | 94.9 |
| $O_2$ | G | 68 | | | | | | | | | | 2678 | 274 | 89.8 |
| | H | 89 | | | | | | | | 143 | 0.0 | 2068 | 1009 | 51.2 |
| 13b | A | 80 | 24 | 1 | | | | | 25 | 306 | 8.2 ± 3.0 | 2966 | 1866 | 37.1 |
| | B | 93 | | | | | | | | | | 5545 | 2617 | 52.8 |
| 1000 r | C | 97 | 48 | 7 | 1 | 3 | | 1 | 60 | 245 | 24.5 ± 5.5 | 4244 | 744 | 82.5 |
| Gas - $O_2$ | D | 97 | 58 | 14 | 1 | 4 | 2 | 1 | 80 | 222 | 36.0 ± 6.5 | 5411 | 599 | 88.9 |
| Post- | E | 95 | 36 | 16 | 3 | 6 | | | 61 | 106 | 57.5 ± 9.7 | 5690 | 203 | 96.4 |
| treatment | F | 62 | 1 | | | | | | 1 | 56 | 1.8 ± 3.6 | 2405 | 124 | 94.8 |
| CO | G | 65 | | | | | | | | | | 2236 | 185 | 91.7 |
| | H | 80 | 1 | 1 | | | | | 2 | 140 | 1.4 ± 2.6 | 2397 | 1036 | 56.8 |
| | | 70 | 15 | | | | | | 15 | 294 | 5.1 ± 2.6 | 3835 | 2542 | 33.7 |

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| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | A | 79 | 15 | | | | | | 15 | 294 | 5.1 ± 2.6 | 3835 | 2542 | 33.7 |
| | B | 87 | 14 | | | | | | 14 | 286 | 4.9 ± 2.6 | 5513 | 3536 | 35.9 |
| 500 r + 500 r | C | 91 | 39 | 5 | 2 | | | | 46 | 266 | 17.3 ± 4.7 | 5480 | 1651 | 69.1 |
| $O_2$ | D | 92 | 55 | 11 | | 3 | | | 69 | 273 | 25.3 ± 5.2 | 6386 | 914 | 85.7 |
| | E | 81 | 9 | 6 | 2 | 1 | | | 18 | 47 | 38.2 ± 14.6 | 4334 | 228 | 94.7 |
| | F | 83 | 1 | 1 | 1 | | | | 3 | 69 | 4.3 ± 4.9 | 2018 | 262 | 87.0 |
| | G | 67 | | | | | | | | | | 2823 | 446 | 84.2 |
| | H | 85 | 1 | | | | | | 1 | 112 | 0.9 ± 1.8 | 3638 | 1543 | 57.6 |
| 14 | A | 94 | 2 | | | | | | 2 | 303 | 0.7 ± 1.0 | 4585 | 3533 | 22.9 |
| | B | 91 | 10 | | | | | | 10 | 297 | 3.4 ± 2.0 | 4996 | 4115 | 17.6 |
| 500 r | C | 96 | 11 | | | | | | 11 | 253 | 4.3 ± 2.6 | 5463 | 3513 | 35.7 |
| $O_2$ | D | 94 | 25 | 2 | | | | | 27 | 333 | 8.1 ± 3.0 | 6047 | 2999 | 50.4 |
| | E | 93 | 24 | 6 | 1 | | | | 31 | 236 | 13.1 ± 4.4 | 4890 | 1424 | 70.9 |
| | F | 93 | 4 | | | | | | 4 | 263 | 1.5 ± 1.6 | 3181 | 1602 | 49.6 |
| | G | 90 | | | | | | | | | | 3544 | 2153 | 39.2 |
| | H | 91 | | | | | | | | 111 | 0.0 | 2633 | 1927 | 26.8 |
| 12 | A | 73 | 62 | 6 | 2 | | | | 70 | 525 | 13.3 ± 3.0 | 15198 | 4106 | 73.0 |
| | B | 89 | 111 | 17 | 7 | 4 | | | 139 | 574 | 24.2 ± 3.6 | | | |
| 2000 r | C | 33 | 14 | 8 | 1 | 1 | 1 | | 25 | 47 | 53.2 ± 14.5 | 19731 | 147 | 99.3 |
| $O_2$ | D | 29 | 2 | 2 | 1 | 3 | | 1 | 9 | 13 | 69.2 ± 25.6 | 21456 | 127 | 99.4 |
| | E | 26 | 4 | 1 | 1 | 1 | | 1 | 8 | 8 | 100.0 | 9688 | 22 | 99.8 |
| | F | 7 | | | | | | | | 1 | 0.0 | 8479 | 117 | 98.6 |
| | G | 8 | 1 | | | | | | 1 | 27 | 3.7 ± 7.2 | 9111 | 2615 | 71.3 |
| | H | 69 | 2 | | | | | | 2 | 150 | 1.3 ± 1.8 | | | |

*All flies were irradiated at 1° to 3°C when 18 to 21 hours of age.

**Age, in days, of male at mating: A = 6 to 7; B = 8 to 9; C = 10 to 11; D = 12 to 13; E = 14 to 15; F = 16 to 17; G = 18 to 19; H = 20 to 21

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that irradiation at this intensity produced more translocations than irradiation at 100 r or less per minute. This difference might be due to a greater concentration of reactive radicals or to restitution in the slow treatment. Muller (10) showed that reattachment in a new arrangement did not occur in sperm until after fertilization. If restitution occurs, it must be too rapid for the CO enzyme poisoning to block it.

Figure 4 compares translocation and dominant lethal rates of a test at 500 r with a test of the 500 r plus a second 500 r given after about one minute's delay to remove the first 500-r sample. In Figure 5, the translocation rates in these five experiments are compared. The peak rate for each test is in stage E which follows after meiosis. Figure 6 shows the corresponding dominant lethal tests. The lethal dominant rate is high during stages G and F which include meiosis, although the recoverable translocations are few. This is the expected result from the cytological determinations by Whiting (85) and Bozeman and Metz (86). Figures 7 and 8 are plotted from the data to show dosage relations in these various stages. Figure 7 shows the information on dominant lethals. The values for series A, which is nearest to mature sperm, might be considered as a case of simple increase of damage to the genetic material with dosage, but series B and C and probably several others could not be so regarded. In addition to the complete destruction of cells in G, which reduces the number of available sperm, there are other differences in radiation damage to the several stages in spermatogenesis. The dynamic biological nature of the system is well illustrated in these curves.

Figure 8 shows that this translocation frequency in stage A differs from the others; it is closest to a sigmoid curve. Stages C and D and most probably E are straight lines which cut the axis between 295 and 330 r. In these susceptible stages of spermeogenesis it would seem that after a dose of about 310 r, additional radiation produces rearrangements in direct proportion to the dosage.

Figure 9, taken from Stone, Haas, Alexander, and Clayton (75), shows the effect of various gas combinations and of the development stage on translocation frequency. All tests were carried out at $0^{o}$ to $3^{o}C$ with an x-ray dose of 2000 r for one minute, except for the pupae which were treated over 20 minutes. The pupae were irradiated about two days after pupation, the period when the amount of cytochrome oxidase is reduced to a very low level in *Drosophila virilis* according to Bodenstein and Sacktor (98). The situation in *Drosophila* resembles that for other insects in this respect, as shown by the work of Williams (99) and others. These pupae take about six days to complete development, so the stage of maximum injury would correspond to E and F in young adults. The genetic system of pupae with the low cytochrome content is damaged as severely as males treated with CO.

Stage E is the most seriously damaged in each test of the oxygen effect, in 4 percent $O_2$, air, or 99.5 percent $O_2$, and at all doses of x-radiation. The effect of the cytochrome oxidase absence (pupae) or poisoning falls a stage earlier in the cycle. If it is an induced delay in development, it cannot be due to greater genetic damage. In these tests the pupae were pretreated in the gas for about 12 hours. Further tests with pupae show that irradiation in nearly pure CO has a considerably greater effect than irradiation in $N_2$. In these tests the effect of oxygen

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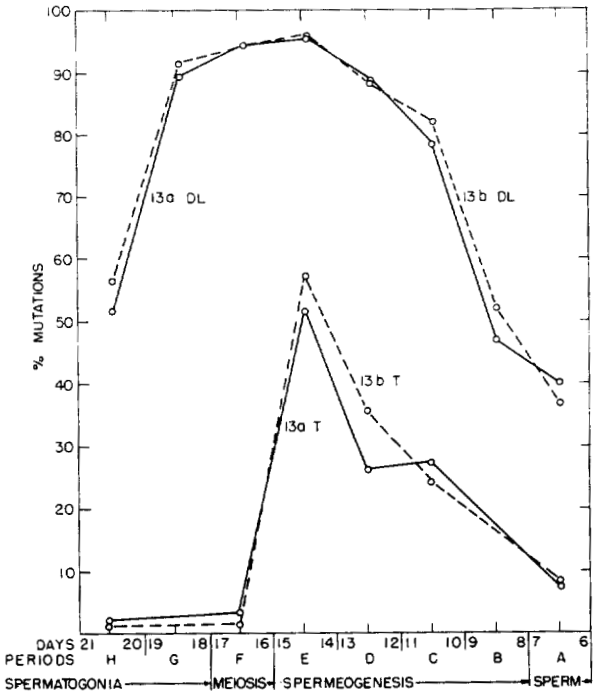


Figure 3. Dominant lethal and translocation damage in oxygen with oxygen and carbon monoxide post-treatments.

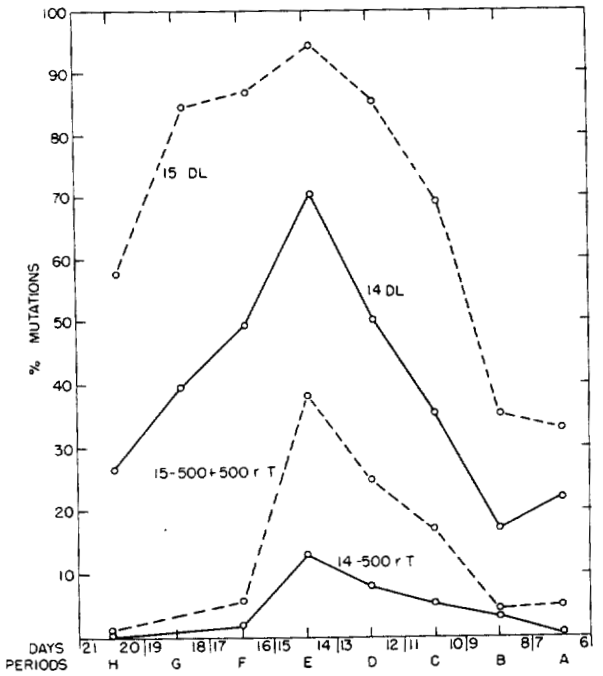


Figure 4. Comparison of dominant lethal and translocation damage at 500 and 1000 r.

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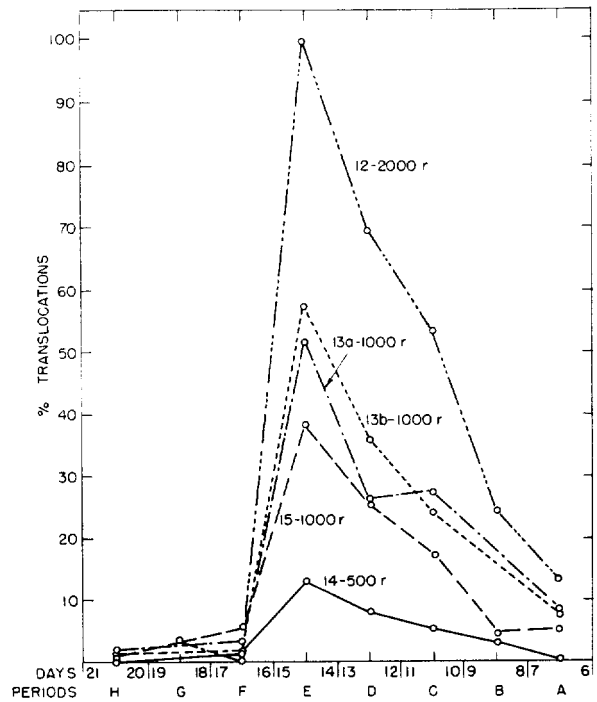


Figure 5. Translocation damage at 2000, 1000, and 500 r.

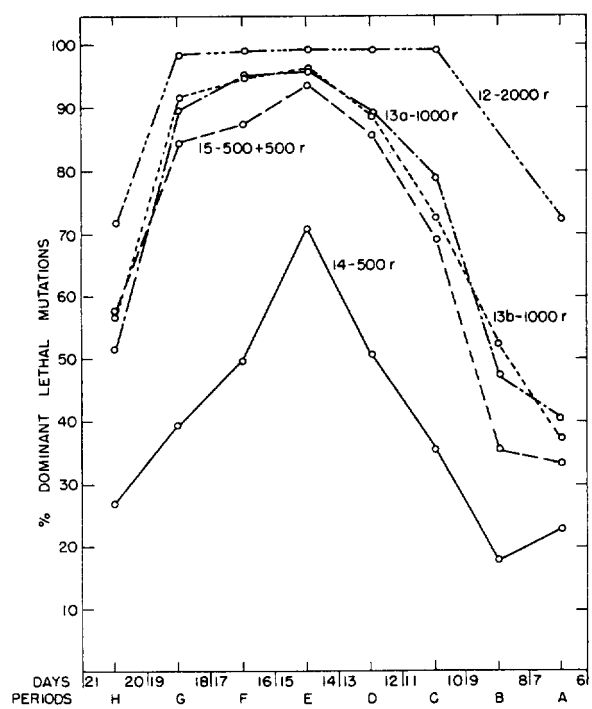


Figure 6. Dominant lethal damage at 2000, 1000, and 500 r.

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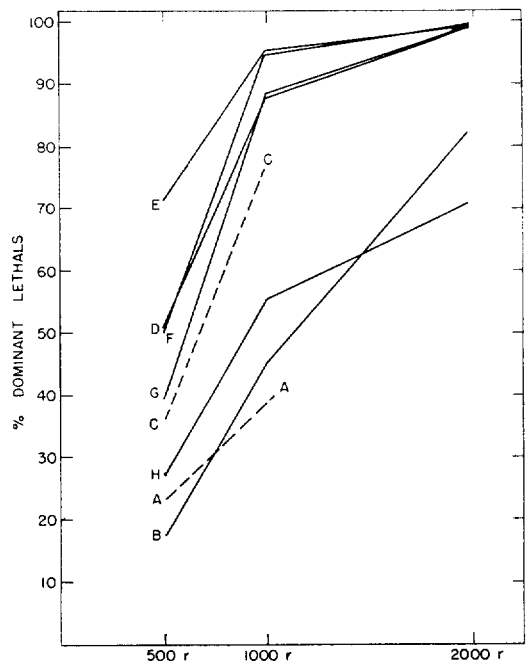


Figure 7. Dominant lethals in relation to dose.

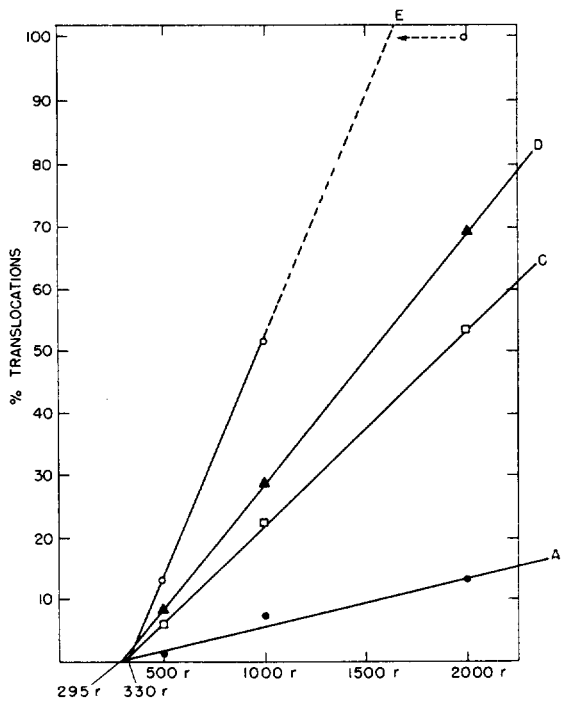


Figure 8. Translocations in relation to dose.

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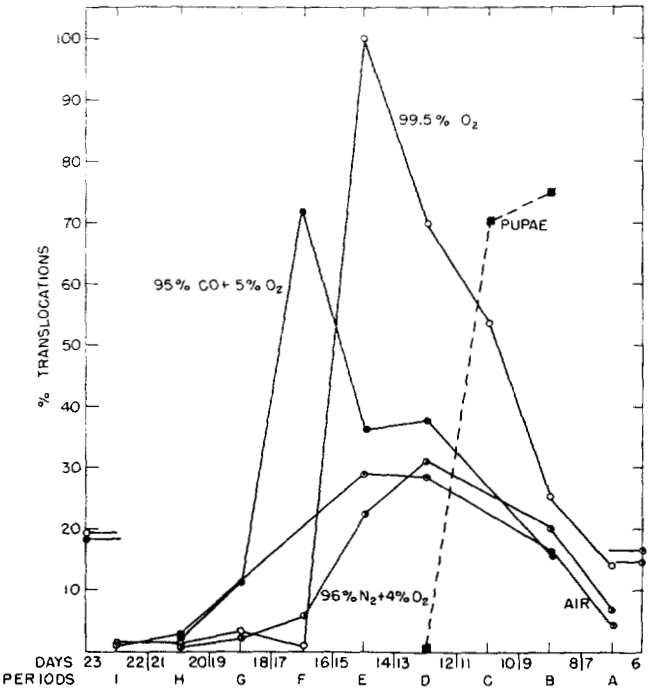


Figure 9. Effect of various gas combinations and of the development stage on translocation frequency.

would be at a minimum. These and other evidences suggest that the cytochrome system has several effects.

The differences between mature sperm and the earlier stages of spermatogenesis lead to some interesting conclusions. The near absence of translocations but presence of dominant lethals not associated with paucity of sperm does not argue for selection against translocations in spermatogonia. Ward and Alexander (100) found no rearrangements accompanying mutations produced by x-rays in spermatogonia; about half the mutations produced in mature sperm were associated with rearrangements. Conditions do not favor translocations and inversions in *Drosophila* spermatogonia, even though mutations and dominant lethals are produced. Radiation of meiotic stages, particularly at metaphase and anaphase, produces few recoverable translocations but many dominant lethals, partly from bridges and loss of acentric fragments during cell division. Whiting (85), Bozeman and Metz (86), and von Borstel (101) show these and other peculiarities, such as stickiness at telophase described recently by Carlson and Harrington (102) and Swanson and Johnston (103). These last authors showed that radiation-induced pycnosis was $O_2$ sensitive and considered this in part the result of the indirect effect of radiation. All these factors and perhaps others explain the high dominant lethal rate in meiosis, period F.

The postmeiotic peak in radiation sensitivity is very interesting and suggestive. Mazia (104) reported that chelating agents such as versene could break the chromosome into small nucleoprotein

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packets. Steffensen (105, 106) showed that magnesium or calcium deficiencies lead to spontaneous chromosome breakage and to an increase in the number of x-ray-induced rearrangements. These observations must be combined with our knowledge of physical and chemical reorganization during spermeogenesis. Cooper (107) reviewed the observations in *Drosophila*. The spermatid is stripped down and excess material eliminated during the elongation process. Furthermore, the chromosomes have much histone protein displaced, replaced, or converted, which results in the protamine protein structure characteristic of animal sperm according to Allfrey, Mirsky, and Stern (108). It seems most probable that this process requires an energy and/or synthesis system dependent on the Krebs' cycle. The cyclophorase system involving the Krebs' cycle was discussed by Green (109) and more recently by Roberts, Abelson, Cowie, Bolton, and Britten (110). The period of reorganization would probably be most sensitive to radiation damage and the cytochrome oxidase system would be useful in protection, perhaps coupled with peroxidase and catalase. In addition, cytochrome would be important in the Krebs' cycle energy systems, involving ATP and electron transport in synthesis. The mature sperm protamine nucleoprotein would be much more resistant to oxidation than the histone nucleoprotein or the transition system.

The majority of workers in the field of radiation genetics now agree that both ultraviolet and ionizing radiations produce part of their genetic effects through chemical mutagens reacting in steps 2 and 3 of Latarjet's and Gray's scheme (27). These chemical mutagens can contribute to all types of mutagenic action. However, especially Schwartz (111) and Baker and Von Halle (112) have claimed that the effect of oxygen was to modify the x-ray-injured chromosome so that restitution would not occur. This view has been criticized on the basis of both the information and the interpretation, especially by Lüning (92, 94). Most of us feel that the views of Schwartz (111) and Baker and Von Halle (112) are too restrictive and that radiation-induced chemical mutagens are similar to other chemical mutagens.

This general view of the action of radiation chemical mutagens as well as their synergistic effect with the direct action of radiation has been discussed by Giles (34), Muller (2), Swanson (113), Zelle and Hollaender (48), Demerec (46), Haas, Dudgeon, Clayton, and Stone (73), Stone, Haas, Alexander, and Clayton (75), Latarjet (56), and Gray (74). Baker (114) presented his summary of the oxygen effect. Baker and Edington (66) showed that the rate of production of both chromosomal abnormalities and mutations in *Drosophila* were reduced in $N_2$ as compared to $O_2$. Whiting (115) showed that radiation damage in *Habrobracon juglandis* measured as dominant and recessive lethal and visible mutations were all reduced in a $N_2$ atmosphere the same way. She concluded that the difference was due to the reduction of radiation-induced chemical mutagens. She opposed the view that the effect could only be on restitution of broken chromosomes.

Barron (116) stressed the importance of the -SH compounds in preventing radiation damage. Mikaelsen (117) showed that such compounds (glutathione, cysteine, and thiourea) reduced the production of chromosomal abnormalities by ionizing radiations. Hollaender and his group

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have tested a series of compounds to determine which will act to protect against radiation damage. These are referred to and discussed in the article by Zelle and Hollaender (48).

The responses of living systems to radiations show the dynamic complexity which makes such studies fascinating and rewarding.

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Discussion

KIMBALL: There is one point which I think should be emphasized in connection with attempts to interpret radiation-induced mutations in terms of chemical mutagens produced by the radiation. It is not sufficient to show that the radiation produces the substance and that the substance is mutagenic. It must also be shown that sufficient quantities of the substance are produced to account for an appreciable fraction of the mutations induced by the radiation. This is not an easy problem, for it involves estimating the quantity of the presumed mutagen in the nucleus when it is added outside the cell and when it is produced by radiation. As Dr. Baker pointed out, Dr. J.Z. Hearon and I have been able to show that $H_2O_2$ cannot be responsible for any appreciable fraction of the mutations produced in *Paramecium* by x-rays, even though there is a marked oxygen effect. Data of Dickey, Cleland, and Lotz (Proc. Natl. Acad. Sci., 35, 581 (1949)) have been interpreted by us to suggest that organic peroxides of the kinds used cannot account for an appreciable fraction of the mutations induced in *Neurospora* by x-rays. I think it should be emphasized that the existence of an oxygen effect does not lead immediately to the conclusion that a chemical mutagen is involved. This is a possible but not a necessary conclusion.

THODAY: Dr. Kimball has raised a point I wanted to make in connection with both Dr. Stone's and Dr. Baker's papers. Dr. Stone rather seemed to bring us back to the feeling that chemical mutagens and radiation mutagens were not so different, and Dr. Baker did mention this question as to whether the amount of peroxide was sufficient to account for radiation mutation.

Now I don't want to defend or deny the peroxide hypothesis, though it was the hypothesis that made us look for the oxygen effect in the first place. But I do want to throw doubt on this quantitative argument against it, on grounds relevant to the question of the equating chemical mutagens and x-rays.

In considering any chemical theory of radiation effects, it is essential that we take into account the question of distribution of the chemical. The concentration of peroxide, for example, along an alpha particle track, Lea once told me, was on the order of four times molar. The concentration in the whole cell may, therefore, be almost negligible, but at the same time there may be very high concentrations in some parts of the cell. I think this gives you all that you need to erect a chemical target theory of radiation mutation. I think we must remember that this is a fundamental difference between radiation mutation and chemical mutation; that with radiation mutation you introduce local concentrations of substances, and with chemical mutation you get what you can get in from outside, and you don't quite know what has got there when it has got there.

KIMBALL: In reply to some of Dr. Thoday's remarks, I agree that the very high concentration of peroxide along an alpha particle track must be considered, although it seems to me that other effects of the great concentration of energy are more likely to be important. However, the main reason for using the peroxide hypothesis for ionizing radiation was the oxygen effect. This means that not the high concentration in alpha particle tracks but the considerably lower concentrations in those tracks whose effectiveness is influenced by oxygen need concern us. In *Paramecium*, we have been able to show that a concentration of $H_2O_2$ estimated to have been nearly 0.1 M within the nucleus was without mutagenic effect when present for about half a minute. It seems to me that this concentration was sufficiently near those in which we are interested that an effect would have been expected if a high concentration of $H_2O_2$ for a brief period was really all that is necessary. This comment applies, of course, only to peroxide in its ground state. Peroxide in an excited state, $HO_2$, etc., are substances which would have to be considered on their own merits.

SOBELS: In connection with the data presented by Dr. Stone, it is perhaps of interest to mention some of my recent results which, I think, have a bearing on similar problems. Previous experiments suggested that adult flies probably

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contain a high catalase activity. Subsequently, the effects of a pretreatment with some catalase-inhibiting substances on various kinds of mutagens were studied.

First, *Drosophila* males were injected with 0.008 M solutions of potassium cyanide prior to x-radiation with doses varying from 980 to 2400 r. Tests for sex-linked lethals were made by means of the "Basc" method. The sensitivity pattern in the testis of the treated males was studied by mating them individually to three fresh "Basc" virgins at time intervals of three days. Compared to the controls, which were only x-rayed, the frequency of sex-linked lethals was significantly increased in germ cells which formed mature sperm four to seven days after treatment. This finding is of interest in connection with the results of Auerbach (1954) who showed that this stage of germ cell development is most sensitive to the mutagenic action of x-rays. It is also in line with Dr. Stone's data that stages corresponding to spermeogenesis in *D. virilis* are most sensitive to the effects of x-rays on chromosome breakage with and without pretreatment with carbon monoxide. Pretreatment with sodium azide similarly produces an enhancing effect on the x-ray-induced mutation rates. These results are tentatively ascribed to an increased production of hydrogen peroxide in the pretreated, irradiated germ cells.

Formaldehyde, also known as a catalase inhibitor, if injected prior to x-rays, also significantly increases the x-ray-induced mutation rates. Unlike cyanide, which is not mutagenic in *Drosophila,* formaldehyde acts as a mutagen by itself. The formaldehyde concentrations used were, however, so low as to cause hardly an appreciable increase of the spontaneous mutation rate.

To test Westergaard's hypothesis (1951) that in the mutagenic action of formaldehyde on *Neurospora* spores peroxides are involved, an experiment was performed in collaboration with Mr. J. Simons, in which flies were treated with cyanide gas prior to injections with formaldehyde. Compared with the controls which received only formaldehyde, the mutation rates in the cyanide-pretreated groups showed a very significant increase.

These results might be tentatively explained if it is assumed that formaldehyde, by acting as a catalase inhibitor, causes an accumulation of organic peroxides. It takes into account the enhancing effects of formaldehyde on x-ray-induced mutagenesis, whereas the enhancing effects of cyanide on formaldehyde-induced mutation rates could then possibly be ascribed to a slower breakdown of the mutagenic peroxides.

In view of the similarity of the effects of x-rays and mustard gas, the influence of a cyanide pretreatment on the rate of mustard gas-induced mutation rates has been studied. Compared to the controls, which, in the same experiment, were subjected to mustard gas only, the frequency of sex-linked lethals after cyanide pretreatment was also significantly increased in those germ cells which formed mature sperm either four to seven, or seven to ten days after treatment. The assumption that peroxides may be involved in the production of the mutagenic effects of mustard gas, therefore, seems at least suggestive.

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