MEASUREMENT AND CONTROL OF SOME DIRECT AND INDIRECT EFFECTS OF X-RADIATION

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D URING the first two decades following MULLER's (1927) proof that irradiation induced genetic changes, most of the effect was considered to result from ionizations (and excitations) within the chromosome itself. LEA (1946) interpreted the evidences accumulated up to that time as indicating that most genetic damage was induced directly, although he believed that ionizing and ultraviolet radiations produced also indirect effects on other components of the cells, which contributed a small part of the total effect. Such a view is no longer tenable. THODAY and READ (1947) showed that the frequency of chromosomal abnormalities induced by X-rays in *Vicia faba* was several times as great in air as it was in nitrogen for the same dosage. KOTVAL and GRAY (1947) concluded that about one-half of the effect of alpha radiation in breaking prophase chromosomes of Tradescantia must be due to energy absorbed in the medium surrounding the chromosome. That same year STONE, WYSS and HAAS (1947) demonstrated that the irradiation of the substrate with ultraviolet light, prior to growing bacteria in the irradiated medium, increased the mutation rate in these microorganisms. WYSS, STONE and CLARK (1947) showed that a similar increase in mutation rates in microorganisms could be accomplished by adding H_2O_2 (one of the active radicals produced in water by ultraviolet, or by X-rays if oxygen is present) to the substrate prior to inoculation.

These investigations provided both inferential and direct evidence that radiation-induced chemical changes occurring outside the chromosomes may subsequently induce genetic changes. The discovery of these radiation-induced chemical mutagens may permit a comparison of the effect of radiation with chemical mutagens, such as those described by AUERBACH and ROBSON (1946).

The present study is an investigation of the effect of changing the environment of mature sperm during irradiation on the production of chromosome rearrangements. The results were reported briefly (HAAS, DUDGEON, CLAYTON and STONE 1952).

MATERIALS AND METHODS

Drosophila virilis was the test organism employed for these experiments. The normal stock was established from a strain collected in Texmelucan,

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Mexico (U.T. Stock No. 1801). The multiple mutant stock used was synthesized from various laboratory mutants, and contained the genes broken (*b*, 188.0) on the second chromosome, tiny bristle (*tb*, 104.0) and gapped (*gp*, 118.5) on the third, cardinal (*cd*, 32.2) on the fourth, and peach (*pe*, 203.0) on the fifth chromosome (CHINO 1941).

A plastic chamber, which held the flies at a constant temperature for sufficiently long periods of time, was used for gaseous treatment and irradiation. The chamber was so constructed that the gaseous atmosphere around the flies was continually being replaced by fresh gas at the same temperature as that of the chamber. Flies to be irradiated were placed in this chamber and held for one hour in the gas mixture at the temperature to be used during the irradiation to insure that they were in equilibrium with the gas. The chamber was then moved into position on the X-ray machine, and the flies were irradiated. After irradiation the gas and temperature treatments were continued for twenty to thirty minutes before removing the flies to a food vial. All testing and growing of Drosophila cultures was carried out in standard U.T. yeast enriched food at $23 \pm 1°C$.

For all tests with the fast dose a 220 kv Westinghouse Constant Potential X-ray machine, operating at 200 kv and 15 ma and having inherent filtration equivalent to 0.25 mm aluminum, was used for irradiation. The fly chamber was placed in position 15.4 cm from the center of the X-ray tube and was held in an apparatus so constructed that the same position was automatically assumed in every experiment. At this distance the output of the machine was 1867 r per minute within the chamber in which the flies were present. The output was checked with the aid of Sievert Ionization Chambers several times during the course of these experiments and the results were always uniform. The time of exposure to the X-radiation was regulated so as to give the total X-ray dose desired.

In several experiments the " slow dose " was administered on the same or a similar machine, in others, on a Victor machine operating at 50 kv and 5 ma with a 1 mm aluminum filter to give about 100 r per minute. As *Drosophila virilis* matures slowly, eight-day-old males were irradiated in the tests.

Most of the gases employed were specially prepared and analyzed mixtures obtained from the Houston Oxygen Company. One was a mixture of 96% nitrogen and 4% oxygen, another of 95.8% carbon dioxide and 4.2% oxygen, and the third had 99.5% oxygen. In preliminary experiments carbon monoxide was generated by heating a mixture of oxalic and sulfuric acids and collected over water. Carbon dioxide was removed to a great extent by bubbling the gas several times through sodium hydroxide solutions. In later experiments carbon monoxide obtained from the Matheson Company was used. The analysis of this gas showed 96.8% CO, 0.36% CO_2, 0.97% H_2, 1.0% N_2, 0.8% saturated hydrocarbons, and traces of iron and sulfur. This gas was mixed over water with the proper amount of CO_2 and/or O_2 to give the desired gas mixture.

Translocations were detected genetically through linkage of genes known

to be located in different linkage groups. The rare translocations which gave very viable aneuploids were not detected. Normal males were irradiated and mated individually within one or two days to several females of the multiple mutant marker stock. The male was discarded three days after mating. A group of ten to twelve F_1 males from each P_1 male were backcrossed individually to several marked females, and translocations were scored by observing the segregations in their backcross progeny. In a number of experiments some of the translocations detected genetically were examined cytologically in the salivary gland nuclei of the second backcross generation. In all doubtful cases, as well as in translocations involving three or more chromosomes and in those involving the Y, an additional test was run by crossing the F_2 phenotypically normal males to stock multiple marker females to recheck linkages.

Previous work on X-ray effects with Drosophila has been performed using comparatively slow X-ray treatment. Large total doses have been given but they have been administered at a rate ranging from 50 to 300 roentgens (r) per minute. The most frequently used rate has been in the neighborhood of 100 r per minute, and at this rate it would take 20 minutes to administer a total dose of 2000 r. If cross-linkages are formed at once, it is possible that during this time some chromosome breaks would not have the opportunity to undergo cross-union due to restitution of the broken chromosome before a second break becomes available. Unless at least two chromosomes cross-join so as to establish a translocation, the breaks would not be detected by our present methods of analysis. The possibility of restitution of the original sequence might not be so great if the total dose were administered in a comparatively short period of time. The cellular mechanisms protecting against the radicals which produce indirect effects also increase the desirability of information concerning the effects of fast irradiation. The X-ray doses in some of these experiments were administered at the rate between 1800 and 1900 r per minute, while others were given at 100 r per minute.

<h2 style="text-align:center">RESULTS</h2>

The experimental results are given in table 1 and figures 1, 2, 3 and 4. In these last two figures, the crosslines indicate twice the standard error for the particular test as given in table 1. In some of these tests the total dose deviated 50 to 100 r units from the 2000 r desired. In these cases the 2000 r rate was estimated by interpolation, using the slope of BAKER's (1949) curve for cold slow irradiation. The interpolation is given in table 1, and these corrected figures (at the 2000 r level) are used in figures 3 and 4. These tests provide information on the relation of radiation damage, here measured as the frequency of translocations induced, to several variables: the rate of irradiation, temperature during irradiation, presence and concentration of chemically active metabolites. These gases, oxygen, carbon monoxide, carbon dioxide, and their mixtures, were present as atmosphere around the Drosophila during irradiation, with nitrogen used as the metabolically inert gas. The pre- and post-

TABLE 1

Rearrangements produced under different conditions.

Exp. Code No.*	Source	Gaseous environment of flies during irradiation	Temp. during irradiation (±2°C)	Total dosage of X-ray (r-units)	X-ray dosage rate per minute (r-units)	No. of sperm tested	Sperm with trans-locations	% sperm showing translocations at	
								Indicated total dosage	2000 r**
1	Baker (1949)	Air	2	998	100	1130	53	4.7 ± 0.6	14.5
2	"	"	"	1996	"	1021	147	14.4 ± 1.1	
3	"	"	"	2986	"	853	258	30.2 ± 1.6	
4	"	"	28	1009	"	550	28	5.1 ± 0.9	11.1
5	"	"	"	2019	"	516	58	11.2 ± 1.4	
6	"	"	"	3025	"	477	104	21.8 ± 1.9	
7	(Current results)	"	5	1800	90	454	55	12.1 ± 1.6	14.1
8	"	"	23	1900	"	455	37	8.1 ± 1.3	8.8
9	"	"	0	500	1818	569	14	2.6 ± 0.7	
10	"	"	3	1000	"	858	49	5.8 ± 0.8	
11	"	"	0	2000	"	703	121	17.2 ± 1.4	17.2
12	"	"	23	2000	"	598	83	13.9 ± 1.4	13.9
13	Baker, Edington (1952)	100% N₂	6	2000	100	512	19	3.7 ± 0.8	3.7
14	"	95% N₂ + 5% O₂	"	2000	"	525	52	9.9 ± 1.3	9.9
15	"	79% N₂ + 21% O₂	"	2000	"	616	99	16.1 ± 1.5	16.1
16	(Current results)	96% N₂ + 4% O₂	0	1976	104	496	25	5.0 ± 1.0	5.2
17	"	" "	23	1960	98	434	28	6.5 ± 1.2	6.8
18	"	" "	0	500	1818	530	11	2.1 ± 0.6	5.2
19	"	" "	2	1000	"	760	40	5.3 ± 0.8	6.8

*The code number serves to locate the corresponding bar graph in figures 2 and 3.

**Where the total dosage in a given experiment was not 2000 r, the percent of sperm showing translocations was interpolated to 2000 r using the slope of Baker's curves at slow dose rate and at the corresponding temperature from figure 1. Such a correction rarely exceeded one percent.

TABLE 1 (continued)

Exp. Code No.*	Source	Gaseous environment of flies during irradiation	Temp. during irradiation (± 2°C)	Total dosage of X-ray (r-units)	X-ray dosage rate per minute (r-units)	No. of sperm tested	Sperm with trans-locations	% sperm showing translocations at	
								Indicated total dosage	2000 r**
20	(Current results)	96% N_2 + 4% O_2	2	2000	1818	505	71	14.1 ± 1.6	14.1
21	'' ''	'' ''	25	2054	1867	569	69	12.1 ± 1.4	11.7
22	Baker, Edington (1952)	100% O_2	6	2000	100	494	85	17.2 ± 1.7	17.2
23	(Current results)	99.5% O_2	3	1957	103	430	69	16.0 ± 1.7	15.3
24	'' ''	''	25	1960	98	509	63	12.4 ± 1.5	12.7
25	'' ''	''	0	2054	1867	505	97	19.2 ± 1.7	18.4
26	'' ''	''	25	2054	1867	517	102	19.7 ± 1.8	18.9
27	'' ''	80% CO_2 + 20% O_2	2	1890	90	508	94	18.5 ± 1.8	19.9
28	'' ''	'' ''	24	1900	95	503	73	14.5 ± 1.6	15.5
29	'' ''	'' ''	14	2054	1867	503	84	16.7 ± 1.6	16.1
30	'' ''	'' ''	25	2054	1867	303	57	18.8 ± 2.1	18.3
31	'' ''	80% CO + 20% O_2	5	2050	102	1048	196	18.7 ± 1.4	18.1
32	'' ''	'' ''	25	1932	92	524	89	17.0 ± 1.7	17.5
33	'' ''	'' ''	0	2054	1867	1099	216	19.6 ± 1.2	19.0
34	'' ''	'' ''	25	2054	1867	485	60	12.4 ± 1.5	11.9
35	'' ''	40% CO + 40% CO_2 + 20% O_2	5	2018	97	521	68	13.0 ± 1.5	12.8
36	'' ''	40% CO + 40% CO_2 + 20% O_2	28	1890	90	523	64	12.2 ± 1.5	13.1
37	'' ''	60% CO + 20% CO_2 + 20% O_2	23	2000	1818	504	45	8.9 ± 1.3	8.9
38	'' ''	95% CO + 5% O_2	3	2054	1867	575	101	17.6 ± 1.6	17.0
39	'' ''	'' ''	25	2054	1867	1033	185	17.9 ± 1.2	17.4
40	'' ''	47.5% CO + 47.5% CO_2 + 5% O_2	2	2000	1818	519	79	15.2 ± 1.6	15.2
41	'' ''	75% CO + 20% CO_2 + 5% O_2	23	2000	1818	664	77	11.6 ± 1.2	11.6

treatment in the gas at the temperature of the test insured, so far as possible, that the gases dissolved in the body fluids of the flies were at equilibrium with the atmosphere of gases surrounding them. The testing procedure used has provided a control to measure the magnitude of the spontaneous translocation rate. With about 10 F_1 progeny from each male tested, it was possible to determine if there were any spontaneous translocations present in the individually tested P_1 males. One cluster was found in which all F_1 males had the same translocation among 2367 P_1 males tested in all the experiments. This is a rate of 4.2×10^{-4}, but, of course, the rate is so low and the test so small that it is subject to a large error.

Most experimental tests were made using 2000 r because of the nature of the material as established by BAKER (1949) who tested *Drosophila virilis* at 1000, 2000 and 3000 r. Some of his material is included, as well as additional material from similar tests on the effect of oxygen (BAKER and EDINGTON 1952). These experiments were carried out using 500, 1000 and 2000 r to establish dosage rate curves, but did not go above 2000 r, because the frequency of complex translocations with more than two chromosomes involved became too high. In table 1 and figures 1–4, each sperm with a translocation (or translocations) is counted as one case. Table 2 gives the data so that two separable translocations are calculated as two cases. The experiments usually involved about 500 F_1 males which were tested for translocations, so the sampling error is larger than desirable.

Temperature effect

In these tests, more translocations were produced when the flies were irradiated at the lower temperature, 0–5°C, than at the higher temperature. This is in agreement with the results of MICKEY (1938) and BAKER (1949). The paired tests (differing only in temperature during irradiation) which did give a higher rate at the warm temperature involve small differences, while the majority of the experiments, including those with large differences between parallel experiments, show a higher rate of rearrangements in the cold. There are twelve pairs of tests which are otherwise comparable but differ in temperature in our 2000 r experiments. In eight sets, irradiation in the cold produced higher translocation rates and in one set the difference is statistically significant. None of the four sets shows significant difference when the rate of translocations is higher in the warm than the cold test. If we disregard other experimental conditions and contrast the translocation rate for the sum of the twelve cold tests with that of the twelve warm tests, the translocation rate is significantly higher at the lower temperature.

Rate effect

One interesting finding was that the rate of irradiation influenced the frequency of translocations produced. The best test to demonstrate the difference between the " fast rate " (over 1800 r per minute) and the " slow rate " (100 r per minute) is in material treated in nitrogen-oxygen mixtures, with only

TABLE 2

Translocation type and chromosome involved.

| Experiment	Number tested	Types of translocations					Chromosomes involved					
		T_2	T_3	T_{2+2}	T_4	T_{2+3}	Y	2	3	4	5	
18	530	10	1				4	6	5	2	6	
9	569	14					4	2	5	10	7	
19	760	38		2			8	22	19	17	18	
10	858	49					11	22	23	19	23	
Total, lower dose	2717	111	1	2			27	52	52	48	54	4.20%
20	505	69	2				23	32	36	27	26	
21	569	67	1	1			11	31	29	38	30	
16	496	24	1				6	11	14	13	7	
17	434	28					10	10	8	14	15	
Total, N_2	2004	188	4	1			50	84	87	92	78	9.63%
11	703	109	10	2			33	56	56	54	53	
12	598	75	7	1			19	43	36	37	39	
7	454	53	2				7	27	25	21	32	
8	455	35	2				8	18	16	15	19	
Total, air	2210	272	21	3			67	144	133	127	143	13.39%
25	505	92	5				28	43	46	49	34	
26	517	93	5	4			28	50	51	36	44	
23	430	65	3	1			15	26	36	30	25	
24	509	60	3				20	26	33	20	30	
Total, O_2	1961	310	16	5			91	145	166	135	133	16.88%
29	503	77	5	2			24	41	34	35	39	
30	303	55	2				11	24	25	30	27	
27	508	88	3	3			27	35	41	39	49	
28	503	68	3	2			16	28	40	33	32	
Total 80 CO_2 + 20 O_2	1817	288	13	7			78	128	140	137	147	16.95%
31	1048	186	6	3	1		49	107	83	86	75	
32	524	84	4	1			17	39	39	39	48	
33	1099	203	11	2			61	115	86	83	98	
34	485	53	5	2			15	32	23	27	25	
38	575	96	3	1	1		25	49	42	37	54	
39	1033	175	7	1	2		50	95	76	82	78	
Total CO + O_2	4764	797	36	10	4		217	437	349	354	378	17.78%
35	521	65	2	1			29	29	25	24	31	
36	523	62	2				21	33	32	26	18	
37	504	43	2				7	25	15	24	21	
40	519	74	4			1	26	30	39	33	34	
41	664	68	8	1			18	35	36	39	34	
Total CO + CO_2 + O_2	2731	312	18	2		1	101	152	147	146	138	12.19%
All 2000 r	15487	2167	108	28	4	1						
Grand total	18204	2278	109	30	4	1	631	1142	1074	1039	1071	= 4957
							12.7	23.0	21.7	21.0	21.6	%
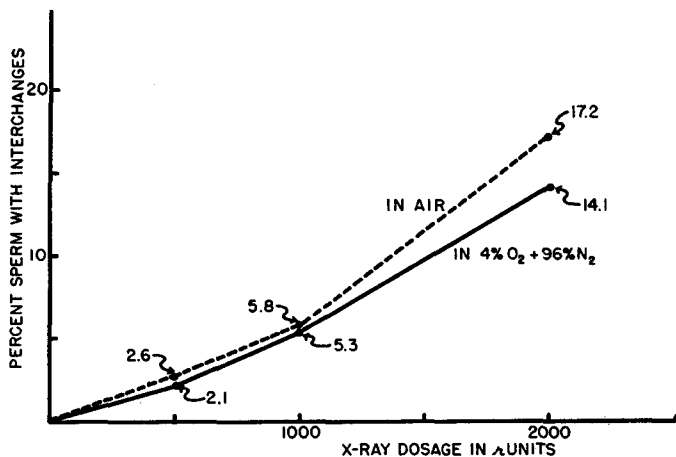
FIGURE 1.—The relationships between dosage and oxygen tension at rate 1867 r per minute.

oxygen of importance as gas environment in the indirect effects (fig. 3). The better data to demonstrate the greater effectiveness of the fast rate of irradiation is the material treated in 4% oxygen plus 96% nitrogen, where the oxygen effect is near the minimum. In these tests the frequency of translocations is two to three times as great if the irradiation is fast. When carbon monoxide
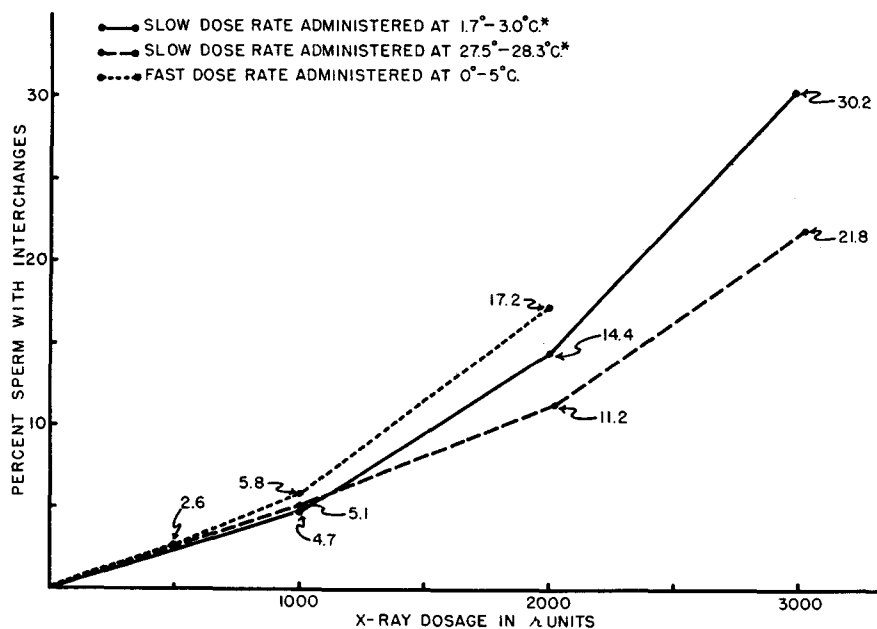

FIGURE 2.—The relationships between dosage, dose rate and temperature in the production of translocations in sperm by X-rays. The fast dose rate in all cases was administered at 1867 r per minute; the slow dose rate at approximately 100 r per minute.

    * Slow dose rate curves are from BAKER 1949.

or carbon dioxide is present in addition to oxygen, the indirect effects produce more complex interactions, which may mask the rate effect. Figure 2 shows that the curve for the fast irradiation in the cold is similar to but has a somewhat steeper slope than those for slow irradiation at the two temperatures used by BAKER (1949). Figure 1 compares the effects of irradiating in air with the effect of irradiating in 4% oxygen plus 96% nitrogen. The higher concentration of oxygen increases the frequency of translocations at the doses of 500, 1000 and 2000 r units. The fast irradiation causes as much genetic damage with only 4% oxygen as slow irradiation causes in air (figs. 1 and 2). There are six paired tests in which Drosophila males were X-rayed 2000 r in nitrogen-oxygen mixtures. The translocation rate was higher in the progeny of Drosophila irradiated rapidly in each test. The difference is statistically significant in 4 percent oxygen or if the average rate for the six fast treatments is compared to that of the six slow treatments.

Oxygen effect

The effect of oxygen is obvious from figures 1 and 3, which include our results together with similar tests with *Drosophila virilis* by BAKER and EDINGTON (1952). Comparisons between the data from the other workers and
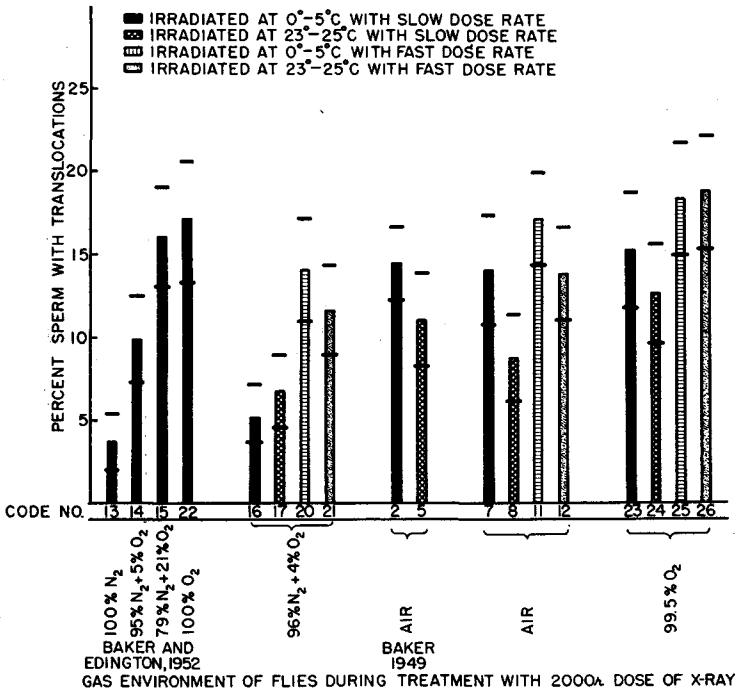
FIGURE 3.—The effect of oxygen, temperature and dose rate on the production of translocations in sperm by 2000 r doses of X-rays. The slow dose rate was approximately 100 r per minute; the fast dose rate approximately 1800 r per minute. Each bar graph is keyed to the data in table 1 by the code number at the base of the graph.

these experiments show good agreement for similar tests. In both slow and fast irradiation these data show that, as the concentration of oxygen is increased during irradiation, the amount of genetic damage increases also. This is true in the series at 0–5°C and that at 23–25°C.

Other gases

The effects of carbon monoxide and of carbon dioxide were investigated in combinations with oxygen. Comparing results obtained in air with those in a mixture of 80% CO_2 plus 20% O_2, the radiation damage is distinctly higher with slow irradiation in the latter. However, the damage is not increased in fast irradiation at 14°C (number 29 on fig. 4), although it seems to be increased in the fast warm test. These findings agree with those of KING and SCHNEIDERMAN (1952) that CO_2 increases radiation damage in Tradescantia. On the other hand air compared with 80% CO plus 20% O_2 shows that there is a large increase in damage in the cold tests, both for slow and fast irradia-
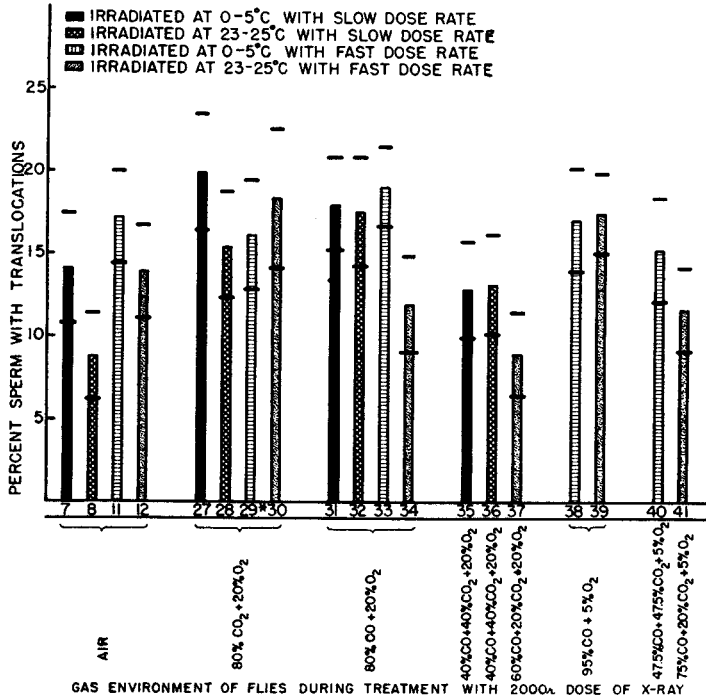
FIGURE 4.—The related effects of carbon dioxide, carbon monoxide, oxygen, temperature and dose rate on the production of translocations in sperm by 2000 r doses of X-ray. The slow dose rate was approximately 100 r per minute; the fast dose rate approximately 1800 r per minute. Each bar graph is keyed to the data in table 1 by the code number at the base of the graph. The results in air, presented at the left, are included for comparison.

* No data for fast irradiation in 80% CO_2 + 20% O_2 at 0°C was available. The data used for construction of this graph was obtained at 14°C under these conditions of gas and dose rate.

tion. There is a large increase in damage in the warm slow test, which is comparable to the results of KING, SCHNEIDERMAN and SAX (1952). On the other hand the warm fast irradiation test shows less damage than obtained in air (number 34 on fig. 4). This apparent reduction is not due to a sampling error, since another test gave 41 translocations in 502 F_1's tested with 2054 r, or a corrected figure of 7.8% translocations. This result was obtained in an early experiment, in which we made carbon monoxide mixed with carbon dioxide, and then removed the latter by bubbling through a NaOH solution. Although our results probably are reliable, the fact that the mixture of CO + $CO_2 + O_2$ gives a low rate of translocations (see 37) caused us to disregard this experiment, in order to remove any possibility that the contaminating effect of appreciable amounts of CO_2 had lowered the value.

In the experiments with 95% CO plus 5% O_2, the fast cold treatment shows no difference from air, but the fast warm treatment (number 39 on fig. 4) may have produced a greater effect than the comparable air experiment, and is certainly significantly above the comparable 80% CO + 20% O_2 test. Every experiment with a mixture of $CO_2 + CO + O_2$ gave values for radiation damage below those obtained in other comparable tests having CO + O_2 or $CO_2 + O_2$ atmospheres. Agents which increase radiation damage are not necessarily additive in their effects.

Location of breaks

There is accurate information on the relative frequency of breakage in a particular chromosome involved in these translocations. Table 2 shows that the Y chromosome is involved in 12.7% of the translocations, chromosome 2 in 23.0%, chromosome 3 in 21.7%, chromosome 4 in 21.0% and chromosome 5 in 21.6%. Stated another way, translocations involving the Y are about six-tenths (0.583) as frequent as those involving an autosome. BAKER (1949) obtained similar results except that a smaller percent of translocations involved the Y. In his sample the Y was involved about four tenths (0.422) as often as the average for the autosomal frequency or 9.55% of 1749 chromosomes involved in his tests. The differences may represent differences in the *Drosophila virilis* stock tested or from sampling. In *Drosophila virilis* the Y chromosome is heterochromatic and as long or longer than the autosomal rods even though the latter have a large heterochromatic segment, one-third to one-half the chromosome (HEITZ 1935). The translocations recovered in these experiments less frequently involve an exchange with a break in the heterochromatin. Some part of the lower frequency of Y chromosome translocations is due to our failure to detect translocations with very viable aneuploid classes. The relative frequency of interchanges involving autosomes is in excellent agreement with expectation; for example, the second chromosome is slightly longer than the others in the salivary gland nuclei and is more frequently involved than any other autosome. A sample of 102 translocations between the Y and one of the major autosomes was analyzed cytologically by GEORGE VLAHAKIS (unpublished). The 25 or 26 breaks in each autosome appeared

to be distributed at random along the chromosome. This small sample would not show the restricted amount of local bunching of breaks demonstrated in the large number of breaks analyzed by KAUFMANN (1946) in *Drosophila melanogaster*.

DISCUSSION

CATCHESIDE (1948) and MULLER (1940, 1954) have reviewed the literature on the genetic effects of radiations. The results presented in table 1 provide certain additional information on chromosome breakage, although only the frequency of sperm with translocations which are viable and fertile is measured rather than the frequency of breaks. There is no sure way to separate the chromosome breaks produced directly by X-rays from those produced secondarily by the chemical mutagens which result from the ionization of water and other components of the biological system outside the chromosomes, or from those produced by the combined direct and indirect effects.

The overwhelming body of evidence from Drosophila, as illustrated by the early work of DEMPSTER (1940), demonstrated that intermittent doses of X-rays produced as many translocations as continuous irradiation. MULLER (1940) gave evidence which agreed with that of DEMPSTER and which showed in addition that the frequency of translocations for the same total dose was as great when the intensity was 0.05 r per minute as when it was 250 r per minute. Consequently it was concluded that breaks accumulate in the sperm and that rearrangements occur after fertilization. There was no evidence for healing through time during sperm storage. These and other tests reviewed by CATCHESIDE (1948) and MULLER (1954) indicate that the number of symmetrical rearrangements depends on the total amount of X-radiation irrespective of the intensity or fractionation of the dose.

In Tradescantia most tests indicate both intensity and fractionation effects. As an example, SAX and LUIPPOLD (1952) presented information on the frequency of two-hit chromosome aberrations which demonstrated an effect of both intensity and fractional dosage. DE SERRÈS and GILES (1953) have recently presented additional evidence on this effect with X-rays. However, GILES, DE SERRÈS and BEATTY (1953) showed that there is no effect due to radiation dose fractionation with fast neutrons. The frequency of chromosomal aberrations is directly proportional to the amount of radiation from neutrons. Our experiments, especially those in figure 3, present evidence that there is an intensity effect when the dosage was given at 2000 r in sixty-six seconds as compared to 2000 r in twenty minutes. We cannot ascribe the intensity effect to differences in wave lengths of X-rays used in different tests, although KIRBY-SMITH and DANIELS (1953) have demonstrated a difference in the effectiveness of X-rays and gamma rays from cobalt[60] in producing chromosome breakage in Tradescantia. In numerous tests by other workers, using both Tradescantia and Drosophila, no such wave length dependence has been demonstrated within the range of wave lengths from X-rays used in our experiments.

We can make a rough comparison of the effects of different physiological conditions of the sperm during irradiation using results from 2000 r units X-radiation (table 2). Although it introduces some bias because of difference in sample size and other factors, we can lump all cultures treated in certain gas combination and compare the percentages of translocations. In 96% nitrogen plus 4% oxygen, 9.63% of 2004 fertile F_1 cultures had translocations present; in air 13.39% of 2210 cultures; in 99.5% oxygen 16.88% of 1961 cultures; in carbon dioxide–oxygen mixtures 16.95% of 1817 cultures; in carbon monoxide–oxygen mixtures 17.78% of 4764 cultures; in carbon monoxide–carbon dioxide–oxygen mixtures only 12.19% of 2731 cultures. Even in these average effects, the low oxygen atmosphere (96% N_2 + 4% O_2) results in least damage, air is lower than the next three combinations of gases, while $CO_2 + CO + O_2$ results in slightly less damage than air. With these size samples, a difference of 2.0% (perhaps less) is statistically significant.

In our experiments radiation damage was usually greater at 3°C than at 25°C. The relations between temperature at the time of irradiation and the oxygen effect are quite interesting. The higher temperature, 25°C, is about optimal for *Drosophila virilis,* while at 3°C the flies are immobilized and their metabolic processes are slowed down, although they are able to survive for considerable periods of time. Oxygen is nearly 1.6 times as soluble in water at 3°C as at 25°C. Although oxygen in the atmosphere may be at equilibrium with that dissolved in the body fluids of the fly at 3°C, the active metabolism at 25°C will tend to reduce the concentration of dissolved oxygen even below two-thirds of the amount present at the lower temperature because of the difference in solubilities. This difference in oxygen present in the cells during irradiation, as well as the greater activity of other biological protective agents at 25°C, might account for much of the temperature differential. Chromosome breakage by the mechanism demonstrated by CONGER and FAIRCHILD (1952) may also contribute, particularly as irradiation may partially disrupt the normal protective mechanisms of the cell. Their tests show that oxygen may act as a chemical mutagen, leading to chromosome breakage. This action is independent of radiations although this effect and combined effects are synergistic with radiations and increase radiation damage.

FABERGÉ (1950a, b) showed that at temperatures above freezing but not at − 192°C the concentration of oxygen affects the amount of damage from X-radiation. NYBOM, LUNDQVIST, GUSTAFSSON and EHRENBERG (1953) published extensive tests of the effect of temperature differences on growth, fertility, mutation rate and chromosome abnormality rate in barley. They tested different doses of X-rays at temperatures of + 20°C and − 190°C. They conclude that X-ray damage is less at low temperature using any of the measures of injury they tested. The low temperature reduces both physiological and genetic damage by eliminating part or most of the indirect effect. This oxygen effect was demonstrated by THODAY and READ (1947), GILES and RILEY (1949, 1950), HAYDEN and SMITH (1949), RILEY, GILES and BEATTY (1952) and numerous others on chromosome breaks. There are two hy-

potheses to explain this effect. The first presumes that oxygen forms mutagenic compounds in tissues during irradiation which increases the amount of damage including the number of breaks. The second assumes that oxygen during irradiation interferes with the restitution process so that more interchanges can occur but that it does not increase the number of breaks. In so far as this is an alternative to the first hypothesis (of course both things may happen) it must assume that breakage is due to the direct action of the X-radiation and not to free radicals and peroxides formed by the ionizations of water and organic materials, for oxygen certainly contributes to these. Most investigators, including MULLER (1954) who reviews the evidence, assume that oxygen contributes chemical mutagens which increase the number of chromosome breaks. However SCHWARTZ (1952) and BAKER and VON HALLE (1953) have advocated that the oxygen prevents restitution and so increases the opportunity for crosslinkages to be formed. Their arguments are not convincing because they cannot justify their basic premise. Before they can restrict the effect of oxygen to a role in reunion, they must assume that although oxygen can break chromosomes and produce rearrangements without irradiation (CONGER and FAIRCHILD 1952), it cannot cause breakage if it is present when the material is irradiated nor can it form active radicals which break chromosomes under these circumstances. The evidences bearing on the role of the direct and indirect effects of radiations are discussed elsewhere (STONE et al. 1954).

We know that random reunion of the segments of broken chromosomes does not occur. As CATCHESIDE (1948) pointed out, several earlier investigations using Drosophila showed an excess of inversions as compared to translocations. In these latter, there is an excess of two-by-two rearrangements over those with all four chromosomes involved together. We have 34 cases where breaks in four chromosomes were present: 30 of these were cases with two separate translocations and only 4 had all four chromosomes linked, table 2. These results agree with those of BAKER (1949) for material irradiated 2000 r. Only one case was found with all five chromosomes involved; it was two translocations, one involving two and the other three chromosomes. The tendency to form two chromosome exchanges more frequently than the 1:2 ratio expected by chance for two-by-two to all four chromosomes might be explained as a result of a proximity limitation. Drosophila resembles Tradescantia in this respect for in the latter LEA and CATCHESIDE (1942) showed that crossunions occur between broken ends lying close together in the nucleus rather than at random. A discussion of the implications of this and other related material is given elsewhere (STONE et al. 1954).

Radiation damage varies with variations in the physiological state of the organism. BONNIER and LÜNING (1950) and LÜNING (1952a, b) showed that the probability that X-radiation would produce chromosomal abnormalities varied with the stage in the chromosome cycle in Drosophila melanogaster just as in other organisms. The effects of oxygen, carbon dioxide, and carbon monoxide in modifying X-ray effects are through the physiological systems

rather than through modification of the ionizations produced by irradiation. DALE (1952) states that the indirect effects of irradiation are very pH dependent. SIZOVA (1936) published a note on the effect of X-rays combined with carbon dioxide or ammonia using *Crepis capillaris* as test organism. Each agent increased chromosomal aberration frequency in combination with X-rays. Later work with different organisms has not always been in agreement. ZIRKLE (1940) showed that lowering the pH with carbon dioxide increased radiation damage.

This effect of CO_2 was consistent at different concentrations when 4.5-hour *Drosophila melanogaster* eggs were irradiated. Eggs irradiated only 2 hours after being laid varied in sensitivity with concentration of CO_2. ZIRKLE (1941) found a similar variation with concentration of CO_2 and of NH_3 in tests using reduction in percentage of cell division in germinating fern spores. In this last paper ZIRKLE showed that there was a difference in response with two intensities of X-rays. ZIRKLE concludes that change in the physiological conditions of the organism influences the radiosensitivity of several different systems which are damaged by irradiation. SCHNEIDERMAN and KING (1953) tested the effect of CO_2 on chromosome damage by X-rays in Tradescantia. In pure CO_2 the number of abnormalities produced by X-rays was equivalent to that in a vacuum. If CO_2 is added to one atmosphere of air, there is a rapid increase in percent of rearrangements above that in air to about 1.8 times at 0.1 atmosphere CO_2. Thereafter the increase in damage with increase in CO_2 is less rapid but consistent, unlike the variations found by ZIRKLE. If the CO_2 was added immediately after irradiation, there was no increase in rearrangement rate. They conclude that the CO_2 influences the indirect effects of irradiation which involve oxygen. Our own results with carbon dioxide show decided variations with different physiological conditions. We have no test of effectiveness in relation to concentration. Some of the variations in radiation damage in the mixtures of CO_2 with O_2 and CO might be due to concentration effects such as ZIRKLE reported.

The relation of the cytochrome system to radiation damage was investigated with carbon monoxide mixtures during irradiation. The effect of this enzyme poison is quickly reversed in Drosophila when the organisms are returned to air so that oxygen must reactivate the cytochrome system as suggested by KING, SCHNEIDERMAN and SAX (1952) from their work in Tradescantia. This is borne out in our material where X-ray induced translocations are more frequent in 95% $CO + 5\%$ O_2 (39) than in 80% $CO + 20\%$ O_2 (34) at the normal temperature for physiological activity in Drosophila. Test 33 indicates that oxygen does not restore the protective capacity of the cytochrome system at $0°C$. When test 32 is compared to 34, we find that some types of injury must require slower irradiation and require time for cumulative action to effect breaks.

Carbon dioxide reduces the radiation damage ordinarily occurring in carbon monoxide–oxygen mixtures in most combinations of temperature and gas mixtures. In fact the frequency of translocations is reduced to the lowest values

for 2000 r in 66 seconds at 25°C, the temperature where most enzyme sys-
tems will be active.

SUMMARY

Investigations were made to determine the extent of X-radiation damage
due to indirect effects by varying the physiological conditions during irradia-
tion of the test organism, *Drosophila virilis*. Genetic damage was measured as
recoverable translocations, which involve breakage and recombination of parts
of two or more chromosomes. The effects of temperature, oxygen, carbon
monoxide, and carbon dioxide in various combinations were tested, using two
rates of irradiation, fast at approximately 2000 r per minute and slow at 100
r per minute.

One spontaneous translocation was found in 2367 tests where such trans-
locations could be detected. Therefore the spontaneous rate is too low to modify
any data appreciably.

In air and other oxygen-nitrogen mixtures, where we can regard nitrogen
as inert and oxygen as an active agent in the indirect effects producing radia-
tion damage, there is a direct relation between the amount of oxygen in the
gas and the genetic changes. As the amount of oxygen increases, the damage
to the chromosomes increases. The greater damage at 0°C compared to 25°C
may be attributed to the greater solubility $(1.6\times)$ of oxygen in water at the
lower temperature, together with a greater saturation of the oxygen receptors
with increased concentration of oxygen.

In tests using oxygen–nitrogen mixtures, the amount of induced genetic
change is much greater with fast irradiation than with slow. If we lump all
paired tests into fast versus slow irradiation, we have over five thousand tested
cultures in each. In eight of the ten pairs, damage was greater at the fast
rate. The average was $15.95 \pm 0.5\%$ for fast versus $13.39 \pm 0.5\%$ for slow
irradiation, with the difference $2.56 \pm 0.7\%$ for all experiments. As there is
no evidence for healing in the sperm, this must be due to the rapid production
of such a high concentration of active radicals in the water and their reactive
derivatives that the natural protective systems, which reduce the damage due
to these chemical effects, are unable to protect the chromosomes.

If nitrogen is replaced by carbon dioxide, the radiation damage is increased,
particularly with slow irradiation. The increase in carbon dioxide concentra-
tion may lower the pH, disrupt the regular oxidation-reduction systems, and
increase the concentration and stability of OH, O_2H and H_2O_2; each would
contribute to radiation damage. Carbon monoxide, which ties up the cyto-
chrome and so interferes with the destruction of oxidative radicals, increases
radiation damage in slow doses and fast doses at 2°C, but not fast doses at
25°C, another illustration of the complexity of the protecting systems. The
fact that both carbon dioxide and carbon monoxide, which can influence only
the indirect chemical effects of radiation, increase damage with slow irradia-
tion to equal that with fast irradiation indicates that the greater damage of
the latter in oxygen–nitrogen mixtures is due to the greater concentration of

active radicals, leading to chemical damage to the genetic system. When all the tests are compared, the indirect effects of radiation account for more than 50 percent of all demonstrable genetic changes in *Drosophila virilis*.

Mixtures of CO, CO_2 and O_2 produce less radiation damage than either $CO + O_2$ or $CO_2 + O_2$, again indicating that the protective systems and interaction of active radicals are very complex problems and that these types of radiation damages are not simply cumulative.

The Y chromosome was involved about six-tenths as frequently as any one major autosome. The Y is heterochromatic and somewhat longer than any autosome in the ordinary metaphase. As from one-third to one-half the length of the autosomes is due to heterochromatin, our tests show a greater rearrangement frequency for the euchromatin.

Among those translocations with four chromosomes involved, the relative frequency of two-and-two translocations to all four chromosome translocations shows that reunion of broken chromosome ends is not at random in *Drosophila virilis*.

Protection against radiation damage by the cell seems to be due to the physiological mechanisms regularly involved in oxidative metabolism such as the —SH compounds and the cytochrome, catalase, and correlated systems.

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