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THE EFFECT OF OXYGEN ON THE FREQUENCY OF SOMATIC RECOMBINATION IN *DROSOPHIA MELANOGASTER*[1]

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ABSTRACT

The influence of oxygen on the frequency of somatic recombination in the yellow singed system on the *X* chromosome of *Drosophila melanogaster* was studied under a variety of experimental conditions. Flies raised from egg to adult in atmospheres containing 70–90% oxygen were found to have significantly more mosaic spots on their abdominal tergites than were observed in flies which developed in air. First instar larvae X-rayed in from 0 to 100% oxygen demonstrated the existence of an oxygen effect for somatic recombination in the cells which form the abdominal hypoderm. The mosaic spot counts, beginning with the lowest numbers which were found in flies X-rayed in nitrogen, increased rapidly with rising oxygen tensions until the percentage in air was reached, then leveled off at the higher concentrations. Post-treatment with nitrogen of larvae X-rayed in air or oxygen created a substantially higher number of mosaic spots than were found when larvae, after being similarly irradiated, were instead placed into air or oxygen.

THE dependence of radiosensitivity on oxygen, the "oxygen effect", first clearly demonstrated in the root tips of *Vicia faba* (THODAY and READ 1947), is found in nearly all forms of life and in many biological systems of genetic interest, including some in Drosophila. A reduced number of recessive sex-linked mutations is produced in male *Drosophila melanogaster* X-rayed in nitrogen compared to flies treated identically in pure oxygen (BAKER and SGOURAKIS 1950). The effectiveness of X-rays in blocking the action of a suppressor gene is decreased when the flies are irradiated in oxygen concentrations below that found in air (GLASS and PLAINE 1952). The ability of anoxia to protect against radiation damage has been noted also in studies of dominant lethality in *D. melanogaster* (BAKER and VON HALLE 1953) and in *D. virilis* (ALEXANDER and BERGENDAHL 1961). In X-rayed *D. virilis* sperm less than one-fourth as many translocations are formed when the males are in nitrogen during treatment than when they are irradiated in oxygen (BAKER and EDINGTON 1952). Analogous results have been obtained in *D. melanogaster* for radiation-produced chromosome breaks (LÜNING 1954) and translocations (ABRAHAMSON 1959).

A high incidence of mosaicism for eye color, bristle shape and hypoderm color

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result when early developmental stages of flies heterozygous for such mutations are treated with ionizing radiation (SHAPIRO 1941; LEFEVRE 1948). Such mosaicism is largely attributable to somatic recombination (BECKER 1957). Of relevance to the present investigation is that in X-rayed diploid yeast the percentage of sectored colonies among those surviving is smaller when the radiation has been given in nitrogen instead of air (MORTIMER *et al.* 1965). Since the sectored colonies are primarily the result of mitotic recombination it is evident that an oxygen effect exists for this genetic phenomenon in diploid strains of *Saccharomyces cerevisiae*. The work reported here was undertaken to determine whether an oxygen effect for somatic recombination can be detected in *D. melanogaster*, a more complex organism. A preliminary report has appeared (STAUFFER 1969).

MATERIALS AND METHODS

Drosophila stocks: Four stocks were employed, one carrying yellow, another singed, a third both yellow and singed, and a fourth, Oregon RC, wild type. The loci of the *X*-linked recessives yellow (*y*) and singed (*sn*^{3}) are at positions 0.0 and 21.0 respectively. Yellow in the homozygous state is expressed by yellow body color and light brown bristles with yellow tips, singed by bristles which are slightly shortened and strikingly gnarled.

The flies were grown in half-pint bottles on the commonly used Drosophila food, consisting of a mixture of corn meal, molasses, agar, yeast, and water, plus a small amount of Tegosept, a mold preventative.

For all experiments one of two types of matings were used: yellow females with singed males or yellow singed females with Oregon RC males. The female offspring of the first cross are *trans* heterozygotes (y +/+ *sn*^{3}), those of the second *cis* heterozygotes (*y sn*^{3}/+/+).

During development of the larvae recombination between the X-chromosomes can occur spontaneously at the four-strand stage, giving rise to the possibility of homozygosity for either or both of the mutant genes among the descendants of the cells in which this event took place. Recombination in cells destined to form bristles or hypoderm is reflected in the appearance of mosaic spots visible in the adult fly. In females of the genotype *y* +/+ *sn*^{3} twin spots, consisting of straight yellow bristles adjacent to black gnarled ones, are formed when somatic recombination takes place between the centromere and the gene singed. Such spots, readily differentiated from the background of straight black bristles, are recorded as single spots. Recombination between singed and yellow results in spots made up of yellow bristles. Double recombination involving two or all four strands, with one event between the centromere and singed and the other between singed and yellow, produces spots of singed bristles. Females with the genotype *y sn*^{3}/ + + are found, most frequently, with spots comprised of bristles with both the yellow color and the gnarled shape, the result of recombination between the centromere and the gene for singed. Recombination between singed and yellow leads to the formation of straight yellow bristles. Double recombination in which two or four strands take part, with an event on each side of singed, produces spots containing singed bristles. In both genotypes double recombination involving three strands results in yellow mosaic spots (STERN 1936).

Experimental procedures: The parental stocks and the flies used in the experiments were kept at a temperature of 25°C, ± 0.5°C. Virgin females with genotypes *y* +/*y* + or *y sn*^{3}/*y sn*^{3} were mated, at two to seven days of age, with the appropriate males.

For the whole-life exposures to various concentrations of oxygen the newly mated flies, in bottles partially filled with Drosophila medium, were placed into a gas-tight compartment within the incubator. Gas mixtures with the desired oxygen concentrations (prepared by mixing either oxygen or nitrogen with air) were led into this compartment at a regulated rate. The offspring were allowed to develop from fertilized egg to adult in a particular gas mixture. They were then killed with either vapor, frozen and storted at —0.5°C.

In the X-ray experiments the appropriately mated flies were given 4 hr in which to lay eggs

on a Petri dish covered with a thin layer of Drosophila medium divided into quadrants. Thirty-one hrs following the termination of the egg laying period the first instar larvae growing on the Petri dish were exposed for 2 hr to a specific oxygen concentration. Two of the quadrants were set aside as controls, the other two were irradiated at a completion of this 2 hr exposure while still in the specific oxygen concentration. Thus the larvae were irradiated 35 hr ± 2 hr, after egg deposition. The X-ray dose employed was 1326 r, given a rate of 102 r per minute (140 kV; 4 mA; 1.5 mm inherent aluminum filtration; 23 cm tube to target distance). Following the X-ray treatment the irradiated larvae and also the unirradiated controls were either subjected to the same or to a different gas mixture for another 2 hr or return directly to air and deposited onto the usual Drosophila food in half-pint bottles. The larvae placed in a post-radiation gaseous environment were, after 2 hr, also returned to air and fresh food. All larvae, irradiated or not, were then permitted to develop without further interference. After hatching the flies were killed and frozen as previously noted.

Preparation of abdomens and recording of mosaic spots: The first step in preparing the frozen flies for study was to dissect the abdomen away from the thorax and to squeeze out and remove most of the abdominal contents. The abdomens were mounted, in an ordered array of ten, on glass slides in "Euparal" and under coverslips according to techniques recommended by HANNAH (1953) and WALEN (personal communication). Gentle pressure on the coverslips flattened the abdomens without seriously distorting the anatomical relationships. By these procedures permanent mounts were made. After a suitable drying period the abdomens were examined under a binocular microscope in transmitted, unfiltered light, generally at a magnification of 150× but at times, when the phenotype of a bristle was in doubt, at a magnification of 430×.

The frequency of somatic recombination was studied by determining the number of mosaic spots found on the abdominal tergites. Only tergites 2 to 6 were examined, for much of tergite 1 was usually lost during dissection and the relatively small tergites 7 and 8, in the genital and anal areas, were usually inadequately flattened and hence were difficult to scrutinize accurately. Each mosaic spot was mapped, bristle by bristle, onto a full page outline of the dorsal aspect of the female abdomen.

The bristles of a mosaic spot, irrespective of phenotype, tended to be strung out laterally, at right angles to the anterior-posterior axis of the fly. Frequently, wild-type bristles were found interspersed with those showing the mutant characteristic. To decide whether a series of bristles represented one spot or two at times seemed to require an arbitrary decision. The situation was especially complex for the flies with genotype $y\ +/+\ sn^s$, for in these the finding of a yellow bristle at some distance from a singed bristle could be interpreted either as a twin spot or as two separate spots, one yellow, the other singed.

In order to standardize the collection of data it was decided to permit, in *trans* heterozygotes, the recording of only one spot per half tergite. Thus, even if the bristles were widely separated but on the same half tergite, the occurrence of a single original genetic event was assumed. For example, two yellow bristles at opposite ends of a half tergite would be scored as a single yellow spot, and a yellow bristle far from a singed bristle would lead to the mapping of a sole twin spot. A yellow bristle and a singed bristle which are not part of twin spots would, if found on the same half tergite, be recorded as a twin spot and consequently result in an overestimation of the incidence of recombination proximal to the gene for singed. This propensity was probably more than compensated for by the fact that some of the yellow and some of the singed spots seen were in reality twin spots of whom only one of the two cell types survived and formed bristles. In flies with the genotype $y\ sn^s/++$ the mosaic spots yellow, singed, and yellow singed were assumed to be the product of separate genetic events and therefore were counted individually. This permitted the recording, in *cis* heterozygotes, of a maximum of three spots, one of each kind, per half tergite since two bristles of the same phenotype, no matter how far apart within a half tergite, were counted as only a single spot.

RESULTS

The standard experiments: In order to determine the number of mosaic spots

TABLE 1

Frequency of mosaic spots in the standard series

Genotype	Experiment*	Yellow	Singed	Twin or yellow singed†	Total	Mean number of spots per abdomen	S.D. of spots per abdomen
$y + / + \ sn^3$	1–6	160 (40.7%)	127 (32.3%)	106 (27.0%)	393 (100%)	.33	.56
$y \ sn^3 / + \ +$	1–6	327 (33.3%)	86 (8.7%)	571 (58.0%)	984 (100%)	.82	.93

* In each experiment 200 abdomens were examined.
† Twin in $y + / + \ sn^3$, yellow singed in $y \ sn^3 / + \ +$.

which are the result of "spontaneous" somatic recombination, groups of *D. melanogaster* were reared under standard conditions within the incubator. The results of these experiments, which were spaced at convenient intervals during the investigation, are given in Table 1. It is evident that the number of mosaic spots is about two and a half times greater in the *cis* than in the *trans* heterozygotes. This inequality is not unexpected since it has been demonstrated that the frequency of somatic recombination in the yellow and singed system is governed by the action of other chromosomal factors, by genes not only on the *X* chromosomes but on the two large autosomes as well (WEAVER 1960). In neither genotype is there an increase or decrease in the mean number of mosaic spots from experiment 1, done at the beginning, to experiment 6, eighteen months later. Chi-square tests of homogeneity do not demonstrate any significant shifts in the frequencies of the different kinds of spots from experiment to experiment. The average number of spots per fly is small, always less than one. The data from these and subsequent experiments follow a Poisson distribution.

The relative frequencies of spot types in the two genotypes are dissimilar. It is generally assumed that these differences result from the fact that in the *trans* heterozygotes the descendants of a cell in which somatic recombination occurred proximal to singed do not always form a twin spot containing both yellow and singed bristles but may instead produce only yellow or singed bristles. Consequently, there are observed excessive numbers of yellow spots and singed spots and fewer twin spots than the genetic events warrant.

It is possible to characterize the two genotypes further than is shown in Table 1 by estimating the proportions of spots ascribable to the different recombination types. In the *cis* heterozygotes the 571 yellow spots are due to single recombination proximal to singed, and the 86 singed spots are due to double recombination taking place simultaneously on both sides of singed. Of all the spots resulting from double recombination of this kind two-thirds are singed and one-third yellow. Therefore, of the 327 yellow spots 43 are due to double recombination, while the remaining 284 are the result of single recombination distal to singed. Double recombination in three-fourths of cases but single recombination in only one-half lead to mosaic spots. Hence a reduction by one-third in the number of spots from double recombination is required to reach a valid result: 2/3 (86 +

43) : 86 doubles. The proportion of spots due to single recombination proximal to singed: single recombination distal to singed: double recombination is 571 : 284 : 86, or, in reduced form, 6.6 : 3.3 : 1. The argument followed above is that of STERN (1936).

In the *trans* heterozygotes the inability to identify all spots resulting from single recombination proximal to singed precludes an independent computation of the spot proportions. Nevertheless, an estimate can be made if it is assumed that the relative frequency of single recombination proximal to singed is equal in the two genotypes. If both daughter cells survive this kind of recombination and are represented by bristles in the hypoderm, a twin spot will be seen in the *trans* and a yellow singed spot in the *cis* heterozygotes. If the descendants of only one daughter cell produce bristles, a yellow spot or a singed spot is formed in the *trans* heterozygotes and a yellow singed spot or an undetectable wild-type "spot" in the *cis* heterozygotes. Thus, if only one daughter cell is represented by bristles, the *trans* will form twice as many mosaic spots as the *cis* genotype. There is a difference of 31% between the sums of the yellow and singed spots in the two genotypes, a difference caused by the presence, in the *trans* configuration, of yellow and singed spots contributed by single recombination proximal to singed. Adding half of this 31 percent, or 15.5 percent, to the 58 percent which Table 1 shows as the percentage of yellow singed spots gives 73.5%, which for the *trans* genotype is an approximation of the percentage of spots due to single recombination proximal to singed. Seventy-three point five percent of the total spot number of 393 equals 289 spots. If both daughter cells initiate bristle formation, these 289 should be twin spots. Since only 106 twin spots are seen there must be 289 − 106, or 183 spots, which arose from single recombination proximal to singed but contain only yellow or singed bristles. The 183 spots must be subtracted from the yellow and singed totals to leave the spots resulting from single recombination distal to singed and double recombination. To try to compensate for the slightly greater viability of the singed phenotype the subtractions are made in proportion to the average number of bristles in the twin spots (2.4 yellow, 2.7 singed): 160 − 86 = 74 yellow spots; 127 − 97 = 30 singed spots. Correcting for the one-third of double recombinants which are yellow and for the variation in detection between single and double recombinants gives the proportion of spots due to single recombination proximal to singed: single recombination distal to singed: double recombination to be 289 : 59 : 30, or 9.6 : 2.0 : 1. Other methods of estimating the spot frequencies in the *trans* genotype have been devised by BROWN *et al.* (1962) and by BAKER and SWATEK (1965).

Life-long exposures to various oxygen concentrations: In this set of experiments flies were kept in various concentrations of oxygen throughout their development. Such prolonged exposures maximized the possibility of detecting any existing influences of oxygen on somatic recombination and also avoided problems posed by differences in sensitivity to oxygen between the several stages of development.

Drosophila is resistant to pure oxygen, even at several atmospheres of pressure (WILLIAMS and BEECHER 1944). The genus is also known to survive for a matter

TABLE 2

Frequency of mosaic spots following life-long exposures to various concentrations of oxygen

Genotype	Percent oxygen*	Numbers of spots				Mean number of spots per abdomen	S.D. of spots per abdomen
		Yellow	Singed	Twin or yellow singed	Total		
$y+/+\ sn^3$	7.5	16	17	17	50	.25	.51
	10	19	22	15	56	.28	.55
	15	32	16	22	70	.35	.63
	25	32	19	13	64	.32	.54
	30	21	23	27	71	.36	.58
	40	30	21	25	76	.38	.62
	50	31	21	21	73	.37	.56
	60	32	24	15	71	.36	.55
	70	42	25	39	106	.53	.72
	80	56	38	40	134	.67	.83
	90	64	47	39	150	.75	.85
Chi-square test of homogeneity of the different spot types: P = .30–.50							
$y\ sn^3/+\ +$	7.5	32	15	81	128	.64	.82
	10	53	15	86	154	.77	.94
	15	44	17	87	148	.74	.89
	25	53	23	126	202	1.01	.95
	30	54	18	96	168	.84	1.02
	40	56	30	124	210	1.05	1.14
	50	58	15	124	197	.99	1.24
	60	50	24	130	204	1.02	1.04
	70	44	25	160	229	1.15	1.01
	80	65	27	172	264	1.32	1.22
	90	91	34	199	324	1.62	1.62
Chi-square test of homogeneity of the different spot types: P = .30–.50							

* At each oxygen concentration in both genotypes 200 abdomens were examined.

of hours in pure nitrogen (ABRAHAMSON 1958). That the range of oxygen tolerance is considerable was ascertained early in the present study: the flies were able to live, with some reduction in activity at the highest and lowest levels, in gaseous mixtures containing as much as ninety and as little as seven and a half percent oxygen. No gross abnormalities in morphology or numbers of offspring were detected in flies raised at oxygen levels from seven and a half through sixty percent. From seventy percent on the individual insects seemed normal but the numbers appearing in the cultures declined. At ninety percent there was a noticeable mortality among the early larval stages. In one of the oxygen concentrations was the time of development prolonged.

Table 2 and Figure 1 show that increased numbers of mosaic spots are found in flies reared in the higher oxygen tensions. This rise in the frequency of mosaicism appears to take place abruptly at seventy percent in the *trans* heterozygotes and more gradually, but at about the same oxygen concentration, in the *cis* heterozygotes. In both genotypes a slight decrease in spot numbers is evident at oxygen levels below the nearly twenty-one percent found in air. A comparison with the standard experiments demonstrates that, at ninety percent, the mean

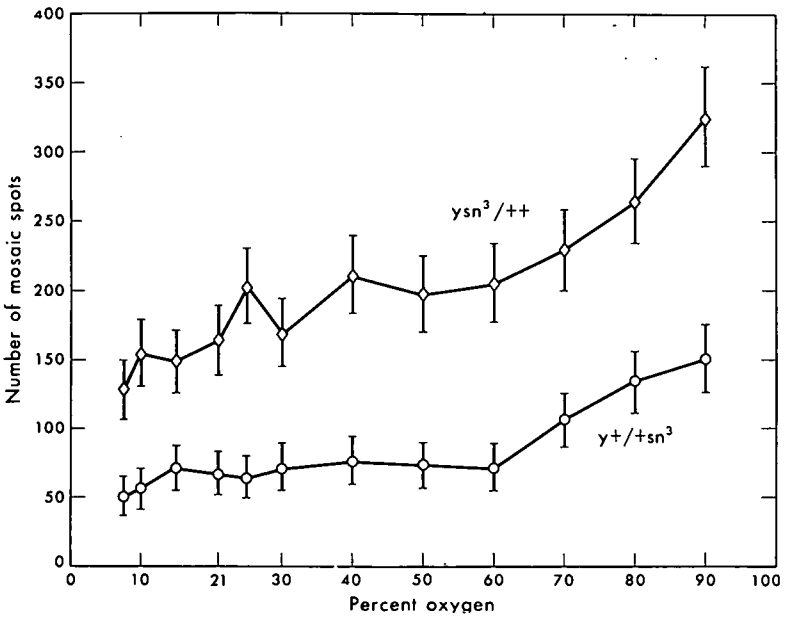

FIGURE 1.—95% confidence limits (Poisson model, see CROW and GARDNER 1959) of the mosaic spot frequencies following life-long exposures to various concentrations of oxygen. (The limits given for air were computed from the means of the standard experiments.)

number of mosaic spots per abdomen in the *trans* heterozygotes is 2.3 and in the *cis* heterozygotes 2.0 times the mean spot totals obtained when the flies are allowed to develop in air. All spot types participate in the increase in spot numbers at seventy percent and higher. The mean frequencies of twin and yellow singed spots in the cultures raised at seventy percent and higher. The mean frequencies of twin and yellow singed spots in the cultures raised at seventy percent or above, 26.0 and 61.4%, respectively, are similar to the corresponding spot frequencies, 27.0 and 58.0%, found in the standard experiments.

Since it seemed possible that oxygen in high concentrations might induce phenocopies of the mutant bristles, Oregon RC flies were reared in ninety percent oxygen. Examination of fifty abdomens of these wild-type flies, however, revealed no grossly abnormal bristles and, specifically, none which might be misclassified as yellow or singed or yellow singed.

X-ray experiments at different oxygen concentrations: First instar larvae were selected for irradiation because of the relative ease with which homogeneous populations of this life stage could be collected. Later larval stages tended to diverge with respect to the rate of development. The X-ray dose chosen, 1326 r, is large enough to insure a detectable increase in mosaicism but not so large as to produce an impractically high mortality. ABBADESSA and BURDICK (1963), on the recommendation of BECKER, used an approximately equal dose, 1350 r, in obtaining an enhancement by a factor of three to four over the natural mosaic spot frequency.

TABLE 3

Frequency of mosaic spots following irradiation in different concentrations of oxygen. Genotype y +/+ sn^3

	Percent oxygen*	Numbers of spots				Mean number of spots per abdomen	S.D. of spots per abdomen
		Yellow	Singed	Twin	Total		
Controls	0†	14	10	9	33	.33	.57
	2	14	10	13	37	.37	.66
	5	13	11	14	38	.38	.63
	10	8	12	5	25	.25	.52
	21	13	10	16	39	.39	.63
	50	12	13	14	39	.39	.57
	100	14	8	6	28	.28	.57
	Chi-square test of homogeneity of the different spot types: P = .50–.70						
X-rayed	0†	29	25	43	97	.97	1.02
	2	60	42	94	196	1.96	1.29
	5	56	64	113	233	2.33	1.37
	10	89	70	133	292	2.92	1.43
	21	102	83	131	316	3.16	1.55
	50	68	89	133	290	2.90	1.49
	100	89	96	144	329	3.29	1.60
	Chi-square test of homogeneity of the different spot types: P = .30–.50						

* At each oxygen concentration in both controls and X-ray experiments 100 abdomens were examined.

† i.e. 100 percent nitrogen.

Inspection of the X-rayed Drosophila prior to dissection did not reveal any abnormalities of the abdomen or elsewhere. None of the flies exhibited deficiencies of pigmentation. No delay in the time required to complete development was noted though this has been reported by BOURGIN and coauthors (1956) for Drosophila larvae given X-ray doses as low as 1000 r.

Two findings of interest are contained in the information displayed in Tables 3 and 4. The first of these, seen in the controls, is the apparent absence of any effect on the rate of somatic recombination by exposures to various oxygen concentrations for periods of two hours. The second, in the X-rayed flies, is the dependence of the rate of somatic recombination on the percent of oxygen present at the time the larvae were X-rayed. The number of mosaic spots found on the tergites is lowest among Drosophila irradiated in pure nitrogen and highest in flies treated in pure oxygen. In the *trans* heterozygotes there are almost 3.4 times as many mosaic spots on the abdomens of flies X-rayed in oxygen as there are on those irradiated under anoxic conditions. The corresponding ratio for the *cis* heterozygotes is 3.2. Figure 2 shows these changes in sensitivity with increasing oxygen tensions.

Varying the oxygen concentrations does not greatly affect the relative frequencies of the different types of spots in either the controls or the X-rayed flies. However, a comparison of the irradiated flies with their controls reveals that among the *trans* heterozygotes those treated with X-rays have a greater proportion of twin spots and relatively fewer yellow spots and singed spots. Out of a

TABLE 4

Frequency of mosaic spots following irradiation in differenet concentrations of oxygen. Genotype y sn^3/+ +

	Percent oxygen*	Numbers of spots: Yellow	Singed	Yellow singed	Total	Mean number of spots per abdomen	S.D. of spots per abdomen
Controls	0†	14	2	49	65	.65	.81
	2	29	13	56	98	.98	1.08
	5	20	10	37	67	.67	.87
	10	16	9	52	77	.77	.97
	21	24	11	37	72	.72	1.00
	50	15	10	50	75	.75	.81
	100	30	17	47	94	.94	.87
	Chi-square test of homogeneity of the different spot types: P = .05–.10						
X-rayed	0†	42	10	93	145	1.45	1.32
	2	71	25	166	262	2.62	1.75
	5	73	24	209	306	3.06	1.94
	10	85	36	244	365	3.65	1.93
	21	115	51	263	429	4.29	2.24
	50	117	42	279	438	4.38	2.33
	100	123	38	309	470	4.70	1.96
	Chi-square test of homogeneity of the different spot types: P = .50–.70						

* At each oxygen concentration in both controls and X-ray experiments 100 abdomens were examined.

† i.e. 100 percent nitrogen.

total of 239 spots in the controls 77 or 32.2% are twin spots, while of 1753 spots on the abdomens of X-rayed flies 791 or 45.1% are twin spots. The comparatively large number of spots in the irradiated flies increases the probability that more than one spot will occur on a half tergite and, under the spot counting rules, tends to produce artificially high twin spot counts. In the *cis* heterozygotes the number of yellow singed spots in the controls is 328 or 59.9% of a total of 548 while in the X-rayed flies 1563 or 64.7% of a total of 2415 spots are yellow singed. The probability of having two or more yellow singed spots on the same half tergite must be higher in the irradiated flies than in the controls. Since in the *cis* heterozygotes the spot counting rules allow a maximum of one spot of each kind per half tergite it is likely that the actual percentage of yellow singed spots in the X-rayed flies exceeds the observed 64.7%.

No phenocopies of the mutant bristles were found in fifty Oregon RC flies exposed to pure oxygen for two hours. Irradiating first instar Oregon RC larvae with 1326 r, in air, did not produce any yellow or singed or yellow singed bristles. In a hundred of these irradiated wild-type flies a few bristles (about one every twenty or thirty abdomens) were partially depigmented or colored lighter than usual. It is unlikely that such bristles could be confused with those exhibiting the yellow phenotype.

Post-radiation exposures to nitrogen, air or oxygen: In order to determine whether the oxygen tension present after irradiation influences the frequency of somatic recombination groups of larvae of the *trans* genotype were placed, within

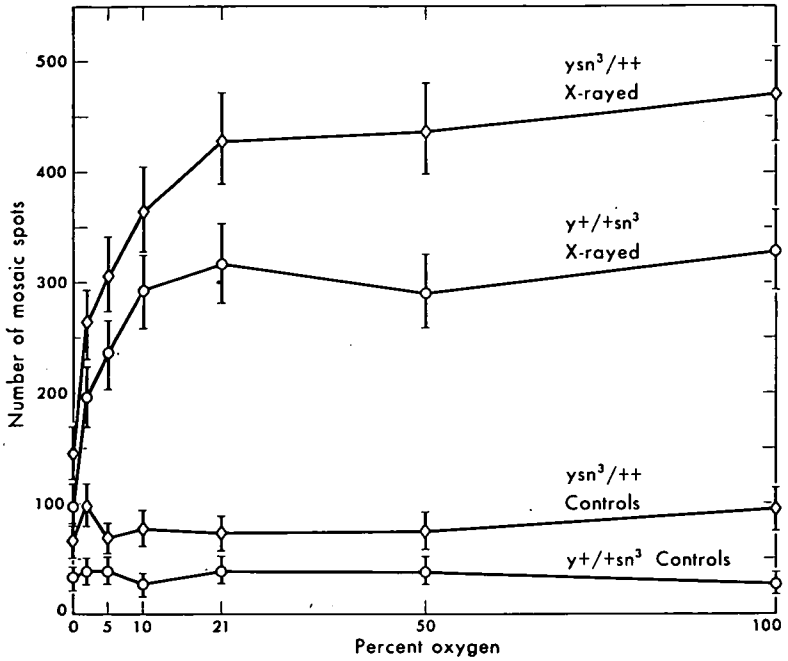

FIGURE 2.—95% confidence limits (Poisson model) of the mosaic spot frequencies following irradiation, with 1326 r of X rays, in different oxygen concentrations.

TABLE 5

Frequency of mosaic spots following irradiation in various concentrations of oxygen and of post-radiation exposures to either nitrogen, air or oxygen. Genotype y +/+ sn³

Percent oxygen during irradiation*	Percent oxygen following irradiation	Numbers of spots				Mean number of spots per abdomen	S.D. of spots per abdomen
		Yellow	Singed	Twin	Total		
0	0	30	37	57	124	1.24	1.02
0	21	29	25	43	97	.97	1.02
0	100	23	34	43	100	1.00	.91
Chi-square test of homogeneity of the different spot types: P = .50–.70							
21	0	141	148	139	428	4.28	1.65
21	21	101	83	131	316	3.16	1.55
21	100	98	98	100	296	2.96	1.58
Chi-square test of homogeneity of the different spot types: P = .05–.10							
100	0	146	130	142	418	4.18	1.60
100	21	89	96	144	329	3.29	1.60
100	100	99	84	126	309	3.09	1.50
Chi-square test of homogeneity of the different spot types: P = .05–.10							

* In each of the nine experiments 100 abdomens were examined.

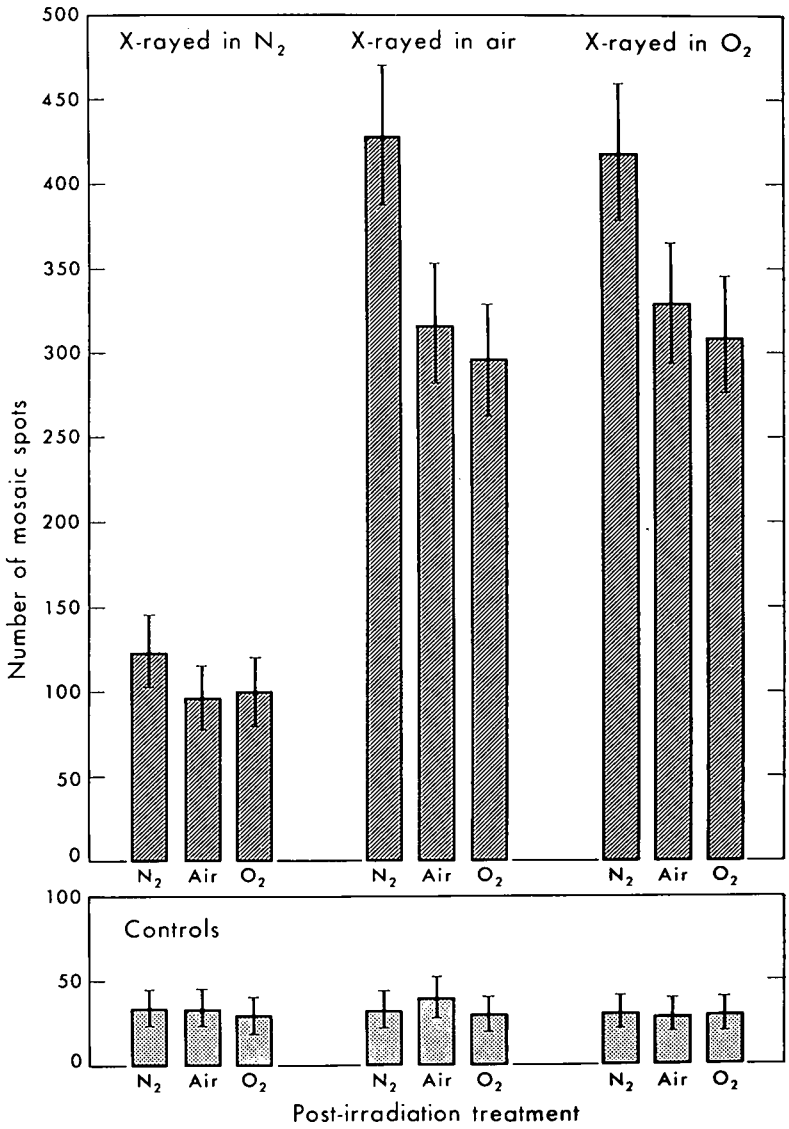

FIGURE 3.—95% confidence limits (Poisson model) of the mosaic spot frequencies following irradiation, with 1326 r of X rays, in different oxygen concentrations and post-radiation treatment with nitrogen, air or oxygen. Genotype $y + / + sn^3$.

a few seconds following the conclusion of the X-ray treatment, into nitrogen, air or oxygen for a period of two hours.

The results are shown in Table 5 and Figure 3. Larvae X-rayed in nitrogen have equal numbers of mosaic spots, regardless of the composition of the post-radiation atmosphere. On the other hand, larvae X-rayed in either air or oxygen and thereafter put into nitrogen are found to have greater numbers of abdominal

spots than do larvae which following irradiation were placed in air or oxygen. This rise in mosaicism is chiefly due to increases in the yellow spots and singed spots. Among the Drosophila X-rayed in air or oxygen those post-treated with oxygen have slightly lower numbers of mosaic spots than those post-treated with air. As was true for the other X-ray experiments the controls for this set also do not show any effects from being exposed to abnormally high or low oxygen concentrations for a few hours.

Bristle numbers and the size of the mosaic spots: When an attempt was made to grow *D. melanogaster* in five percent oxygen it was noted that in the few flies which reached the adult stage the number of bristles on the tergites were markedly reduced. This observation prompted the counting of bristles on randomly selected abdomens (ten in the standard experiments, five in each of the others). The mean bristle count in the standard experiments is 518 for the *trans* and 492 for the *cis* genotype. These figures are a little higher than the mean of 464 bristles which STERN (1936) reported for tergites one through six. Four hundred ninety-eight and 449 bristles, respectively, are found in the *trans* and *cis* heterozygotes given life-long exposures to oxygen levels of seventy percent or higher. In the X-ray experiments there are 501 bristles in the *trans* controls and 487 in the irradiated *trans* flies. The corresponding bristle counts for the *cis* heterozygotes are 487 and 463. In view of the small numbers of abdomens examined and the considerable variability in bristle numbers from fly to fly it is doubtful whether the slight decreases noted in the treated flies are of significance. Of interest is that the bristle counts in the *cis* genotype are consistently lower than those obtained in the *trans*.

The two genotypes differ also with respect to mosaic spot size. In the standard experiments the mean size of all spots in the *trans* heterozygotes is 2.7 bristles, that in the *cis* heterozygotes only 1.6 bristles. This disparity is largely due to the fact that twin spots cannot contain fewer than two bristles. The average twin spot consists of 5.1 bristles (2.4 yellow and 2.7 singed), the average yellow singed spot 1.7 bristles. The sizes of the yellow and singed spots, however, are similar though not identical: 1.7 and 1.7 bristles in the *trans* and 1.5 and 1.3 bristles in the *cis* heterozygotes. The whole life exposures to different oxygen concentrations appear to have no influence on spot size, but X-irradiation causes a uniform increase in all spot types. Thus, in the X-rayed *trans* heterozygotes the sizes of yellow, singed, and twin spots are 2.2, 2.5, and 6.6 bristles (3.4 yellow and 3.2 singed), and in the irradiated *cis* heterozygotes the yellow, singed, and yellow singed spots contain 1.6, 1.6, and 2.4 bristles, respectively. Comparable increases in the size of mosaic spots in X-rayed Drosophila have been previously described (ABBADESSA and BURDICK 1963).

DISCUSSION

Developmental studies of the abdominal hypoderm of Drosophila have demonstrated that both of the bilateral nests of imaginal cells from which each tergite is derived contain about eight cells, which throughout larval life probably remain

in the G_2 stage of the cell cycle (GARCIA-BELLIDO and MERRIAM 1971). If eight imaginal cells lead to the creation of all the hypodermal structures on a half tergite, including the approximately forty-eight bristles, then on the average one cell is responsible for one-eight of this complement, or six bristles. Thus, in the genotype y +/+ sn^s, somatic recombination occurring in one of the eight cells during its first division should produce a twin spot with six bristles, three yellow and three singed. Similarly, in the genotype y sn^s/+ +, a yellow singed spot with three bristles should be formed, the smaller spot size reflecting the fact that half of the descendants of the parent cell will be wild type. The average spot size in the standard experiments, 5.1 bristles per twin and 1.7 bristles per yellow singed spot, is a little smaller than might be anticipated on the basis of the reasoning above. The observation that many twin spots have fewer than six and many yellow singed spots less than three bristles suggests that spontaneous somatic recombination can occur during or prior to the first division of the imaginal cells but that often it takes place considerably later.

In the X-ray experiments the larvae were irradiated during the first instar, long before the surge of mitotic activity which in early pupal life forms the adult hypoderm. If, as it seems reasonable to assume, genetic damage is manifested in the first division following irradiation, twin and yellow singed spots with about six and three bristles, respectively, should be observed. The finding of a mean of 6.6 bristles for the twin and 2.4 bristles for the yellow singed spots closely approximates these expectations. However, these averages are based on a wide spectrum of spot sizes, including a substantial proportion of very small spots and some considerably larger than are found in unirradiated flies. The larger spots can be explained either by somatic recombination occurring in more than one of the eight cells or by assuming that, if not all eight cells remain viable after irradiation, the survivors compensate by covering a larger fraction of the half tergite. The presence of very small spots implies that part of the X-ray damage is latent and not expressed until after several cell divisions have taken place. An alternate explanation of the very small spots is that cells homozygous for one or both of the mutant genes may divide less frequently than wild-type cells.

The whole-life exposures demonstrate that oxygen levels several times as high as any which *D. melanogaster* could have experienced during its evolution can cause somatic recombination. The X-ray experiments show a typical oxygen effect. A life-long exposure to ninety percent oxygen is roughly equivalent, with respect to the quantity of induced mosaicism, to 1326 r given anoxic first instar larvae. In flies exposed during development to at least seventy percent oxygen or treated with X-rays all mosaic spot types are elevated but in the irradiated flies the numbers of twin and yellow singed spots are disproportionately large, indicating a relatively great increase in the rate of somatic recombination proximal to singed. These results agree with the findings of BECKER (1969) that the heterochromatin of the X chromosome is more radiosensitive than the euchromatin. The mechanisms by which oxygen and X-rays bring about somatic recombination remain obscure but it seems unlikely that only a very limited segment of the X chromosome will be found to be primarily involved, as is known to be the case

for somatic mosaicism caused by the dominant Drosophila mutants known as Minutes (RITOSSA, ATWOOD and SPIEGELMAN 1966).

The data obtained by varying the oxygen content of the post-radiation atmosphere can be interpreted as supporting the existence of an oxygen dependent system capable of repairing, at least partially, the damage created by X rays. It seems probable that the appearance of as much as a fourth of the potential mosaic spots is prevented by the availability of sufficient oxygen. The finding that post-radiation exposure of the larvae to nitrogen increases principally yellow and singed spots, and not twin or yellow singed spots, raises the possibility that oxygen-dependent repair takes places preferentially in the euchromatin and only to a slight extent, if at all, in the proximal heterochromatin.

It is a pleasure to thank Dr. CURT STERN for his guidance and stimulating discussions during the course of this investigation. I would also like to thank WILLIAM HOGAN for his help in making possible the use of a computer to perform many of the routine computations.

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THE EFFECT OF OXYGEN ON THE FREQUENCY OF SOMATIC RECOMBINATION IN *DROSOPHIA MELANOGASTER*[1]

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ABSTRACT

The influence of oxygen on the frequency of somatic recombination in the yellow singed system on the *X* chromosome of *Drosophila melanogaster* was studied under a variety of experimental conditions. Flies raised from egg to adult in atmospheres containing 70–90% oxygen were found to have significantly more mosaic spots on their abdominal tergites than were observed in flies which developed in air. First instar larvae X-rayed in from 0 to 100% oxygen demonstrated the existence of an oxygen effect for somatic recombination in the cells which form the abdominal hypoderm. The mosaic spot counts, beginning with the lowest numbers which were found in flies X-rayed in nitrogen, increased rapidly with rising oxygen tensions until the percentage in air was reached, then leveled off at the higher concentrations. Post-treatment with nitrogen of larvae X-rayed in air or oxygen created a substantially higher number of mosaic spots than were found when larvae, after being similarly irradiated, were instead placed into air or oxygen.

THE dependence of radiosensitivity on oxygen, the "oxygen effect", first clearly demonstrated in the root tips of *Vicia faba* (THODAY and READ 1947), is found in nearly all forms of life and in many biological systems of genetic interest, including some in Drosophila. A reduced number of recessive sex-linked mutations is produced in male *Drosophila melanogaster* X-rayed in nitrogen compared to flies treated identically in pure oxygen (BAKER and SGOURAKIS 1950). The effectiveness of X-rays in blocking the action of a suppressor gene is decreased when the flies are irradiated in oxygen concentrations below that found in air (GLASS and PLAINE 1952). The ability of anoxia to protect against radiation damage has been noted also in studies of dominant lethality in *D. melanogaster* (BAKER and VON HALLE 1953) and in *D. virilis* (ALEXANDER and BERGENDAHL 1961). In X-rayed *D. virilis* sperm less than one-fourth as many translocations are formed when the males are in nitrogen during treatment than when they are irradiated in oxygen (BAKER and EDINGTON 1952). Analogous results have been obtained in *D. melanogaster* for radiation-produced chromosome breaks (LÜNING 1954) and translocations (ABRAHAMSON 1959).

A high incidence of mosaicism for eye color, bristle shape and hypoderm color

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result when early developmental stages of flies heterozygous for such mutations are treated with ionizing radiation (SHAPIRO 1941; LEFEVRE 1948). Such mosaicism is largely attributable to somatic recombination (BECKER 1957). Of relevance to the present investigation is that in X-rayed diploid yeast the percentage of sectored colonies among those surviving is smaller when the radiation has been given in nitrogen instead of air (MORTIMER *et al.* 1965). Since the sectored colonies are primarily the result of mitotic recombination it is evident that an oxygen effect exists for this genetic phenomenon in diploid strains of *Saccharomyces cerevisiae*. The work reported here was undertaken to determine whether an oxygen effect for somatic recombination can be detected in *D. melanogaster*, a more complex organism. A preliminary report has appeared (STAUFFER 1969).

MATERIALS AND METHODS

Drosophila stocks: Four stocks were employed, one carrying yellow, another singed, a third both yellow and singed, and a fourth, Oregon RC, wild type. The loci of the *X*-linked recessives yellow (y) and singed (sn^3) are at positions 0.0 and 21.0 respectively. Yellow in the homozygous state is expressed by yellow body color and light brown bristles with yellow tips, singed by bristles which are slightly shortened and strikingly gnarled.

The flies were grown in half-pint bottles on the commonly used Drosophila food, consisting of a mixture of corn meal, molasses, agar, yeast, and water, plus a small amount of Tegosept, a mold preventative.

For all experiments one of two types of matings were used: yellow females with singed males or yellow singed females with Oregon RC males. The female offspring of the first cross are *trans* heterozygotes ($y\ +/+\ sn^3$), those of the second *cis* heterozygotes ($y\ sn^3/+/+$).

During development of the larvae recombination between the X-chromosomes can occur spontaneously at the four-strand stage, giving rise to the possibility of homozygosity for either or both of the mutant genes among the descendants of the cells in which this event took place. Recombination in cells destined to form bristles or hypoderm is reflected in the appearance of mosaic spots visible in the adult fly. In females of the genotype $y\ +/+\ sn^3$ twin spots, consisting of straight yellow bristles adjacent to black gnarled ones, are formed when somatic recombination takes place between the centromere and the gene singed. Such spots, readily differentiated from the background of straight black bristles, are recorded as single spots. Recombination between singed and yellow results in spots made up of yellow bristles. Double recombination involving two or all four strands, with one event between the centromere and singed and the other between singed and yellow, produces spots of singed bristles. Females with the genotype $y\ sn^3/+\ +$ are found, most frequently, with spots comprised of bristles with both the yellow color and the gnarled shape, the result of recombination between the centromere and the gene for singed. Recombination between singed and yellow leads to the formation of straight yellow bristles. Double recombination in which two or four strands take part, with an event on each side of singed, produces spots containing singed bristles. In both genotypes double recombination involving three strands results in yellow mosaic spots (STERN 1936).

Experimental procedures: The parental stocks and the flies used in the experiments were kept at a temperature of 25°C, ± 0.5°C. Virgin females with genotypes $y\ +/y\ +$ or $y\ sn^3/y\ sn^3$ were mated, at two to seven days of age, with the appropriate males.

For the whole-life exposures to various concentrations of oxygen the newly mated flies, in bottles partially filled with Drosophila medium, were placed into a gas-tight compartment within the incubator. Gas mixtures with the desired oxygen concentrations (prepared by mixing either oxygen or nitrogen with air) were led into this compartment at a regulated rate. The offspring were allowed to develop from fertilized egg to adult in a particular gas mixture. They were then killed with either vapor, frozen and storted at —0.5°C.

In the X-ray experiments the appropriately mated flies were given 4 hr in which to lay eggs

on a Petri dish covered with a thin layer of Drosophila medium divided into quadrants. Thirty-one hrs following the termination of the egg laying period the first instar larvae growing on the Petri dish were exposed for 2 hr to a specific oxygen concentration. Two of the quadrants were set aside as controls, the other two were irradiated at a completion of this 2 hr exposure while still in the specific oxygen concentration. Thus the larvae were irradiated 35 hr ± 2 hr, after egg deposition. The X-ray dose employed was 1326 r, given a rate of 102 r per minute (140 kV; 4 mA; 1.5 mm inherent aluminum filtration; 23 cm tube to target distance). Following the X-ray treatment the irradiated larvae and also the unirradiated controls were either subjected to the same or to a different gas mixture for another 2 hr or return directly to air and deposited onto the usual Drosophila food in half-pint bottles. The larvae placed in a post-radiation gaseous environment were, after 2 hr, also returned to air and fresh food. All larvae, irradiated or not, were then permitted to develop without further interference. After hatching the flies were killed and frozen as previously noted.

Preparation of abdomens and recording of mosaic spots: The first step in preparing the frozen flies for study was to dissect the abdomen away from the thorax and to squeeze out and remove most of the abdominal contents. The abdomens were mounted, in an ordered array of ten, on glass slides in "Euparal" and under coverslips according to techniques recommended by HANNAH (1953) and WALEN (personal communication). Gentle pressure on the coverslips flattened the abdomens without seriously distorting the anatomical relationships. By these procedures permanent mounts were made. After a suitable drying period the abdomens were examined under a binocular microscope in transmitted, unfiltered light, generally at a magnification of 150× but at times, when the phenotype of a bristle was in doubt, at a magnification of 430×.

The frequency of somatic recombination was studied by determining the number of mosaic spots found on the abdominal tergites. Only tergites 2 to 6 were examined, for much of tergite 1 was usually lost during dissection and the relatively small tergites 7 and 8, in the genital and anal areas, were usually inadequately flattened and hence were difficult to scrutinize accurately. Each mosaic spot was mapped, bristle by bristle, onto a full page outline of the dorsal aspect of the female abdomen.

The bristles of a mosaic spot, irrespective of phenotype, tended to be strung out laterally, at right angles to the anterior-posterior axis of the fly. Frequently, wild-type bristles were found interspersed with those showing the mutant characteristic. To decide whether a series of bristles represented one spot or two at times seemed to require an arbitrary decision. The situation was especially complex for the flies with genotype y +/+ sn^3, for in these the finding of a yellow bristle at some distance from a singed bristle could be interpreted either as a twin spot or as two separate spots, one yellow, the other singed.

In order to standardize the collection of data it was decided to permit, in *trans* heterozygotes, the recording of only one spot per half tergite. Thus, even if the bristles were widely separated but on the same half tergite, the occurrence of a single original genetic event was assumed. For example, two yellow bristles at opposite ends of a half tergite would be scored as a single yellow spot, and a yellow bristle far from a singed bristle would lead to the mapping of a sole twin spot. A yellow bristle and a singed bristle which are not part of twin spots would, if found on the same half tergite, be recorded as a twin spot and consequently result in an overestimation of the incidence of recombination proximal to the gene for singed. This propensity was probably more than compensated for by the fact that some of the yellow and some of the singed spots seen were in reality twin spots of whom only one of the two cell types survived and formed bristles. In flies with the genotype y sn^3/++ the mosaic spots yellow, singed, and yellow singed were assumed to be the product of separate genetic events and therefore were counted individually. This permitted the recording, in *cis* heterozygotes, of a maximum of three spots, one of each kind, per half tergite since two bristles of the same phenotype, no matter how far apart within a half tergite, were counted as only a single spot.

RESULTS

The standard experiments: In order to determine the number of mosaic spots

TABLE 1

Frequency of mosaic spots in the standard series

Genotype	Experiment*	Yellow	Singed	Twin or yellow singed†	Total	Mean number of spots per abdomen	S.D. of spots per abdomen
$y +/+ sn^3$	1–6	160 (40.7%)	127 (32.3%)	106 (27.0%)	393 (100%)	.33	.56
$y sn^3/+ +$	1–6	327 (33.3%)	86 (8.7%)	571 (58.0%)	984 (100%)	.82	.93

* In each experiment 200 abdomens were examined.
† Twin in $y +/+ sn^3$, yellow singed in $y sn^3/+ +$.

which are the result of "spontaneous" somatic recombination, groups of *D. melanogaster* were reared under standard conditions within the incubator. The results of these experiments, which were spaced at convenient intervals during the investigation, are given in Table 1. It is evident that the number of mosaic spots is about two and a half times greater in the *cis* than in the *trans* heterozygotes. This inequality is not unexpected since it has been demonstrated that the frequency of somatic recombination in the yellow and singed system is governed by the action of other chromosomal factors, by genes not only on the *X* chromosomes but on the two large autosomes as well (WEAVER 1960). In neither genotype is there an increase or decrease in the mean number of mosaic spots from experiment 1, done at the beginning, to experiment 6, eighteen months later. Chi-square tests of homogeneity do not demonstrate any significant shifts in the frequencies of the different kinds of spots from experiment to experiment. The average number of spots per fly is small, always less than one. The data from these and subsequent experiments follow a Poisson distribution.

The relative frequencies of spot types in the two genotypes are dissimilar. It is generally assumed that these differences result from the fact that in the *trans* heterozygotes the descendants of a cell in which somatic recombination occurred proximal to singed do not always form a twin spot containing both yellow and singed bristles but may instead produce only yellow or singed bristles. Consequently, there are observed excessive numbers of yellow spots and singed spots and fewer twin spots than the genetic events warrant.

It is possible to characterize the two genotypes further than is shown in Table 1 by estimating the proportions of spots ascribable to the different recombination types. In the *cis* heterozygotes the 571 yellow spots are due to single recombination proximal to singed, and the 86 singed spots are due to double recombination taking place simultaneously on both sides of singed. Of all the spots resulting from double recombination of this kind two-thirds are singed and one-third yellow. Therefore, of the 327 yellow spots 43 are due to double recombination, while the remaining 284 are the result of single recombination distal to singed. Double recombination in three-fourths of cases but single recombination in only one-half lead to mosaic spots. Hence a reduction by one-third in the number of spots from double recombination is required to reach a valid result: 2/3 (86 +

43) : 86 doubles. The proportion of spots due to single recombination proximal to singed: single recombination distal to singed: double recombination is 571 : 284 : 86, or, in reduced form, 6.6 : 3.3 : 1. The argument followed above is that of STERN (1936).

In the *trans* heterozygotes the inability to identify all spots resulting from single recombination proximal to singed precludes an independent computation of the spot proportions. Nevertheless, an estimate can be made if it is assumed that the relative frequency of single recombination proximal to singed is equal in the two genotypes. If both daughter cells survive this kind of recombination and are represented by bristles in the hypoderm, a twin spot will be seen in the *trans* and a yellow singed spot in the *cis* heterozygotes. If the descendants of only one daughter cell produce bristles, a yellow spot or a singed spot is formed in the *trans* heterozygotes and a yellow singed spot or an undetectable wild-type "spot" in the *cis* heterozygotes. Thus, if only one daughter cell is represented by bristles, the *trans* will form twice as many mosaic spots as the *cis* genotype. There is a difference of 31% between the sums of the yellow and singed spots in the two genotypes, a difference caused by the presence, in the *trans* configuration, of yellow and singed spots contributed by single recombination proximal to singed. Adding half of this 31 percent, or 15.5 percent, to the 58 percent which Table 1 shows as the percentage of yellow singed spots gives 73.5%, which for the *trans* genotype is an approximation of the percentage of spots due to single recombination proximal to singed. Seventy-three point five percent of the total spot number of 393 equals 289 spots. If both daughter cells initiate bristle formation, these 289 should be twin spots. Since only 106 twin spots are seen there must be 289 − 106, or 183 spots, which arose from single recombination proximal to singed but contain only yellow or singed bristles. The 183 spots must be subtracted from the yellow and singed totals to leave the spots resulting from single recombination distal to singed and double recombination. To try to compensate for the slightly greater viability of the singed phenotype the subtractions are made in proportion to the average number of bristles in the twin spots (2.4 yellow, 2.7 singed): 160 − 86 = 74 yellow spots; 127 − 97 = 30 singed spots. Correcting for the one-third of double recombinants which are yellow and for the variation in detection between single and double recombinants gives the proportion of spots due to single recombination proximal to singed: single recombination distal to singed: double recombination to be 289 : 59 : 30, or 9.6 : 2.0 : 1. Other methods of estimating the spot frequencies in the *trans* genotype have been devised by BROWN *et al.* (1962) and by BAKER and SWATEK (1965).

Life-long exposures to various oxygen concentrations: In this set of experiments flies were kept in various concentrations of oxygen throughout their development. Such prolonged exposures maximized the possibility of detecting any existing influences of oxygen on somatic recombination and also avoided problems posed by differences in sensitivity to oxygen between the several stages of development.

Drosophila is resistant to pure oxygen, even at several atmospheres of pressure (WILLIAMS and BEECHER 1944). The genus is also known to survive for a matter

TABLE 2

Frequency of mosaic spots following life-long exposures to various concentrations of oxygen

Genotype	Percent oxygen*	Numbers of spots: Yellow	Singed	Twin or yellow singed	Total	Mean number of spots per abdomen	S.D. of spots per abdomen
$y + / + \ sn^3$	7.5	16	17	17	50	.25	.51
	10	19	22	15	56	.28	.55
	15	32	16	22	70	.35	.63
	25	32	19	13	64	.32	.54
	30	21	23	27	71	.36	.58
	40	30	21	25	76	.38	.62
	50	31	21	21	73	.37	.56
	60	32	24	15	71	.36	.55
	70	42	25	39	106	.53	.72
	80	56	38	40	134	.67	.83
	90	64	47	39	150	.75	.85
Chi-square test of homogeneity of the different spot types: P = .30–.50							
$y \ sn^3 / + +$	7.5	32	15	81	128	.64	.82
	10	53	15	86	154	.77	.94
	15	44	17	87	148	.74	.89
	25	53	23	126	202	1.01	.95
	30	54	18	96	168	.84	1.02
	40	56	30	124	210	1.05	1.14
	50	58	15	124	197	.99	1.24
	60	50	24	130	204	1.02	1.04
	70	44	25	160	229	1.15	1.01
	80	65	27	172	264	1.32	1.22
	90	91	34	199	324	1.62	1.62
Chi-square test of homogeneity of the different spot types: P = .30–.50							

* At each oxygen concentration in both genotypes 200 abdomens were examined.

of hours in pure nitrogen (ABRAHAMSON 1958). That the range of oxygen tolerance is considerable was ascertained early in the present study: the flies were able to live, with some reduction in activity at the highest and lowest levels, in gaseous mixtures containing as much as ninety and as little as seven and a half percent oxygen. No gross abnormalities in morphology or numbers of offspring were detected in flies raised at oxygen levels from seven and a half through sixty percent. From seventy percent on the individual insects seemed normal but the numbers appearing in the cultures declined. At ninety percent there was a noticeable mortality among the early larval stages. In one of the oxygen concentrations was the time of development prolonged.

Table 2 and Figure 1 show that increased numbers of mosaic spots are found in flies reared in the higher oxygen tensions. This rise in the frequency of mosaicism appears to take place abruptly at seventy percent in the *trans* heterozygotes and more gradually, but at about the same oxygen concentration, in the *cis* heterozygotes. In both genotypes a slight decrease in spot numbers is evident at oxygen levels below the nearly twenty-one percent found in air. A comparison with the standard experiments demonstrates that, at ninety percent, the mean

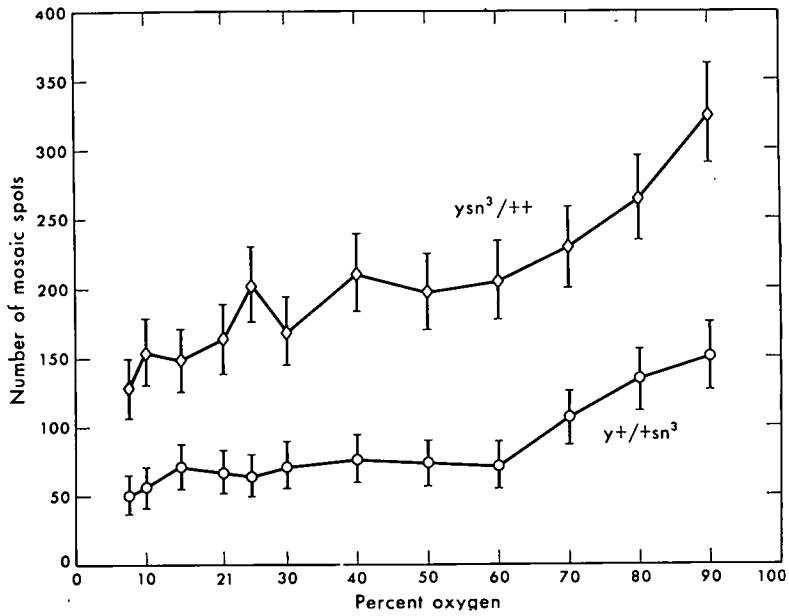

FIGURE 1.—95% confidence limits (Poisson model, see CROW and GARDNER 1959) of the mosaic spot frequencies following life-long exposures to various concentrations of oxygen. (The limits given for air were computed from the means of the standard experiments.)

number of mosaic spots per abdomen in the *trans* heterozygotes is 2.3 and in the *cis* heterozygotes 2.0 times the mean spot totals obtained when the flies are allowed to develop in air. All spot types participate in the increase in spot numbers at seventy percent and higher. The mean frequencies of twin and yellow singed spots in the cultures raised at seventy percent and higher. The mean frequencies of twin and yellow singed spots in the cultures raised at seventy percent or above, 26.0 and 61.4%, respectively, are similar to the corresponding spot frequencies, 27.0 and 58.0%, found in the standard experiments.

Since it seemed possible that oxygen in high concentrations might induce phenocopies of the mutant bristles, Oregon RC flies were reared in ninety percent oxygen. Examination of fifty abdomens of these wild-type flies, however, revealed no grossly abnormal bristles and, specifically, none which might be misclassified as yellow or singed or yellow singed.

X-ray experiments at different oxygen concentrations: First instar larvae were selected for irradiation because of the relative ease with which homogeneous populations of this life stage could be collected. Later larval stages tended to diverge with respect to the rate of development. The X-ray dose chosen, 1326 r, is large enough to insure a detectable increase in mosaicism but not so large as to produce an impractically high mortality. ABBADESSA and BURDICK (1963), on the recommendation of BECKER, used an approximately equal dose, 1350 r, in obtaining an enhancement by a factor of three to four over the natural mosaic spot frequency.

TABLE 3

Frequency of mosaic spots following irradiation in different concentrations of oxygen. Genotype y +/+ sn^3

	Percent oxygen*	Numbers of spots				Mean number of spots per abdomen	S.D. of spots per abdomen
		Yellow	Singed	Twin	Total		
Controls	0†	14	10	9	33	.33	.57
	2	14	10	13	37	.37	.66
	5	13	11	14	38	.38	.63
	10	8	12	5	25	.25	.52
	21	13	10	16	39	.39	.63
	50	12	13	14	39	.39	.57
	100	14	8	6	28	.28	.57
	Chi-square test of homogeneity of the different spot types: P = .50–.70						
X-rayed	0†	29	25	43	97	.97	1.02
	2	60	42	94	196	1.96	1.29
	5	56	64	113	233	2.33	1.37
	10	89	70	133	292	2.92	1.43
	21	102	83	131	316	3.16	1.55
	50	68	89	133	290	2.90	1.49
	100	89	96	144	329	3.29	1.60
	Chi-square test of homogeneity of the different spot types: P = .30–.50						

* At each oxygen concentration in both controls and X-ray experiments 100 abdomens were examined.

† i.e. 100 percent nitrogen.

Inspection of the X-rayed Drosophila prior to dissection did not reveal any abnormalities of the abdomen or elsewhere. None of the flies exhibited deficiencies of pigmentation. No delay in the time required to complete development was noted though this has been reported by BOURGIN and coauthors (1956) for Drosophila larvae given X-ray doses as low as 1000 r.

Two findings of interest are contained in the information displayed in Tables 3 and 4. The first of these, seen in the controls, is the apparent absence of any effect on the rate of somatic recombination by exposures to various oxygen concentrations for periods of two hours. The second, in the X-rayed flies, is the dependence of the rate of somatic recombination on the percent of oxygen present at the time the larvae were X-rayed. The number of mosaic spots found on the tergites is lowest among Drosophila irradiated in pure nitrogen and highest in flies treated in pure oxygen. In the *trans* heterozygotes there are almost 3.4 times as many mosaic spots on the abdomens of flies X-rayed in oxygen as there are on those irradiated under anoxic conditions. The corresponding ratio for the *cis* heterozygotes is 3.2. Figure 2 shows these changes in sensitivity with increasing oxygen tensions.

Varying the oxygen concentrations does not greatly affect the relative frequencies of the different types of spots in either the controls or the X-rayed flies. However, a comparison of the irradiated flies with their controls reveals that among the *trans* heterozygotes those treated with X-rays have a greater proportion of twin spots and relatively fewer yellow spots and singed spots. Out of a

TABLE 4

Frequency of mosaic spots following irradiation in differenet concentrations of oxygen. Genotype y sn^3/+ +

	Percent oxygen*	Numbers of spots: Yellow	Singed	Yellow singed	Total	Mean number of spots per abdomen	S.D. of spots per abdomen
Controls	0†	14	2	49	65	.65	.81
	2	29	13	56	98	.98	1.08
	5	20	10	37	67	.67	.87
	10	16	9	52	77	.77	.97
	21	24	11	37	72	.72	1.00
	50	15	10	50	75	.75	.81
	100	30	17	47	94	.94	.87
	Chi-square test of homogeneity of the different spot types: P = .05–.10						
X-rayed	0†	42	10	93	145	1.45	1.32
	2	71	25	166	262	2.62	1.75
	5	73	24	209	306	3.06	1.94
	10	85	36	244	365	3.65	1.93
	21	115	51	263	429	4.29	2.24
	50	117	42	279	438	4.38	2.33
	100	123	38	309	470	4.70	1.96
	Chi-square test of homogeneity of the different spot types: P = .50–.70						

* At each oxygen concentration in both controls and X-ray experiments 100 abdomens were examined.

† i.e. 100 percent nitrogen.

total of 239 spots in the controls 77 or 32.2% are twin spots, while of 1753 spots on the abdomens of X-rayed flies 791 or 45.1% are twin spots. The comparatively large number of spots in the irradiated flies increases the probability that more than one spot will occur on a half tergite and, under the spot counting rules, tends to produce artificially high twin spot counts. In the *cis* heterozygotes the number of yellow singed spots in the controls is 328 or 59.9% of a total of 548 while in the X-rayed flies 1563 or 64.7% of a total of 2415 spots are yellow singed. The probability of having two or more yellow singed spots on the same half tergite must be higher in the irradiated flies than in the controls. Since in the *cis* heterozygotes the spot counting rules allow a maximum of one spot of each kind per half tergite it is likely that the actual percentage of yellow singed spots in the X-rayed flies exceeds the observed 64.7%.

No phenocopies of the mutant bristles were found in fifty Oregon RC flies exposed to pure oxygen for two hours. Irradiating first instar Oregon RC larvae with 1326 r, in air, did not produce any yellow or singed or yellow singed bristles. In a hundred of these irradiated wild-type flies a few bristles (about one every twenty or thirty abdomens) were partially depigmented or colored lighter than usual. It is unlikely that such bristles could be confused with those exhibiting the yellow phenotype.

Post-radiation exposures to nitrogen, air or oxygen: In order to determine whether the oxygen tension present after irradiation influences the frequency of somatic recombination groups of larvae of the *trans* genotype were placed, within

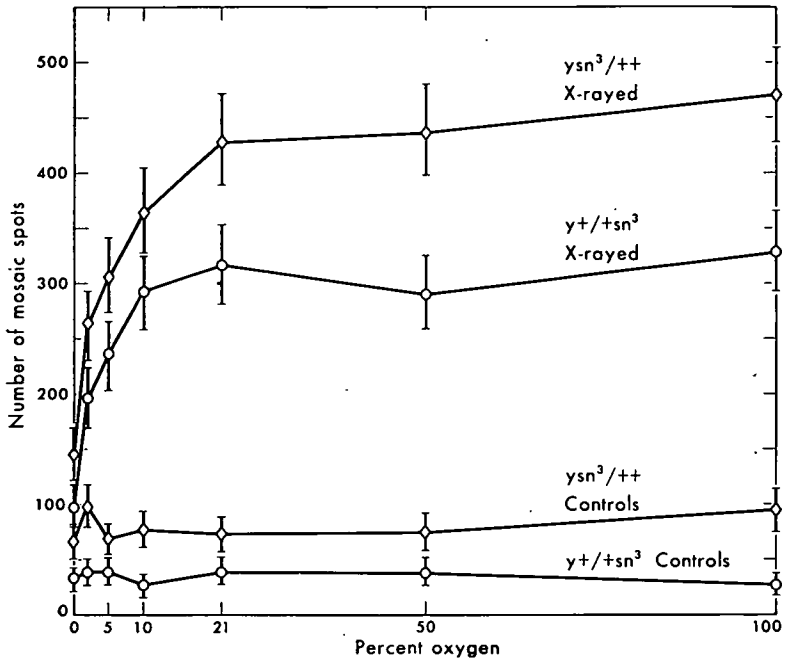

FIGURE 2.—95% confidence limits (Poisson model) of the mosaic spot frequencies following irradiation, with 1326 r of X rays, in different oxygen concentrations.

TABLE 5

Frequency of mosaic spots following irradiation in various concentrations of oxygen and of post-radiation exposures to either nitrogen, air or oxygen. Genotype y +/+ sn³

Percent oxygen during irradiation*	Percent oxygen following irradiation	Numbers of spots				Mean number of spots per abdomen	S.D. of spots per abdomen
		Yellow	Singed	Twin	Total		
0	0	30	37	57	124	1.24	1.02
0	21	29	25	43	97	.97	1.02
0	100	23	34	43	100	1.00	.91
Chi-square test of homogeneity of the different spot types: P = .50–.70							
21	0	141	148	139	428	4.28	1.65
21	21	101	83	131	316	3.16	1.55
21	100	98	98	100	296	2.96	1.58
Chi-square test of homogeneity of the different spot types: P = .05–.10							
100	0	146	130	142	418	4.18	1.60
100	21	89	96	144	329	3.29	1.60
100	100	99	84	126	309	3.09	1.50
Chi-square test of homogeneity of the different spot types: P = .05–.10							

* In each of the nine experiments 100 abdomens were examined.

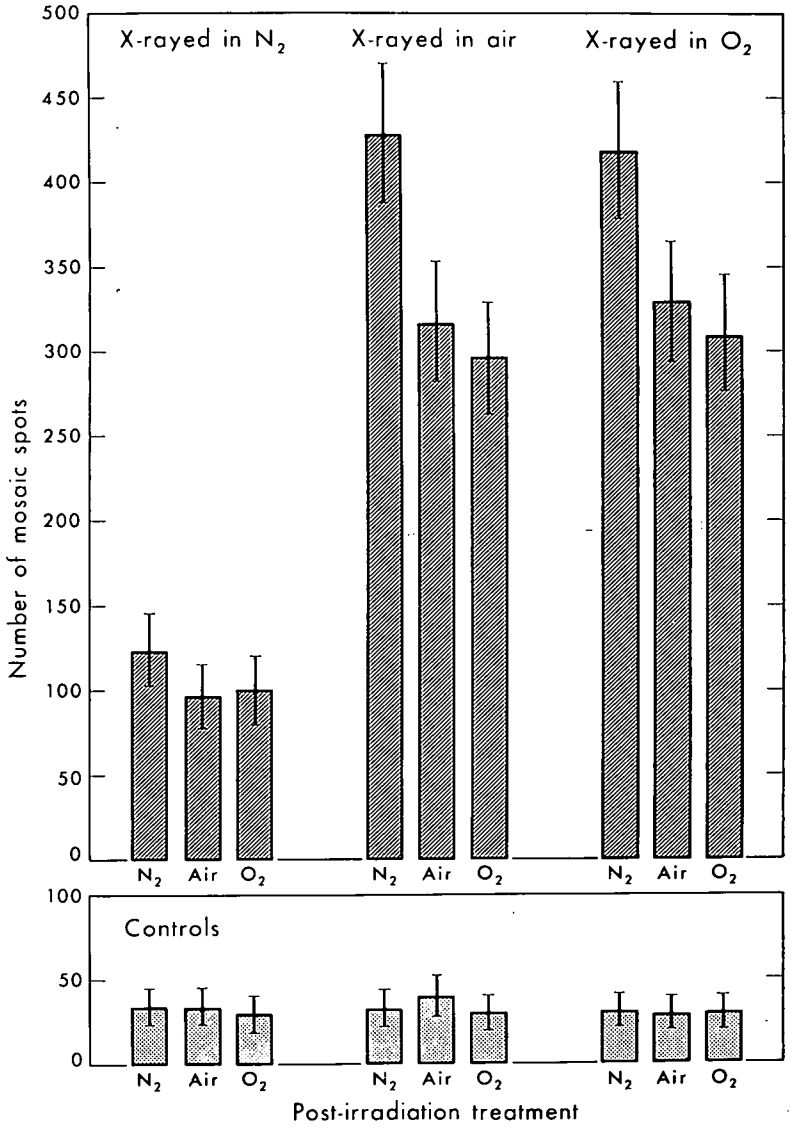

FIGURE 3.—95% confidence limits (Poisson model) of the mosaic spot frequencies following irradiation, with 1326 r of X rays, in different oxygen concentrations and post-radiation treatment with nitrogen, air or oxygen. Genotype $y +/+ sn^3$.

a few seconds following the conclusion of the X-ray treatment, into nitrogen, air or oxygen for a period of two hours.

The results are shown in Table 5 and Figure 3. Larvae X-rayed in nitrogen have equal numbers of mosaic spots, regardless of the composition of the post-radiation atmosphere. On the other hand, larvae X-rayed in either air or oxygen and thereafter put into nitrogen are found to have greater numbers of abdominal

spots than do larvae which following irradiation were placed in air or oxygen. This rise in mosaicism is chiefly due to increases in the yellow spots and singed spots. Among the Drosophila X-rayed in air or oxygen those post-treated with oxygen have slightly lower numbers of mosaic spots than those post-treated with air. As was true for the other X-ray experiments the controls for this set also do not show any effects from being exposed to abnormally high or low oxygen concentrations for a few hours.

Bristle numbers and the size of the mosaic spots: When an attempt was made to grow *D. melanogaster* in five percent oxygen it was noted that in the few flies which reached the adult stage the number of bristles on the tergites were markedly reduced. This observation prompted the counting of bristles on randomly selected abdomens (ten in the standard experiments, five in each of the others). The mean bristle count in the standard experiments is 518 for the *trans* and 492 for the *cis* genotype. These figures are a little higher than the mean of 464 bristles which STERN (1936) reported for tergites one through six. Four hundred ninety-eight and 449 bristles, respectively, are found in the *trans* and *cis* heterozygotes given life-long exposures to oxygen levels of seventy percent or higher. In the X-ray experiments there are 501 bristles in the *trans* controls and 487 in the irradiated *trans* flies. The corresponding bristle counts for the *cis* heterozygotes are 487 and 463. In view of the small numbers of abdomens examined and the considerable variability in bristle numbers from fly to fly it is doubtful whether the slight decreases noted in the treated flies are of significance. Of interest is that the bristle counts in the *cis* genotype are consistently lower than those obtained in the *trans*.

The two genotypes differ also with respect to mosaic spot size. In the standard experiments the mean size of all spots in the *trans* heterozygotes is 2.7 bristles, that in the *cis* heterozygotes only 1.6 bristles. This disparity is largely due to the fact that twin spots cannot contain fewer than two bristles. The average twin spot consists of 5.1 bristles (2.4 yellow and 2.7 singed), the average yellow singed spot 1.7 bristles. The sizes of the yellow and singed spots, however, are similar though not identical: 1.7 and 1.7 bristles in the *trans* and 1.5 and 1.3 bristles in the *cis* heterozygotes. The whole life exposures to different oxygen concentrations appear to have no influence on spot size, but X-irradiation causes a uniform increase in all spot types. Thus, in the X-rayed *trans* heterozygotes the sizes of yellow, singed, and twin spots are 2.2, 2.5, and 6.6 bristles (3.4 yellow and 3.2 singed), and in the irradiated *cis* heterozygotes the yellow, singed, and yellow singed spots contain 1.6, 1.6, and 2.4 bristles, respectively. Comparable increases in the size of mosaic spots in X-rayed Drosophila have been previously described (ABBADESSA and BURDICK 1963).

DISCUSSION

Developmental studies of the abdominal hypoderm of Drosophila have demonstrated that both of the bilateral nests of imaginal cells from which each tergite is derived contain about eight cells, which throughout larval life probably remain

in the G_2 stage of the cell cycle (GARCIA-BELLIDO and MERRIAM 1971). If eight imaginal cells lead to the creation of all the hypodermal structures on a half tergite, including the approximately forty-eight bristles, then on the average one cell is responsible for one-eight of this complement, or six bristles. Thus, in the genotype y +/+ sn^3, somatic recombination occurring in one of the eight cells during its first division should produce a twin spot with six bristles, three yellow and three singed. Similarly, in the genotype y sn^3/+ +, a yellow singed spot with three bristles should be formed, the smaller spot size reflecting the fact that half of the descendants of the parent cell will be wild type. The average spot size in the standard experiments, 5.1 bristles per twin and 1.7 bristles per yellow singed spot, is a little smaller than might be anticipated on the basis of the reasoning above. The observation that many twin spots have fewer than six and many yellow singed spots less than three bristles suggests that spontaneous somatic recombination can occur during or prior to the first division of the imaginal cells but that often it takes place considerably later.

In the X-ray experiments the larvae were irradiated during the first instar, long before the surge of mitotic activity which in early pupal life forms the adult hypoderm. If, as it seems reasonable to assume, genetic damage is manifested in the first division following irradiation, twin and yellow singed spots with about six and three bristles, respectively, should be observed. The finding of a mean of 6.6 bristles for the twin and 2.4 bristles for the yellow singed spots closely approximates these expectations. However, these averages are based on a wide spectrum of spot sizes, including a substantial proportion of very small spots and some considerably larger than are found in unirradiated flies. The larger spots can be explained either by somatic recombination occurring in more than one of the eight cells or by assuming that, if not all eight cells remain viable after irradiation, the survivors compensate by covering a larger fraction of the half tergite. The presence of very small spots implies that part of the X-ray damage is latent and not expressed until after several cell divisions have taken place. An alternate explanation of the very small spots is that cells homozygous for one or both of the mutant genes may divide less frequently than wild-type cells.

The whole-life exposures demonstrate that oxygen levels several times as high as any which *D. melanogaster* could have experienced during its evolution can cause somatic recombination. The X-ray experiments show a typical oxygen effect. A life-long exposure to ninety percent oxygen is roughly equivalent, with respect to the quantity of induced mosaicism, to 1326 r given anoxic first instar larvae. In flies exposed during development to at least seventy percent oxygen or treated with X-rays all mosaic spot types are elevated but in the irradiated flies the numbers of twin and yellow singed spots are disproportionately large, indicating a relatively great increase in the rate of somatic recombination proximal to singed. These results agree with the findings of BECKER (1969) that the heterochromatin of the X chromosome is more radiosensitive than the euchromatin. The mechanisms by which oxygen and X-rays bring about somatic recombination remain obscure but it seems unlikely that only a very limited segment of the X chromosome will be found to be primarily involved, as is known to be the case

for somatic mosaicism caused by the dominant Drosophila mutants known as Minutes (RITOSSA, ATWOOD and SPIEGELMAN 1966).

The data obtained by varying the oxygen content of the post-radiation atmosphere can be interpreted as supporting the existence of an oxygen dependent system capable of repairing, at least partially, the damage created by X rays. It seems probable that the appearance of as much as a fourth of the potential mosaic spots is prevented by the availability of sufficient oxygen. The finding that post-radiation exposure of the larvae to nitrogen increases principally yellow and singed spots, and not twin or yellow singed spots, raises the possibility that oxygen-dependent repair takes places preferentially in the euchromatin and only to a slight extent, if at all, in the proximal heterochromatin.

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