NONRANDOM DISTRIBUTION OF LETHALS INDUCED BY TRITIATED THYMIDINE IN DROSOPHILA MELANOGASTER¹

W. D. KAPLAN, H. D. GUGLER, K. K. KIDD, AND V. E. TINDERHOLT²

Department of Biology, City of Hope Medical Center, Duarte, California

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THE use of tritiated thymidine (H^3T) has, in recent years, become quite widespread in many types of biological research. Because thymidine is a specific precursor of DNA, its direct and exclusive incorporation into chromosomes permits analysis of their behavior and metabolism during the mitotic cycle. Its incorporation is revealed through the use of radioautography, for which purpose tritiated thymidine has exceptional qualities, permitting resolutions at the chromatid level. The excellent radioautographs reflect the physical properties of the radioactive H^3 atom.

A comparison of the characteristics of the beta emission of C^{14} and H^3 provides an insight into the relevant properties of the tritium atom. The beta particle emitted by H^3 has a maximum energy of 18 kev, and an average energy of 6 kev, while the average energy of the C^{14} beta particle is 50 kev. The corresponding path lengths in water are 0.8 to 1.0 μ for the H^3 , and approximately 50 μ for the C^{14} beta particle (NELMS 1956). The amount of energy necessary for the formation of one ion pair is estimated at 0.0325 kev (LAPP and ANDREWS 1954). The H^3 particle can thus lead, on the average, to the formation of about 180 ion pairs over a distance of 0.9 μ . BACQ and ALEXANDER (1955) list the ionization density of H^3 beta particles at 200/ μ , whereas for C^{14} the density is 30/ μ . When H^3 atoms are present in the molecular structure of the chromosomes, each beta emission will accordingly produce 10 to 20 ionizations within the first 0.1 μ of path traversed. According to LEA (1955), 15 to 20 ionizations within 0.1 μ of a chromatin thread are required to produce a chromatid break with a probability of one.

Thus, although in terms of energy levels the beta particles emitted by C^{14} would be expected to have greater genetic effect, the special properties of the H^3 beta particle would seem to prove more interesting. LAJTHA and OLIVER (1959) and PLAUT (1959) have already pointed this out in stressing the fact that virtually all the H^3 beta energy will be dissipated within the nucleus of origin.

The ability of tritiated thymidine to produce sex-linked recessive lethals in *Drosophila* males has already been reported (KAPLAN and SISKEN 1960). Since then its mutagenic properties have been further investigated: in mice, by BATEMAN and CHANDLEY (1962) who measured the frequency of induced dominant

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² Deceased.

lethals; in *Drosophila* by STRØMNAES (1962) who found after injection into adults a measureable level of sex-linked recessives but no induction of dominant lethals; and in bacteria, by PERSON and LEWIS (1962).

We have continued our studies of the mutagenic properties of H^3 -thymidine and, because of the properties of the H^3 beta particles, have localized the induced mutations along the length of the X chromosome. The distribution of lethals appears to be nonrandom.

MATERIALS AND METHODS

The Canton-S strain used in this work has been maintained in our laboratory in mass culture for nine years.

For this study, males were treated in one of two ways: as larvae by feeding upon a food substrate containing the isotope, and as adults, by injection. The H^3T obtained from the Schwartz Laboratories, Mt. Vernon, New York, had a specific activity of 1.9 c/mm.

Larval feeding: To insure maximum incorporation of isotope into germ cell chromosomes, the H^3T was not added to the standard fly culture medium, which is rich in yeast, but was made up as follows: 0.1 ml H^3T , 0.5 ml 3 percent agar solution, and 0.5 ml of a modified Eagle's tissue culture medium (SISKEN and KINOSITA 1961). The total activity present within this volume and kind of food was 100 microcuries. Sixteen-hour first instar larvae were kept upon this substrate for 6 to 8 hours, then removed to the standard fly culture medium. The feeding was restricted in order to insure a single chromosome replication cycle in the presence of isotope within any individual nucleus of the germ cell population. However, we cannot be sure whether this was indeed so since some labeled food and yeast cells remain in the gut for about 18 hours after removal to unlabeled food; presumably this may be available for incorporation into germ cells.

Injection into adults: The volume of undiluted H^3T injected into each male was approximately 0.025 microliters, giving a total activity of 0.025 microcuries per fly. In the control experiment, an equivalent volume of unlabeled thymidine was injected into each male. Since OFTEDAL (1959) has shown that a considerable amount of DNA synthesis goes on in the somatic cells of *Drosophila* imago during the first 12 hours of life, males were not injected until 24 hours old. This would serve to limit incorporation of H^3T into the nuclei of germ cells.

Mating regimen and detection of sex-linked recessive lethals: In order to measure the rate of induced sex-linked lethals, the Basc, or Muller-5 (M-5), test was carried out. Upon eclosion males fed as larvae were mated individually in vials to three M-5 females each. After three days, these females were removed to "shakeover" vials to continue their egg-laying, and in a second fresh vial the males were presented with another group of three females each. At the end of the sixth day this process was repeated. Thus three broods were obtained from each treated male, providing samples of chromosomes from three different generations of germ cells.

Injected males were subjected to the same mating schedule starting one day after injection.

Localizations: After detection, each lethal was maintained as a balanced lethal stock over *FM6* (GRELL and LEWIS 1956) until localization was possible. Since the accurate determination of the map position of individual lethals upon the X chromosome is an important factor of this work, our method will be described in detail.

In order to minimize error from variations within culture conditions, a standardized procedure was established. Two crosses were made as follows:

$$\begin{array}{l}
 P \quad \frac{\text{lethal}}{FM6} \times \text{marker stock} \quad (\text{see below}) \\
 \\
 F_1 \quad \frac{\text{lethal}}{\text{marker}} \times M-5
 \end{array}$$

Two groups of eight newly-hatched virgin lethal/marker females were mated to 12 M-5 males in each of two half-pint culture bottles. M-5 males were used to mark clearly the X chromosome derived from the male parent to distinguish it from the chromosome bearing the lethal mutation carried by the female parent. After the first two days, the flies were transferred to bottles of freshly-prepared food each successive day until two series of five bottles each had been obtained. Thus, ten cultures were established for each localization. Restriction of the egg-laying period insured uncrowded conditions to reduce as far as possible selection resulting from differential viability among the crossover types recovered. Counts were made daily, the last one 17 days after establishment of each culture.

The marker stock used for the initial determination contained the following seven recessive genes: scute, echinus, crossveinless, *cut*⁶, vermilion, garnet², and forked. Thus the chromosome is divided into seven regions; all but the one to the right of forked are defined by easily-classified left and right markers. Table 1 delineates the regions; marker loci are from BRIDGES and BREHME (1944).

In regions 1, 2, and 3, the lethal cannot lie more than 5 crossover units to the right or left of a marker gene. In 1,000 males, the error may not be greater than plus or minus 1 crossover unit. With respect to regions 4, 5, 6, and 7, the distance from the lethal to the closer of the two markers may possibly be greater than 5 crossover units. In such instances a second marker stock was used for more precise determinations. These stocks and the regions involved are defined in Table 2.

A yellow², scute, white-ivory stock was used to distinguish between lethals that localize to the left or to the right of the white locus on the distal end of the X chromosome.

Usually, it was necessary to classify the flies with respect to all crossover types only in the first two bottles. At this point, the region in which the lethal lies has become apparent and any unusual features such as crossover suppression for any region can be observed. When no irregularities were present, and when the lethal lay close enough to a particular marker to provide reliable data, classification of all crossover types was discontinued and only crossovers relevant to that particular region were scored. On the other hand, when it appeared that too large an error would characterize the localization because of the distance of the lethal from the closer of the two marker genes, classification stopped and the localization was set up with the appropriate second marker stock. By this scheme, reliable data were obtained and the time involved was shortened.

Radioautography: Males were selected for radioautography at random from the population of treated males used to measure the induced rate of mutation. Before the matings were set up, six males were taken from each brood and their testes used to furnish a picture of mature sperm available within their respective broods. Testes of larval-fed males of the first brood revealed the extent of sperm labeling in one-day-old males prior to mating. From the second brood, sperm was derived from males mated individually to three females for a period of three days. The third brood sperm came from males mated with two groups of three females each, for two three-day periods. The males injected as adults differed in one respect: the first matings

TABLE 1

Markers and chromosome regions: all-purpose stock

Region	Limits	Distance
1	<i>sc</i> (0.0) – <i>ec</i> (5.5)	5.5
2	<i>ec</i> (5.5) – <i>cv</i> (13.7)	8.2
3	<i>cv</i> (13.7) – <i>ct</i> ⁶ (20.0)	6.3
4	<i>ct</i> ⁶ (20.0) – <i>v</i> (33.0)	13.0
5	<i>v</i> (33.0) – <i>g</i> ² (44.4)	11.4
6	<i>g</i> ² (44.4) – <i>f</i> (56.7)	12.3
7	<i>f</i> (56.7) – (70.0)	13.3 (approx.)

TABLE 2

Markers and chromosome regions: specific region stocks

Region	Limits	Distance
Stock: $ct^n oc lz^3 v$ (cut-notch, ocelliless, lozenge ³ , vermilion)		
4a	ct^n (20.0) – oc (23.1)	3.1
4b	oc (23.1) – lz^3 (27.7)	4.6
4c	lz^3 (27.7) – v (33.0)	5.3
Stock: $v dy fw^{34e} g^2$ (vermilion, dusky, furrowed ^{34e} , garnet ²)		
5a	v (33.0) – dy (36.2)	3.2
5b	dy (36.2) – fw^{34e} (38.3)	2.1
5c	fw^{34e} (38.3) – g^2 (44.4)	6.1
Stock: $v dy g^2 sd$ (vermilion, dusky, garnet, ² scalloped)		
5a	v (33.0) – dy (36.2)	3.2
5bc	dy (36.2) – g^2 (44.4)	8.2
6a	g^2 (44.4) – sd (51.5)	7.1
Stock: $(\gamma cv v) f car$ (forked, carnation)		
7a	f (56.7) – car (62.5)	5.8
7b	car (62.5) – right end (70)	7.5
Stock: $\gamma^2 sc w^i$ (yellow, ² scute, white-ivory)		
1a	γ^2 (0.0) – sc (0.0+)	0
1b	sc (0.0+) – w^i (1.5)	1.5

were set up when they were two days old. This scheme is diagrammed in Figure 1. Concomitantly, the spermathecae and seminal receptacles of females of each brood were radioautographed to show the extent of labeling of the transferred sperm populations. Quantitative data derived from preparations of sperm in female structures proved less satisfactory, as individual sperm heads are less readily visualized. Consequently, only data derived from the radioautographs of testes will be presented, although observations of sperm in female storage organs support data derived from testes.

The stripping film technique of preparing radioautographs was employed according to the method described by TAYLOR (1956). Testes of adult males were dissected into tap water in a depression slide, removed directly into a drop of tap water on a clean slide, pulled apart, and the sperm bundles freed and allowed to disperse into the fluid medium. After 5 minutes, a drop of 5 percent formalin was placed on the slide next to the drop containing the testes, a bridge was formed to connect the two drops, and fixation of the tissue allowed to proceed for 10 minutes. After fixation, the tissue was squashed by placing a siliconized coverslip on the slide and press-

FIGURE 1.—The scheme used for matings and selection of males for radioautographs.

ing. The expressed liquid was absorbed with filter paper. The coverslip was floated off in water, the slide washed and Feulgen-stained, and the radioautography procedure started. With few exceptions, the film was exposed for 21 days before development.

The formalin fixation technique described herein was found to permit the best spreading of sperm heads for our purposes. Acetic acid-alcohol fixatives either fragmented the bundles or coagulated them, making it difficult to carry out relevant observations. Our objective was to preserve the identity of the sperm bundles to permit counting of individual sperm heads as well as the number of reduced photographic granules lying above them.

Determination of dominant lethals: Included in this report is one experiment in dominant lethality. For this purpose, larval males were fed labeled food and upon eclosion were mated according to the scheme described earlier. Four broods were examined. After each three-day period, the females were allowed to lay eggs individually overnight, and the performance of each female mated to each treated male was recorded. The number of eggs laid was counted, and 26 hours later, the number hatching. Controls were handled identically except that the thymidine present in the food was nonlabeled. In this experiment and in Blv III, the larvae were kept on the labeled food for 6 hours, removed, the food substrate was mixed with an equal volume of normal *Drosophila* food rich in yeast, and the larvae were returned to this mixture until pupation when they were transferred to fresh vials containing unlabeled food. In this way, although diluted by the presence of unlabeled thymidine, the isotope was available for incorporation during several chromosome reduplication cycles. Thus it was possible to obtain some sperm bundles more heavily labeled than those with more limited exposure to H^3T .

RESULTS

(A) *Frequency of mutation. Restricted larval feeding:* Three experiments were completed during which larval feeding upon labeled food was restricted to from 6 to 8 hours; these are RFL I, Blv I, and Blv II. The genetic and radioautographic data derived are summarized in Table 3.

In each experiment, the significantly elevated mutation rates occur in the first

TABLE 3
Genetic and radioautographic data: recessive lethals

		Chromosomes tested	Sex-linked recessive lethals	Lethals percent	Sperm bundles labeled percent	Labeled sperm per bundle	Grain counts per sperm head
RFL I	Brood 1	625	5	0.80			
	2	1085	3	0.30			
	3	1198	2	0.17			
	4	816	0	...			
Blv I	Brood 1	952	7	0.73	80	2-32	14.1 $\pm$ 5.3
	2	998	3	0.30	28	1-8	13.5 $\pm$ 5.3
	3	900	2	0.22	4	1-2	12
Blv II	Brood 1	2716	14	0.52	52	8-64	21.0 $\pm$ 8.5
	2	2612	1	0.04	16	1-4	19.6 $\pm$ 10
	3	2471	1	0.04	11	1-2	10.8 $\pm$ 2.8
Control	Brood 1	1289	1	0.08			
	2	1253	0	...			
	3	1068	0	...			
	4	736	0	...			

brood. These mutations were carried by a sperm population in which at least half the sperm bundles were labeled. After the inseminations of the first brood, labeled sperm bundles in the treated males dropped to as few as 16 percent. In neither Blv I nor Blv II had the extent of incorporation per sperm cell fallen; they were as heavily-labeled as those in the respective first broods, but the number of labeled bundles and the number of labeled sperm within each bundle was considerably lower, as illustrated in Table 3.

The decrease in each successive brood of labeled bundles and number of labeled sperm within each bundle is a consequence of the dichotomous pattern of sperm production which prevails in *D. melanogaster* (TIHEN 1946); a single spermatogonium in six divisions produces a bundle of 64 sperm. Since these divisions are synchronous within the cells of individual cysts, incorporation of H^3T within the cells of a given bundle is also synchronous. The first meiotic division is the last of the series during which the chromosomes are replicated and, therefore, the last divisions which may result in labeled chromosomes. When incorporation occurs at this point, each sperm head of the mature bundle will be labeled. The further removed from this stage, or the closer to the original spermatogonial division that labeling occurs, the fewer sperm heads of the bundle will contain the isotope. This aspect of the problem is of interest in itself and will be the subject of a later report.

Extended larval feeding: In the experiment designated Blv III, H^3T was available in the food during most of the larval life. Since many chromosome replication cycles took place during the availability of isotope, a higher percentage of sperm bundles could be labeled than in earlier experiments. The data are summarized in Table 4.

The high variability of grain counts within the first brood is accounted for by the presence of at least three different sperm populations which comprise the brood. While grain counts varied from as low as 17 to a peak of 72, labeling within individual bundles remained consistent. Three levels of incorporation were present, centering about 19, 35, and 55 counts per head. Since 100 percent of the bundles were labeled, these data indicate that for some of the bundles, their last reduplication cycle as primary spermatocytes was the only one occurring in the presence of the isotope. Another portion of the bundles went through more than one cycle in the presence of H^3T , some two, others three, but no attempt has been made to establish a precise correlation.

TABLE 4

Genetic and radioautographic data: recessive lethals. Blv III

Brood	Chromosomes tested	Sex-linked recessive lethals	Lethals percent	Sperm bundles labeled percent	Labeled sperm per bundle	Grain counts per sperm head
1	1537	37	2.4	100	64	26.7 $\pm$ 18.8
2	1354	70	5.2	100	64	40.8 $\pm$ 4.2
3	1679	22	1.3	90	16-64	11.2 $\pm$ 6.2

Although the third brood shows a falling off in level of incorporation and, correspondingly, in induced rate of mutation when compared to Broods 1 and 2, these mutation rates are still higher than any reported in the earlier experiments. These observations strengthen the idea that the effects of H^3T are delimited within germ cells that have been labeled since the sperm available for Brood 3 of Blv III are more heavily labeled than any of the aforementioned first broods. Figure 2 illustrates the difference in degree of incorporation between the first broods of Blv II and Blv III.

Adult injections: The two experiments involving injection of adults were the first completed in this study, RA 1 and RA 2. The data are summarized in Table 5. Table 6 summarizes data from the control experiment, and details the slightly different brood pattern. At the time these experiments were carried out, no radioautographs were prepared. However, our studies on the availability of labeled sperm following injection of adults have shown that the first labeled sperm bundles to appear have label in all 64 sperm heads and are available ten days after injection. This corresponds with the last day of the third brood in the present study. The third and fourth broods show the most significant elevation in mutation rates. These findings on the timing of spermatogenesis agree with the report of CHANDLEY and BATEMAN (1962).

Fewer sperm bundles were labeled after injection, presumably because thymidine is available for such a brief period of time. Since this in turn results in fewer induced mutations, injection as a technique for inducing mutations was abandoned in favor of larval feeding.

Dominant lethals: The correlation between extent of labeling and rate of induced mutation is strikingly illustrated by the data derived from the experiment on dominant lethals summarized in Table 7. In this case, film was exposed for only seven days.

The first two broods were heavily labeled. Although 100 percent of the sperm bundles were labeled in the first brood, some bundles contained unlabeled sperm heads, although none of the bundles were less than one-half labeled. This indicates that the germ cells comprising this brood of sperm were probably not more than one or two generations removed from primary spermatocytes at the time the isotope was available, but that most replicated in the presence of isotope as primary spermatocytes. The second-brood sperm appeared to be 100 percent labeled both in respect to number of bundles and the number of sperm heads within each bundle, indicating that isotope was available during several replications or that isotope was present when this population went through the first meiotic division. Since the number of grain counts over sperm comprising the second brood was higher on the average than in the first brood, both conditions are probably true.

The point to be emphasized here is that despite the high general activity produced in the testes by the feeding regimen employed, the induced mutations do not occur at random within the several broods, but only in those that are heavily labeled. When labeling falls off, as it does in Brood 3 and more markedly in Brood 4, the rate of mutation drops as well until in the fourth brood the level is

FIGURE 2a-d.— Photographs by Zeiss Photomicroscope, phase contrast (8×100). Top: Brood 1 sperm bundle from Blv II. a: tissue level; b: photographic film level. Bottom: Brood 1 sperm bundles from Blv III. c: tissue level; d: photographic film level.

FIGURE 2e.—Silver grains above sperm heads in seminal receptacle.

TABLE 5

Recessive lethals recovered: adult injection

		Chromosomes	Lethals	Lethals percent
RA1	Brood 1	641	1	0.16
	2	769	2	0.26
	3	734	7	0.95
	4	639	1	0.16
RA2	Brood 1	737	0	...
	2	1074	1	0.09
	3	834	2	0.24
	4	501	2	0.40

TABLE 6

Control rate following injection of adults

Brood	Regimen	Chromosomes	Number lethals	Percent lethals
1	5 females/male Day 2-5	955	1	.10
2	3 females/male Day 5-7	565	0	..
3	3 females/male Day 7-10	653	0	..
4	2 females/male Day 10-13	716	1	.14
Total:		2889	2	.07

TABLE 7

Genetic and radioautographic data: dominant lethals induced by tritiated thymidine

Brood	Number of eggs	Percent dominant lethals	Percent sperm bundles labeled	Labeled sperm per bundle	Grain counts per sperm head
1	3055	65.6	100	<64-64	22.8 $\pm$ 4.7
2	1981	69.1	100	64	36.4 $\pm$ 5.3
3	4386	23.9	50	16-32 $\pm$	6.3 $\pm$ 3.3
4	2364	6.8	20	1-4	2.3 $\pm$ 1.0
Controls					
1	3179	1.8			
2	2702	1.2			
3	2330	4.8			
4	1741	6.3			

back to that of the controls. This, added to the data above, indicates quite clearly that the genetic effects of H^3T are circumscribed within those nuclei in which the beta emissions occur.

(B) *Localization of mutants:* Because of the physical properties of the H^3 atom it was of interest to determine the distribution of induced mutations to test whether a more refined limitation of effect, than within labeled broods, might be present. The localizations are listed in Table 8 and the distribution of the mutations is mapped in Figure 3.

The 40 lethals plotted in Figure 3 were derived from Experiments RFL I, Blv I, Blv II, and RA 2. The total number of lethals recovered from these experiments, as listed in Tables 3 and 5, is 43. The discrepancy results from the following considerations: in Blv I, Brood 1, lethal Lv 3 is a translocation; in Blv I, Brood 2, lethal Lv 8 is an inversion; and in RA 2, Brood 2, a lethal was lost during balancing.

Table 8 lists 41 lethals. A 11 and A 12, listed individually within the table, are derived from the same male and localize to the same locus. They are plotted as a single lethal in Figure 3, at 63.0.

Five of the lethals warrant special comment. Lv 2 and Lv 4, from the same experiment and brood but from different males, have localized close to the forked locus; the former at 54.7 ± 1.1 , the latter 54.9 ± 1.3 . Dp(1,3)126 (BRIDGES and BREHME 1944) failed to cover the lethal in both cases. It is unlikely that either lies to the right of 54.5, since the duplication covers the region from there to 57.0.

FIGURE 3.—The distribution of lethals induced by H^3 -thymidine along the X chromosome of *Drosophila melanogaster*.

TABLE 8
Localization of lethals

Experiment	Brood	Male	Lethal	Locus $\pm$ 2sd
RFL I	1	6	A 2	29.8 $\pm$ 1.1
	1	10	A 3	36.3 $\pm$ 0.15
	1	21	A 4	43.5 $\pm$ 0.5
	1	23	A 5	28.8 $\pm$ 1.1
	1	25	A 6	0.4 $\pm$ 0.4
	2	24	A 7	64.0 $\pm$ 1.0
	2	25	A 8	48.1 $\pm$ 1.3
	2	35	A 10	0.0 $\pm$ 0.1
	3	4	A 11*	62.9 $\pm$ 0.5
	3	4	A 12*	63.1 $\pm$ 0.6
	3	16	A 13	41.4 $\pm$ 1.1
RA 2	3	24	B 1	33.0
	3	13	B 2	41.7 $\pm$ 1.5
	4	27	B 3	37.5 $\pm$ 1.5
	4	12	B 4	64.0 $\pm$ 0.8
Blv I	1	18	Lv 1	62.5 $\pm$ 0.3
	1	31	Lv 2	54.7 $\pm$ 1.1
	1	52	Lv 4	54.9 $\pm$ 1.3
	1	7	Lv 5	0.0 $\pm$ 0.1
	1	30	Lv 6	21.0 $\pm$ 0.9
	1	47	Lv 7	65.6 $\pm$ 0.9
	2	34	Lv 9	65.7 $\pm$ 1.0
	3	40	Lv 10	0.3 $\pm$ 0.5
	2	41	Lv 12	15.2 $\pm$ 1.0
	3	11	Lv 13	32.2 $\pm$ 0.7
Blv II	1	3	Lv 14	31.5 $\pm$ 0.7
	1	18	Lv 15	48.1 $\pm$ 1.0
	1	28	Lv 16	51.2 $\pm$ 1.2
	1	29	Lv 17	28.3 $\pm$ 0.5
	1	44	Lv 18	44.8 $\pm$ 0.3
	1	51	Lv 19	31.7 $\pm$ 0.7
	1	6	Lv 20	43.0 $\pm$ 0.6
	1	12	Lv 21a	32.7 $\pm$ 0.3
	1	12	Lv 21b	0.0 $\pm$ 0.2
	1	12	Lv 21c	57.5 $\pm$ 0.6
	1	16	Lv 22	64.8 $\pm$ 0.7
	1	27	Lv 23	24.5 $\pm$ 0.8
	1	48	Lv 24	33.6 $\pm$ 0.6
	1	49	Lv 25	57.3 $\pm$ 0.5
	2	17	Lv 26	0.4 $\pm$ 0.4
	3	35	Lv 27	50.6 $\pm$ 1.5

* A 11 and A 12 are considered as one mutation in Table 3 and plotted as one locus, 63.0 ± 0.3 , in Figure 3.

Lv 1 is shown at 62.5 ± 0.3 . From a total of 1,250 males, not a single $+^{car}$ fly was recovered, using the *f car* stock. Hence we cannot be sure whether the lethal is to the right or left of carnation or whether it is an allele of carnation. Females heterozygous for the lethal and *car* are not phenotypically carnation.

Lethal A 4, heterozygously with forked, produces forked females. Dp(1,3)126 suppresses the forked phenotype in females but does not suppress the action of the lethal in males. Genetic tests have shown that the lethal is separable from forked, both mutations having been induced in the same chromosome.

Lethal B 1 produces the vermilion phenotype when present heterozygous with

vermilion in females. No attempt was made to test for double mutation in this case; however, if two mutations are present, the lethal is close to the vermilion locus.

Figure 3 illustrates the nonrandomness of the lethal distribution. Of greater interest, however, is a comparison of this distribution with data obtained by X rays or gamma irradiation. Accordingly, our findings have been compared with those of IVES (1959) and SPENCER and STERN (1948). In both cases, the area of high mutability was found to be between 55 and 60. In neither case is the 28–34 area one of high mutability, nor the region between 62 and 66, as is true for the H³T-induced lethals. Similarly the relative paucity of mutations we observed in the region between 1.0 and 25 contrasts strikingly with the distribution obtained by IVES and by SPENCER and STERN. SPENCER and STERN point out, however, that the region between 9.4 and 12.7 on their map is the largest blank region. Similarly SLIZYNSKA and SLIZYNSKI (1943) reported a low point in the region 5–10 in their study of the distribution of 89 chemically induced sex-linked lethals.

By means of 2×2 contingency tables our distribution was compared for homogeneity with the X-ray induced lethals of SPENCER and STERN. Regions 1–25, 28–34, and 62–66 were compared with the following results:

Region	χ^2	Degrees of freedom	P
1–25	8.29	1	<.004
28–34	6.54	1	<.01
62–66	6.04	1	<.01

The data presented by MULLER (1928) and OLIVER (1932) also show a lack of high responsiveness in the 60–66 region and the 30–35 region, as grouped in their data, and are characterized by a high mutability of 50–55.

Among the lethals recorded here there are most certainly some that have arisen spontaneously. Table 8 identifies each lethal with respect to male and brood of origin, and it may thus be seen which lethals arose in the broods with the lowest frequencies of labeled sperm. However, one may not conclude categorically that these lethals represent those from unlabeled germ cells, since in each brood labeled sperm were present.

Our data show that sex-linked recessive lethals induced by the incorporation of H³T appear to be nonrandomly distributed. Moreover, their distribution differs from those reported by other investigators who used gamma rays or X rays.

DISCUSSION

The distribution of induced mutations obtained in this study may be explained in several ways. Two which are readily testable will be considered. The first concerns the possibility that different regions of the X chromosome vary among themselves with regard to the time of DNA synthesis. Asynchrony of DNA synthesis between the different chromosomes of individual nuclei has been reported (LIMA-DE-FARIA 1959) as well as variation in time of synthesis in different regions of individual chromosomes (PRESCOTT and BENDER 1963). If this were the underlying factor in the distribution reported herein, it would be expected that in Blv III, the nonrandomness will have been blurred or disappeared

entirely. Since more than one chromosome reduplication cycle in the presence of isotope has occurred during the maturation cycle of the treated sperm bundles, localized differences would be expected to cancel out. The lethals induced in this experiment have not all been localized to date; the data when complete will be reported separately. However, it seems unlikely that in four different experiments, RFL I, Blv I, Blv II, and RA 1, the same portion of the cycle would have occurred when isotope was available.

The second explanation of the distribution we observed is that this pattern reflects varying frequencies of thymine bases along the X chromosome. The two regions of high mutability parallel regions in which thymine occurs with relatively high frequency with respect to neighboring regions. Conversely, the 1-25 region is one in which thymine bases occur with low frequency compared with the other regions of the chromosome. Because of the low mean path of the beta particle emitted from the H^3 atom, radiation effects are restricted to the chromosome region from which they are emitted.

This second hypothesis is now being tested with tritiated deoxycytidine, and tritiated deoxyadenosine. A different distribution pattern would be expected as a result of using the former compound, while a pattern similar to that obtained with H^3T would be expected with the latter compound.

No distribution of X-ray induced mutations is available for the Canton-S stock used in this laboratory. However, the distribution of lethals induced by H^3 deoxycytidine may serve as a control in itself. If the two distributions do not differ, the hypothesis may then be discarded; but if a different distribution emerges, each may act as a control for the other.

Whether the mutations result from radiation damage caused by the action of beta rays upon the chromosome or from chemical changes in the transmutation process cannot be decided at this point. PERSON and LEWIS (1962) suggest that in strain 15 of *E. coli* the mutagenic action stems from effects other than radiation damage, but experimental evidence differentiating the two effects is unavailable. Neither is an attempt being made at this point to analogize from the structure of the DNA molecule to the structure of the chromosome. The "hot spots" reported here are of an entirely different order of magnitude or refinement from the situation discussed by FREESE (1959). Our data are derived from the genetic techniques available in *Drosophila melanogaster*, which do not afford resolution at the level possible within the *rII* region of phage T4. Further work along these lines, however, may provide relevant information concerning the arrangement of DNA molecules within the chromosome.

This paper is dedicated to the memory of VICTOR E. TINDERHOLT who played an important role in the early development of this work.

SUMMARY

Mutations have been induced in *Drosophila* males by tritiated thymidine fed to larvae or injected into adults. Radioautographs of testes of treated males demonstrated a correlation between the occurrence of mutations and the presence of labeled sperm bundles. In addition to the presence of sex-linked recessive lethals, dominant lethals were also measurable. Mapping of the induced sex-linked

recessive lethals disclosed a nonrandom distribution which differed from reported distributions of mutations induced by X rays and gamma rays. Distribution of mutations induced by H³T appears to reflect the varying frequency with which thymine bases occur along the length of the *Drosophila* X chromosome.

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