DISTRIBUTION OF LETHALS INDUCED BY TRITIATED DNA PRECURSORS IN *DROSOPHILA MELANOGASTER*^{1,2}

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AN earlier study showed that in *Drosophila melanogaster*, the distribution of sex-linked recessive lethals induced by tritiated thymidine (H^3 TDR) is a nonrandom one (KAPLAN, GUGLER, KIDD, and TINDERHOLT 1964), different in several respects from those obtained by SPENCER and STERN (1948) and by IVES (1959) following the use of X- or gamma-irradiation as the inducing agents.

Mutations were recovered following restricted larval feeding upon labeled food and correlated with labeled sperm populations. Owing to the properties of the H^3 atom, virtually all of the beta energy is dissipated within the nucleus of origin (PLAUT 1959; LAJTHA and OLIVER 1959). Thus, although it is possible to label the testes heavily, mutations have not been recovered from all broods. By use of radioautography it was shown that the broods characterized by the presence of mutations were those for which the available sperm were labeled.

The nonrandom distribution of induced lethals suggests that an even finer resolution may be possible than the limitation of induced mutations to labeled sperm. We have hypothesized that because of the physical properties of the beta particle emitted by the H^3 atom, radiation effects are generally restricted to the chromosome region from which they have been emitted. The two regions of high mutability uncovered by our technique may parallel regions of high thymine frequency with respect to neighboring regions of the chromosome. Conversely, the region of the X chromosome from 1–25, in which few mutations were found, appeared to represent a rather extensive region of low thymine frequency.

The work reported in this paper was carried out to test this hypothesis by using tritiated deoxycytidine (H^3 CDR) as the labeled nucleic acid precursor. Since thymine and cytosine are not hydrogen-bonded in the nucleic acid molecule, regions of high or low mutability induced by feeding H^3 CDR to *D. melanogaster* larvae might be expected to be different from those uncovered by the use of H^3 TDR.

A preliminary report has been made (KAPLAN *et al.* 1965).

MATERIALS AND METHODS

The techniques are essentially the same as those reported by KAPLAN *et al.* (1964). Canton-S males were treated by feeding them, as first instar larvae, upon food labeled with H^3 CDR. The

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tritiated compound (New England Nuclear Corporation) had a specific activity of 1.06 curies per millimole. The food was prepared as follows: 0.5 ml of 3% agar solution, 0.5 ml of a modified Eagle's tissue culture medium (SISKEN and KINOSITA 1961), and 0.2 ml of H^3 TDR; the total activity was 168 microcuries. Two volumes of this food were prepared for each experiment, and 100 16-hour-old first instar larvae were placed upon each for eight hours, then removed to the standard fly culture medium, rich in yeast, to complete development. Adult males were collected as they emerged and when 24 hours old were mated to females appropriate for the detection of sex-linked lethals.

Mating regimen and detection of sex-linked lethals: Induced lethals were detected by the Basc (Muller-5) technique. Three 3-day broods were established by mating the treated males, individually in vials, to three virgin females per brood. In one experiment, CDR III, only two 3-day broods were set up.

Localizations: The same care to minimize environmental variation and differential viability of the recovered crossover types was taken as in the previous study. Ten culture bottles were established for each localization, providing at least 1,000 males for measuring the rate of recombination. The marker stocks were selected to provide distances of no more than five crossover units to the right or left of the lethal tested.

Radioautographs: Males selected at random were used for radioautographs. Testes of Brood 1 males taken before the first mating, one day after eclosion, provide a picture of sperm available for Brood 1 fertilizations. Testes prepared after the first 3-day mating period present information on sperm used in Brood 2 matings. Similarly, Brood 3 radioautographs picture the sperm populations available for Brood 3 matings. Squashes were fixed in 2.5% formaldehyde, or by simply placing the slide after squashing upon a slide warmer at 60°C for one minute. The latter method was preferable for materials to be treated with DNase, as formaldehyde fixation reportedly interferes with the action of this enzyme. Alcohol fixation was avoided since it causes sperm bundles to clump. Kodak AR-10 stripping film was used after the preparations were stained by the Feulgen method.

Both enzymes, DNase and RNase, were obtained from the Worthington Biochemical Laboratories, Freehold, New Jersey. DNase digestion proceeded at 37°C for three hours according to the method of KUNITZ (1950). RNase digestion proceeded for 3 hours at 45°C according to the method of McDONALD (1948).

RESULTS

A. Incorporation of isotope: To determine how much of the label, if any, was incorporated into RNA, differential enzymatic digestions were carried out using deoxyribonuclease and ribonuclease together and separately. Labeled sperm remained after DNase and RNase digestion and the label above sperm heads was as heavy after the slides had been treated with enzyme as it was with no enzyme treatment whatever. However, the nuclei of spermatogonia, spermatocytes, and early spermatids were unlabeled after DNase treatment, whereas label was present after RNase treatment. Subsequent studies using the Feulgen reaction as a measure of the presence of DNA disclosed that mature sperm heads are resistant to the action of DNase, probably because of the cell membrane. Since the immature stages of spermatogenesis became Feulgen-negative after digestion with the enzyme, and radioautographs revealed no label, we concluded that no incorporation of deoxycytidine into RNA was detectable by the methods used. These findings agree with the chemical studies carried out by REICHARD, CANELLAKIS, and CANELLAKIS (1961) in which it was shown that ribonucleotides may be converted to deoxyribonucleotides which are then incorporated into DNA, the reac-

FIGURE 1.—Feulgen-positive sperm heads following digestion with DNase. Feulgen-negative nuclei in the testis wall above the sperm heads.

tion being nonreversible. Figure 1 illustrates the Feulgen-positivity of DNase-treated sperm. No Feulgen-positive nuclei are visible in the testis itself.

Two experiments, CDR I and CDR III, in which first instar larvae were fed labeled food, have been carried out. The radioautographic data are presented in Table 1.

First brood sperm of experiments CDR I and CDR III are almost totally labeled. Only two nonlabeled bundles were observed, but these two are of especial significance as will be discussed below. In the sperm expressed from the seminal vesicles, about 6.5% of the sperm heads were unlabeled. Some mature bundles contained one to four unlabeled sperm heads, which indicates that labeling occurred before the primary spermatocyte stage, probably during the spermatogonial division which gave rise to the 16 primary spermatocytes, or the spermatogonial generation before that. Bundles in which all 64 sperm were labeled had presumably incorporated label during interphase of the first meiotic division. Unlabeled bundles are those which had completed the DNA synthesis of the first meiotic division before the label had become available. This may serve as evidence that primary spermatocytes are present in the larval testis sometime before the end of the first instar.

It is generally believed that only spermatogonia are found in first instar larvae (BODENSTEIN 1950). However, the data from the pattern of labeling observed in the testes of adults which were fed labeled food as first instar larvae, as well as observations of labeling in larval gonads, indicate that spermatocytes may well be present before the end of the first instar.

In radioautographs prepared following H^3 TDR feeding for 6 hours to 24-hour

TABLE 1

Radioautographic data

	Labeled/unlabeled	Number labeled sperm/bundle	Grain counts
Brood 1 males			
Seminal vesicle sperm*	92/8		10.3 ± 0.4
	114/6		15.1 ± 0.9
Mature bundles	60/2	60-64	14.6 ± 2.9
			9.4 ± 0.8
			11.6 ± 1.6
			8.2 ± 0.2
			13.3 ± 0.8
			14.1 ± 1.0
	2/0	32	
	29/0	60-64	10.2 ± 0.5
			9.6 ± 0.8
			9.3 ± 0.7
			8.1 ± 0.9
			9.3 ± 0.5
			8.1 ± 0.7
Immature bundles	16/0	16-64	3.5 ± 0.8
Brood 2 males			
Mature bundles	47/0	50-60	3.7 ± 0.5
			2.9 ± 0.5
			2.8 ± 0.7
			2.9 ± 0.2
Immature bundles	15/0	32-60	4.7 ± 0.7
			2.6 ± 0.4
			3.5 ± 0.3
Brood 3 males			
Mature bundles	24/3	16-20	1.77 ± 0.13

* Labeled/unlabeled ratio applies to individual sperm heads in case of seminal vesicle sperm since bundle arrangement no longer exists.

larvae, incorporation of the label is restricted to the anterior region of the larval testis, as shown in Figure 2. The size gradient observed in these gonads is observed, also in larvae fixed shortly before the end of the first molt. The large unlabeled posterior cells are spermatocytes which have synthesized the DNA in preparation for the first meiotic division before the availability of label. The same topographical pattern and pattern of incorporation is shown in 48-hour and 72-hour larvae.

In the second and third broods of both experiments, all bundles were labeled but none was found with all 64 sperm labeled. In these cells, labeling occurred no later than one generation preceding the formation of primary spermatocytes. Grain counts of second and third brood sperm are lower than first brood counts, not necessarily because of lesser incorporation. Because incorporation has taken place at earlier spermatogonial generations, the sperm bundles end up with fewer labeled chromosomes. This decrease in each successive brood of labeled sperm within each bundle is a consequence of the dichotomous pattern of sperm pro-

FIGURE 2.—Labeled gonial cells in anterior region of gonad of 24-hour larvae fed H^3TDR for 6 hours. Fixation immediately upon removal from labeled food. A: tissue level. B: grain level.

duction characterizing spermatogenesis in *D. melanogaster* (TIHEN 1946); a single spermatogonium in a series of six divisions produces a bundle of 64 sperm. Since these divisions are synchronous within the cells of an individual cyst, incorporation of H^3CDR within the cells of a given cyst is also synchronous. Variability in amount of incorporated isotope is related to the time of DNA synthesis relative to the availability of isotope.

The rather striking uniformity of grain counts within individual bundles illustrates this point. Whereas there are marked differences among the means of the several bundles, the variability within individual bundles is low.

The first meiotic division is the last of the series within which new DNA is synthesized and, therefore, the last division which may be associated with chromosomal labeling. 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.

B. Frequency of mutation: In the two experiments, CDR I and CDR III, larval feeding upon labeled food was restricted to 8 hours. CDR II is a control in which unlabeled CDR was used in place of the labeled compound. To achieve the same concentration of CDR as existed in the radioactive food, the concentration of unlabeled CDR was 4.2×10^{-2} mg per 1.2 ml of food.

Mutation rates recovered in experiments CDR I and CDR III are shown in Table 2. Both for Brood 1 and for Brood 2 the data from these experiments may be pooled for statistical computations since they are compatible with each other.

TABLE 2

Genetic data. Larval feeding of tritiated deoxycytidine

Experiment	Brood	Chromosomes tested	Sex-linked recessive lethals	Percent lethals			
CDR I	1	1536	10	0.65			
	2	1523	4	0.26			
	3	1408	2	0.14			
CDR III	1	3008	31	1.03			
	2	2292	10	0.44			
CDR II (control)	1	2456	4	0.16			
	2	1866	0	...			
	3	1800	1	0.06			
		6122	5	0.08			
Statistical data							
		χ^2	χ_e	m	p	df	P
Brood 1 (pooled) <i>vs.</i> control		14.8				1	0.0001
Brood 2 (pooled) <i>vs.</i> control*		5.1	2.3	4.9	0.3	1	0.025>P>0.005

* Calculated according to method of FISHER and YATES (1948) for 2×2 contingency tables where smallest expectation is less than 100. YATES's correction for continuity applied.

Comparison with the control shows that mutation rates of both these broods are significantly elevated above the respective control rates.

These mutation rates are associated with sperm populations almost entirely labeled. Although the grain counts for Brood 2 sperm are lower than in the case of Brood 1, at least half the sperm within each bundle were labeled. The falling off to the control mutation rate within Brood 3 is correlated with the appearance of nonlabeled bundles, and with fewer labeled sperm within each labeled bundle.

C. *Localization of mutants:* The sex-linked recessive lethals recovered were localized and the distribution compared with the one obtained following the use of H^3 TDR (KAPLAN *et al.* 1964). The lethals are given in Table 3; the two distributions are plotted in Figure 3. The lethals derived from experiment CDR I have been given the prefix DCA; the prefix DCB used to identify the lethals recovered from experiment CDR III. The first Arabic number indicates the brood in which the mutant was found, and the numeral after the dash identifies the male in which the mutant sperm was produced. The association of more than one mutation with a male in any one brood is denoted by use of lower-case letters. Thus DCB 1-39d indicates that this lethal occurred within experiment CDR III, within the first brood, and was the fourth recovered from male 39 within that brood.

A total of 57 mutations was scored for the two experiments, CDR I and CDR III. However, Table 3 shows 49 lethals, a discrepancy brought about by the loss of four lethals before localization, identified as DCA 2-16, DCA 2-26, DCB 1-15, and DCB 1-28. In addition, five lethals are chromosomal rearrangements, and one, DCB 1-35a, was found to be a double lethal and is listed twice in the table.

The rearrangements as determined in the salivary gland chromosomes are as follows: DCB 1-2b: Translocation between the X and 2L. Breaks are at bands 6F and 26C. DCB 1-45a: Translocation between the X and 2R. Breaks are at 8B

TABLE 3

Localization of lethals

Locus $\pm 2sd$	Lethal	Locus $\pm 2sd$	Lethal
0.06 $\pm$ 0.1	DCB 1-47	45.4 $\pm$ 0.5	DCA 1-23
0.07 $\pm$ 0.1	DCA 1-3	48.1 $\pm$ 1.1	DCB 1-23
0.2 $\pm$ 0.1	DCB 1-35a*	48.5 $\pm$ 1.0	DCA 1-17
0.3 $\pm$ 0.3	DCB 1-49a	51.8 $\pm$ 0.3	DCB 1-2c
0.5 $\pm$ 0.4	DCB 1-15	52.3 $\pm$ 1.1	DCA 2-25
0.7 $\pm$ 0.4	DCB 1-9	54.7 $\pm$ 0.7	DCA 1-2b
1.2 $\pm$ 0.4	DCB 1-11	56.5 $\pm$ 0.2	DCA 1-5
1.7 $\pm$ 0.8	DCB 1-39b	56.5 $\pm$ 0.2	DCB 2-29c
2.3 $\pm$ 0.8	DCB 1-28	56.7 $\pm$ 0.3	DCB 1-43b
5.1 $\pm$ 0.4	DCA 1-26	58.2 $\pm$ 0.7	DCB 1-2a
14.6 $\pm$ 0.5	DCB 1-30	59.1 $\pm$ 1.4	DCB 1-35a*
15.3 $\pm$ 0.6	DCA 1-19	59.3 $\pm$ 0.8	DCA 2-15
16.7 $\pm$ 1.3	DCB 1-50	59.7 $\pm$ 0.9	DCB 1-39a
17 $\pm$	DCB 1-14	59.9 $\pm$ 1.0	DCB 1-43a
21.6 $\pm$ 0.1	DCA 3-6	60.4 $\pm$ 0.7	DCB 1-41
27.5 $\pm$ 0.2	DCB 1-39c	60.7 $\pm$ 0.7	DCB 2-16
31.4 $\pm$ 0.6	DCA 1-2a	62.1 $\pm$ 0.3	DCB 1-44
35.4 $\pm$ 0.5	DCB 1-39d	62.2 $\pm$ 0.3	DCB 2-39
35.5 $\pm$ 0.9	DCB 1-35d	62.3 $\pm$ 0.3	DCB 2-29a
40.3 $\pm$ 1.0	DCB 1-16	62.9 $\pm$ 0.3†	DCB 2-35b
42.2 $\pm$ 0.9	DCA 1-30a†	63.5 $\pm$ 0.6†	DCB 1-35b
41.9 $\pm$ 0.9	DCA 1-30b†	64.5 $\pm$ 0.7†	DCB 2-35a
43.0 $\pm$ 0.6	DCB 2-42	64.7 $\pm$ 0.8†	DCB 1-35c
43.7 $\pm$ 0.3	DCB 1-45b	64.7 $\pm$ 0.7	DCA 3-19
		68.7 $\pm$ 0.8	DCB 2-19

* Two loci listed for DCB 1-35a.

† Cluster of two.

‡ Cluster of four.

and 46B. DCB 1-49b: Translocation between X and 3L. Breaks are at bands 4F and 62A. DCB 1-32: Inversion. Breaks are at bands 13B and 16A. DCB 2-29b: Lost before cytological characterization. Crossover suppression, however, extended from about 39 to the right of forked, 56.7.

Of the 49 listings in Table 3, 45 have been plotted in Figure 3. This is because of two clusters, one a cluster of two and one of four: DCA 1-30a and b; and DCB 1-35b and c, and DCB 2-35a and b. Thus two points have been plotted for six lethals. A cluster of two derived from the work with H³TDR was, similarly, plotted as one point.

In the case of DCB 2-35b, only seven crossovers between the lethal and carnation, which lies to its left, were found in the 1,589 flies counted. Between DCE 2-35a and carnation, 37 crossovers in a total of 1,807 flies were recovered. The difference between these two is significant at the 1% level. However, there is no significant difference in the number of crossovers between carnation and DCB 2-35b and DCB 1-35b, nor between DCB 1-35b and DCB 1-35c. Because all four were recovered from the progeny of the same treated male, and tests of

TRITIATED DEOXYCYTIDINE

TRITIATED THYMIDINE

COMBINED DISTRIBUTION

FIGURE 3.—Distribution of lethals induced by H^3 CDR and H^3 TDR, plotted separately and combined. One lethal, B1, reported in the paper on H^3 TDR, has been removed from its original locus at 33. The lethal has been found to be separable from a visible mutation at the vermilion locus and is located at 27.4.

allelism were not possible, all four were scored as having derived from a single event. Partially labeled bundles were observed among the sperm available for first brood fertilizations. Therefore, it is reasonable to assume that incorporation had taken place one or two spermatogonial generations preceding the first meiotic division. In this way a mutation occurring shortly after incorporation in one X chromosome in a group of four or eight spermatogonia would be present in 16 or eight sperm cells of the bundle of 64.

The lethal DCB 1-14 deserves a few words of special comment. We show its occurrence at 17 since both of the complementary crossover classes resulting from recombination between crossveinless and cut were recovered. Severe crossover suppression from the distal end of the chromosome to crossveinless was observed but was not correlated with any observable cytological rearrangement. Although normal recombination was observed in the females scored, the pattern observed in scored males cannot be explained by the presence of two lethals. A factor exists which apparently reduces viability of the recombination classes containing both crossveinless from the marker chromosome and the sc^+ gene from the treated chromosome.

In Figure 3, the distributions of lethals induced by H^3 CDR and H^3 TDR are

plotted in intervals of one crossover unit. Thus a lethal localizing to 59.3 has been plotted between 59 and 60, as would any other falling within that interval. The H^3CDR distribution is a nonrandom one.

When the chromosome is divided into ten equal classes, each containing seven crossover units, 4.5 is the mean class-frequency for uniform distribution. The actual distribution is 10, 0, 4, 2, 1, 3, 6, 3, 13, and 3. The chi-square test gives 34.3 ($P < 0.001$). As the class interval decreases, the departure from uniformity becomes greater. Moreover, the first region, 0-7 may be omitted because of the special characteristics of the left end of the *Drosophila* X and the distribution within the remaining nine regions compared with the expected uniform distribution. This yields a chi-square of 27.6 ($P < 0.01$).

Although the H^3TDR distribution, as well as the H^3CDR distribution, has been shown to be nonrandom, it is important to determine whether the two differ from each other or whether they may be regarded as the same. A comparison yields a chi-square value of 15.9, which is significant at the 4% level.

When individual chromosomal regions of the two distributions are compared, we find that from 0-25 the two are essentially the same. Between 28-34, more lethals are induced by H^3TDR , a difference significant at the 1% level. From 35-55, the two distributions are almost identical. Between 56-62.5, the locus of carnation, 13 lethals have been induced by H^3CDR whereas only three were produced by treatment with H^3TDR , a difference significant at the 2% level. The number of lethals recovered in this region is also higher than that reported by SPENCER and STERN after X rays. All lethals within this group, with one exception, lie to the left of carnation. Genetically, they cannot lie to its right. Lethal LVI, within the H^3TDR distribution, is listed at 62.5. Since no crossovers between it and carnation were recovered in a total of 1,250 males counted, it is scored, in statistical computations, as occurring between 56 and 62.5, and also scored among the lethals lying to the right of carnation.

After treatment with H^3TDR , a greater proportion of the lethals induced in the X chromosome was shown to lie within the region 62-66 than after treatment with X rays. There is, however, no significant difference between the H^3TDR and the H^3CDR distributions with respect to this region (2/45 *vs.* 7/40), nor is the H^3CDR distribution reported here different from the one of SPENCER and STERN. (Statistical data for the relevant analyses are presented in Table 4.)

DISCUSSION

STURTEVANT (1913, 1915) pointed out very early in studies of linkage relationships that regional differences in crossover frequencies could distort the actual relative spacial distances separating genetic factors. MULLER and PAINTER (1932), developing this idea further, showed that regions of equal physical length may be differentiated from one another not only in regard to their crossover frequency, but also in regard to their mutation frequency. Thus, although in terms of recombinational relations, the region from yellow to white constitutes only about 2.5% of the X chromosome, on the basis of mutation frequency it

TABLE 4
Statistical data

Region	Comparison*	χ^2	χ_c	m	p	df	P
56-62.5	cytidine <i>vs.</i> X ray	10.1				1	<0.001
28-34†	thymidine <i>vs.</i> X ray	4.5	2.6	3.1	1.2	1	0.025>P>0.005
28-34†	cytidine <i>vs.</i> thymidine	5.3	2.3	4.2	0.46	1	0.025>P>0.005
56-62.5†	cytidine <i>vs.</i> thymidine	5.1	2.3	7.5	0.47	1	0.025>P>0.005
0-1	(cytidine + thymidine) <i>vs.</i> X ray	6.7				1	<0.01
28-34	cytidine <i>vs.</i> mutator	12.2				1	<0.001
56-end	cytidine <i>vs.</i> mutator	11.1				1	<0.001
Complete							
distribution	cytidine <i>vs.</i> thymidine	15.9				8	<0.04
	Randomness of cytidine distribution	34.3				9	<0.001

* Cytidine—distribution of H³CDR-induced lethals; thymidine—distribution of H³TDR-induced lethals; X-ray—distribution of X-ray-induced lethals (SPENCER and STERN 1948); mutator—distribution of lethals induced by Ives's (1959) *hi* gene.

† Calculated according to the method of FISHER and YATES (1948), with YATES's correction for continuity applied.

accounts for 10–20%. Recently RUDKIN (1965) published a “DNA map” of the X chromosome in order to introduce DNA content as a basis on which to express the relative frequencies with which mutations are observed in different chromosome regions. He finds that mutations have been recovered within the ten regions into which he has divided the X chromosome in proportion to the DNA content. On the basis of RUDKIN's findings and in consideration of the physical properties of the beta emissions from the tritium atom, the distribution of lethals recovered after the use of tritiated DNA precursors would be expected to reflect the regional DNA content along the length of the X chromosome.

The separate distributions for tritiated TDR and CDR do not fulfill this expectation. Each differs from SPENCER and STERN's (1948) X-ray distribution, and each differs from the other. This may indicate that the relative mutabilities are not related to DNA content or it may mean that the sensitive regions are those which contain an unusually high frequency of one or the other pyrimidine.

A way of testing our pattern of lethal mutations in relation to DNA content would be to feed a combination of H³TDR and H³CDR to *Drosophila* larvae, then plot the distribution of recovered lethals. For the present, we are constrained to add the two distributions of the independently induced lethals and compare the sum to the one obtained by SPENCER and STERN, who used X rays. Since cytosine and thymine are not hydrogen-bonded within the DNA molecule, the summing of the lethal distributions recovered independently after the use of each would seem to be a valid operation. The data of SPENCER and STERN are used for several reasons. Their distribution of lethals represents the mutational response of the X chromosome to radiation delivered from an external source. The lethals were localized by a technique closely approximating our own; therefore, their data and ours are biased in the same way by differences in crossover

frequency within the different regions of the chromosome. Moreover, RUDKIN used these data in his study of mutabilities of chromosome regions.

The combined distribution of the H^3 TDR and H^3 CDR induced lethals is presented at the bottom of Figure 3. This distribution differs from SPENCER and STERN's only in the region 0-1, where ours is higher; there are no significant differences in the other regions. Nor is the portion of chromosome from 1-15 different in the frequency of recovered lethals from the comparable region in SPENCER and STERN's data. The region 5-15 has been consistently one of low response to X rays, gamma-rays, and to chemicals (MULLER 1928, SLIZYNSKA and SLIZYNSKI 1943; SPENCER and STERN 1948; IVES 1959).

While the region 28-34 is higher in its response after H^3 TDR than after H^3 CDR or X rays, the distribution after H^3 CDR is not lower than that of X rays. Similarly, for the region 56-62.5, H^3 CDR produces a higher frequency in that region than does H^3 TDR, but H^3 TDR does not differ significantly in its effect from X rays.

The left tip of the X from 0-1 is always a high response region. Our data show a higher response than that of SPENCER and STERN. However, MULLER and PAINTER (1932) indicated that this region of the chromosome, on the basis of their "mutation map," constitutes 10 to 20% of the chromosomal material. In our study, 15% of the lethals lie within that region.

Thus, by combining data derived from the use of two different labeled DNA precursors, we find the induced sex-linked lethals are distributed along the X chromosome in a pattern that does not differ from that obtained after X-irradiation; yet each differs from the other in two regions; 28-34 and 56-62.5. In the former is found a higher frequency of lethals after the use of H^3 TDR than after X rays or H^3 CDR. In the latter region the frequency is higher after H^3 CDR than either H^3 TDR or X rays. These differences, we suggest, reflect differences in base frequencies within these regions sufficient to result in a higher probability of mutation in 56-62.5 than in other regions when H^3 CDR is used.

Differences of this nature, as pointed out by RUDKIN (1965), may be sought in short regions, particularly single bands. It is, therefore, noteworthy that in such relatively large regions as we have used to compare the H^3 TDR and the H^3 CDR distributions, a level of significance of 4% has been attained.

IVES's study on the mutator gene *hi* disclosed that in several ways the mutations induced under the influence of this recessive gene were different in their distribution along the length of the X chromosome than was the case for his lethal mutations induced by gamma- or X-irradiation. He pointed out that this difference in distribution presumably reflects a general difference between the mutator effects and radiation effects. The mutagenic effect of a genetic mutator such as *hi* is much more specific and biochemically selective than is the action of radiations. On the other hand, lethals produced by radiation would be expected more nearly to approximate the distribution of available genetic material.

IVES's *hi* gene has since been lost and it is no longer possible to restudy its effect, but it is of interest to compare his mutator distribution with the two that we obtained. We found that the H^3 TDR distribution did not differ in any of the

regions from Ives's mutator distribution, whereas the H³CDR does differ significantly in the 28–34 and from forked (56.7) to the right end. It is not possible to test the H³CDR distribution relative to 56–62.5 since Ives's data are given for the region from forked to the right end. This leads us to suggest that Ives's mutator gene in some way influenced the thymine or adenine metabolism or possibly their pairing relationships.

In conclusion it may be pointed out that this hypothesis has some relevance to the question of gene duplication. The presence of a chromosomal region with high thymine (or cytosine) frequencies suggests that in the evolution of new units by the duplication of preexisting ones, it may have been possible to produce a region of high thymine (or cytosine) frequency from the original unit characterized by an unusually large number of thymine (or cytosine) bases.

SUMMARY

Sex-linked recessive lethals have been induced in males by feeding tritiated deoxycytidine to first instar larvae. The lethals have been localized and their distribution compared to the one previously obtained with tritiated thymidine. The two independently produced distributions differ from each other at the 4% level of significance. Two regional differences have been noted, one of high mutability after tritiated thymidine and one of high mutability after tritiated deoxycytidine. Data combined from the two treatments closely parallel the distribution of lethals induced by X rays and gamma-rays, suggesting that the combined distribution reflects the regional content of DNA along the X chromosome. The differences in lethal distributions produced by the two DNA precursors suggest that significant local variations in thymine and cytosine content may exist within chromosomal DNA.

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