Hughes, Ann M. and Philip E. Hildreth.
University of California, Berkeley, California. The production of a new mutant in D. melanogaster by low doses of tritium irradiation.

On two separate occasions, during the course of an investigation in which D. melanogaster larvae were being raised on tritiated medium, apparently identical mutants affecting adult coloration were recovered. Since these mutants originated among only a few thousand flies and only in the treated series, it appeared that tritium might be repeatedly causing mutations at a specific locus. Therefore, further experiments were conducted in two series (1961-62, 1965-66) in order to test whether this one type of mutant could be routinely produced.

Homozygous yellow (y/y) females that had mated with $y/B^{S}Yy^{+}$ males oviposited on control or tritium-supplemented medium where the eggs hatched, the larvae and pupae developed. For the treated series, the standard cornmeal-molasses-agar-yeast medium contained either 0.1 or 1.0 μc ^{3}H per gram. On the basis of the table of Tolbert (1960) and assumptions of rapid equilibration between the larval body water and that of the medium, the two concentrations of tritium used would give a maximum total body irradiation of 0.5 and 5.0 r per fly during the time from hatching through eclosion. The actual irradiation is probably less.

The F_1 males ($y/B^{S}Yy^{+}$) were then individually mated to virgin yellow nontreated females and the offspring raised on control medium. The expected offspring would again be yellow females and nonyellow Bar-eyed ($y/B^{S}Yy^{+}$) males. Among the second-generation offspring exceptional males were recovered; they had bar-shaped eyes and wildtype-colored wings as in the expected class, but their bristles were yellow and bodies yellowish (but not as yellow as y/Y males) instead of wildtype. The mutant has been transmitted with the Y chromosome ($B^{S}Yy^{61d}$) through many generations. The results are shown in Tables I and II.

In order to determine whether other types of irradiation would produce similar mutation, experiments involving the above mating procedure, but using X rays as the irradiation source, were carried out. In the first experiment, virgin males were given 2800 r by use of a 250-kV X-ray machine with a 0.5-mm Cu and a 1.0-mm Al filter. The second experiment repeated the first except that a smaller dose of X-ray was used. (A 150-kV machine with an inherent filtration of 1.5 mm Al was used to give the males 1000 r.) In the third experiment, prepupae were collected from culture bottles, irradiated with the 150-kV machine (735 r), and then allowed to pupate and eclose. In these experiments the expected offspring were the same as in the tritium experiments. Among 25,120 males from the control and 22,476 from the treated series, no mutants were recovered. Therefore it appears likely that it is not irradiation per se but some specific property of tritium that caused the mutations.

According to Dr. Irwin I. Oster, who kindly did salivary gland analyses, the $B^{S}Yy^{61d}$ chromosome was not different from the original $B^{S}Yy^{+}$ chromosome. Thus no gross chromosomal aberration was associated with the mutation. It is likely that the change was within the y^{+} locus, although an undetected alteration in some other region of the $B^{S}Yy^{+}$ chromosome cannot be ruled out.

To determine interactions with other alleles, males carrying $B^{S}Yy^{61d}$ were crossed with the following stocks: y, y^{2S}, y^{62a} sc cv and y f:, br ec/y^{3d}, y^{2} cv v f and M(1)n/FM6 y^{31d}.

When heterozygous, y^{61d} acted as a dominant gene over all alleles in regard to wing color, over y^{2} and y^{3d} in regard to body color.

The alleles which normally produce dark bristles are dominant over y^{61d}. In hemizygous condition, both y^{3d} and y^{61d} produce yellow bristles, yet $y^{3d}/B^{S}Yy^{61d}$ males have dark bristles.

Combining the control and the X-ray totals, the mutation rate is 0.016/1000. Since both mutants were found in the same culture, the minimum number of mutant events could be 0.008/1000. In the tritium-treated group, the total mutation rate is 0.148/1000, and the minimum mutant event rate 0.058/1000. In addition, in the tritium treatment, in one culture a gonosomal mosaic for this mutant was recovered, and in another culture one mutant in the third generation. At present there is no explanation for the lack of recovery of mutants in each experiment in the 1965-66 series, as was observed in 1961-62. (The work described in this paper was sponsored, in part, by AEC Contract No. W-7405-eng-48.)

Tolbert, B.M.: Self-Destruction in Radioactive Compounds. Nucleonics, Aug. 1960:74-75.

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Table I. The occurrence of $B^{S}Yy^{61d}$ mutant in Drosophila grown on medium containing tritium (1961-62).

Experiment	Date	Treatment	Number cultures	Total males	Total mutants	Mutant events[a]
I	6/61	Control	199	6487	0	0
		0.5 r	189	6044	5	2
II	8/61	Control	385	15574	0	0
		0.5 r	388	15954	1	1
III	1/62	Control	240	7260	2	1
		0.5 r	263	8474	6	3
		5.0 r	246	7419	1	1
IV	4/62	Control	199	7817	0	0
		0.5 r	206	7673	0	0
		5.0 r	394	13903	10	2
Totals		Control	1023	37138	2	1
		0.5 r	1046	38145	12	6
		5.0 r	640	21322	11	3

a. In some cultures, more than one mutant was recovered. Thus, "mutant event" refers to the number of cultures in which one or more mutants were found.

Table II. The occurrence of $B^{S}Yy^{61d}$ mutant in Drosophila grown on medium containing tritium (1965-66)

Experiment	Date	Treatment	Number cultures	Total males	Total mutants	Mutant events[a]
V	10/65	Control	28	7941	0	0
		20 r[b]	69	18085	0	0[c]
VI	11/65	Control	200	15745	0	0
	12/65	0.5 r	495	41178	0	0
VII	2/66	Control	117	9853	0	0
		0.5 r	338	26270	0	0
VIII	3/66	Control	100	2923	0	0
		1 r	319	10463	1[d]	1

a. In some cultures, more than one mutant was recovered. Thus "mutant event" refers to the number of cultures in which one or more mutants were found.
b. Tritium solution was inadvertently contaminated with ^{14}C.
c. One mutant found in third generation.
d. Phenotypically like original mutant, but germ cells $B^{S}Yy^{+}$.

Hijikuro, S. Osaka University, Osaka, Japan. Studies on the content of beta-alanine and the body color of Drosophila.

Seki (1962, DIS 36:115) first reported that beta-alanine was detected in the pupal sheaths of wild type strain of D. virilis, but not in those of dark color mutant strain, ebony (eb). Furthermore, Fukushi (1966, Japan. J. Genetics: In press) revealed the fact that the pupal sheaths of a pale body color mutant, yellow (y), of D. melanogaster contained more beta-alanine than those of the wild type.

In the present study, the content of beta-alanine in adult flies (whole body) was analyzed after acid hydrolysis with an amino acid analyzer. In a wild type strain Oregon-R) and y of D. melanogaster, molar ratios of beta-alanine to leucine were 0.05 and 0.08, respectively. In D. virilis, a wild type strain (Pasadena) contained the same amount of beta-alanine as y. The ratio was 0.06. The content of beta-alanine in ebony mutants of both species was negligible.

These results suggest some relationship between the body color and the content of beta-alanine in adult flies as well as in pupal sheaths.