THE INDUCTION OF MINUTE MUTATIONS IN DROSOPHILA WITH TRITIUM-LABELLED THYMIDINE

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SEVERAL studies have been performed with the aim of inducing mutations by tritiated compounds. In *Drosophila*, KAPLAN and SISKEN (1960) investigated the induction of sex-linked recessive lethals in sperm from males which had lived on food containing tritium-labelled thymidine from the time they were 16 hour old larvae until pupation. STRÖMNÆS (1962) has also studied the induction of such lethals by injecting tritium-labelled cytidine and thymidine into 6 to 24 hour old male imagoes. The data presented in both papers suggest a significant increase in the frequency of sex-linked recessive lethals after treatment with tritiated compounds. The present authors decided, therefore, to test the ability of tritiated thymidine to induce dominant Minute mutants in *Drosophila*. The experiment was designed in such a way that other changes could be studied simultaneously.

MATERIALS AND METHODS

All the males used were from an Iso-Amherst stock. The females employed were all virgins of a yellow, achaete, scute, prune, singed (*y ac sc pn sn*) stock; all the genetic markers are located on the X-chromosome. The adult males were 1 to 24 hours old when injected with thymidine. After injection the males were stored for a day and a half without females. This was done to insure that only those males without apparent ill effect from the operation were used in these experiments. Individual males were then crossed with eight females for a period of three days. Then the females were transferred to half-pint milk bottles to oviposit for six days. Each male was then given a new set of eight females for another three day mating period. This was repeated six times. The female offspring from all these broods except Brood 2, which was discarded, were counted and scrutinized for Minutes and for mosaic females. The number of wild-type males and the number of mutations at the marked loci have been recorded. All presumptive Minutes and many of the mosaic females were tested at least one more generation.

The tritium-labelled thymidine had an activity of 2.7 c per mmole, or 1 mc per ml in distilled water. The amount of thymidine injected in the abdomen of the males was about 0.08 mm³. Injection was as close to the testis as possible, and was done in such a manner that it produced a readily visible swelling of the abdomen. However, great care was taken to prevent the injected fluid from escaping. The injection apparatus consisted of a small-bore rubber tubing connected at one end to a syringe and at the other end to a metal holder fitted with the glass injection

needle. The whole system except the glass needle was filled with sterile distilled water. The needle was made by drawing glass tubing to capillary size in a gas flame, and was then, in a microflame, redrawn to the desired bore. All needles were inspected and broken under the dissecting microscope to obtain a bevel. A hole punctured by a sharp, beveled needle would close up after injection, while a blunt needle would leave the puncture open so that the injected solution would run out.

The entire procedure in the control series was the same as that described, except that unlabelled thymidine was employed. It was prepared by dissolving 2.2 mg thymidine in 25 ml of distilled water which was reboiled just before use. The tritium-labelled as well as the unlabelled thymidine were purchased from Schwarz Laboratories, Inc., Mt. Vernon, New York.

According to BRIDGES and BREHME (1944) dominant Minute loci are found on all four chromosomes of *Drosophila melanogaster*. Most Minutes are small deficiencies which are lethal in homozygous condition. In heterozygous condition, Minutes are readily recognizable since the bristles are shorter and thinner than normal. The bristle effect is often accompanied by a more drastic pleiotropic effect which in some flies comes to expression as rough eyes, spread wings, abnormal wing veins, and so on. Some Minute flies often may exhibit a longer developing time than normal, reduced fertility, or complete sterility. In our material, several types of bristle deformities were found. Some of these looked like forked, singed, javelin or Stubble. The Minutes may have very much reduced bristle size or may be closer to normal. Since two investigators scored Minutes, in our experiments an error in classification might easily have been introduced, and thereby bias the final result. This was appreciated at the beginning of our investigation, and all precautions were taken to minimize errors of this type. Thus, a close cooperation of the two investigators was established at the start in order to obtain uniformity in classification and scoring. In the first scoring, all variations in bristle phenotype were scored as Minute or deviant. These were all tested a second generation, and then the final scoring was made. This procedure revealed that there were very few mistakes in the initial scoring. Although it was soon established that the scoring was uniform, the routine of testing all variants from normal bristle phenotype was employed. When flies were completely sterile, they were scored on the basis of their phenotype only. About two thirds of the F_1 Minutes were fertile and transmitted the Minute character.

RESULTS

A total of 1,420 males was injected. Of these, 1,344 males survived more than 36 hours, yielding a survival rate of 95 percent. Of the surviving males, 1,068 were selected at random for further study: 562 had been injected with tritium-labelled thymidine, and 506 with unlabelled thymidine.

Since all the males were approximately of the same age, from the same stock and given an injection, it was anticipated that they would have the same survival rate whether the injected fluid included labelled or unlabelled thymidine. The male survival rate is given in Table 1. Here it may be seen that survival of males

TABLE 1

Survival values and fecundity rates for males injected with thymidine

Brood No.	Days	Number of F ₁ females	Number of P ₁ males	Male survival percent	Male fecundity
<i>Tritium-labeled thymidine</i>					
1	1-3	144,849	562	100	258
3	7-9	74,556	494	88	151
4	10-12	54,960	397	71	138
5	13-15	42,144	310	55	136
6	16-18	25,930	203	36	128
<i>Unlabelled thymidine</i>					
1	1-3	134,046	506	100	265
3	7-9	82,614	457	90	181
4	10-12	61,156	394	78	155
5	13-15	44,896	320	63	140
6	16-18	23,182	220	43	105

Survival value is that portion of the initial number of males that survive through later broods. Fecundity is calculated as number of F₁ daughters per P₁ male.

receiving labelled thymidine is less in the third and later broods than in the series using unlabelled thymidine. A chi-square test indicates that the differences in the fourth and later broods are statistically significant. The same table summarizes male fecundity, as represented by the number of F₁ daughters per male. It can be seen that all males in the control series (unlabelled thymidine) have a higher fecundity than the males in the labelled series (tritiated thymidine) for all broods except the last. None of the differences are statistically significant in individual brood comparisons. Overall, however, the unlabelled males seem to be more fecund.

The total number of F₁ daughters and the numbers of Minute females, wild-type males, mosaic females and flies with hemithorax are given for each brood in Table 2. In the series where labelled thymidine was used, the overall Minute frequency was about 0.71×10^{-3} (Table 2). The overall frequency of Minutes in the control series is about 0.66×10^{-3} , and the frequencies in the individual broods did not deviate significantly from this value as shown by chi-square test. Although the labelled series exhibited a higher frequency of Minutes than the control series, the overall difference was not significant at the 5 percent level. However, by inspecting the frequencies for the individual broods, it can be seen that the sixth brood in the labelled thymidine series had a higher frequency than those obtained for any other brood. The difference from each other brood considered individually was significant.

The wild-type males arise when a nullo-X-chromosome egg is fertilized by an X or XY bearing sperm. For each brood and for all broods, the frequency of wild-type males was less in the labelled series than in the unlabelled series. Although the differences between the two series were not statistically significant, the data support that fewer wild-type males occurred in the labelled series. In conformity with the data obtained for Minutes, the lowest frequency of wild-type males was found in the sixth brood of the labelled series.

TABLE 2

Minute females, wild-type males, mosaic females and flies with hemithorax among the offspring from males injected with thymidine

Brood No.	Days	Number of F_1 females	Minutes Number $\times 10^{-3}$	Wild-type males Number $\times 10^{-3}$	Mosaic females Number $\times 10^{-3}$	Hemithorax Number $\times 10^{-3}$
<i>Tritium-labelled thymidine</i>						
1	1-3	144,849	105 0.72	119 0.82	51 0.35	15 0.10
3	7-9	74,556	49 0.66	63 0.85	43 0.58	19 0.25
4	10-12	54,960	32 0.58	48 0.87	15 0.27	15 0.27
5	13-15	42,144	26 0.62	43 1.02	16 0.38	16 0.38
6	16-18	25,930	30 1.16	18 0.69	7 0.27	19 0.73
Total		342,439	242 0.71	291 0.85	132 0.39	84 0.25
<i>Unlabelled thymidine</i>						
1	1-3	134,046	86 0.64	123 0.92	34 0.25	10 0.07
3	7-9	82,614	56 0.68	77 0.93	18 0.22	21 0.25
4	10-12	61,156	40 0.65	65 1.06	12 0.20	15 0.25
5	13-15	44,896	31 0.69	50 1.11	12 0.27	9 0.20
6	16-18	23,182	14 0.60	25 1.08	4 0.17	4 0.17
Total		345,894	227 0.66	340 0.98	80 0.23	59 0.17

Since the P_1 females carried several sex-linked recessive markers, it was possible to examine the offspring for mosaic females. There was overall a significantly higher frequency of mosaic females in the labelled series than in the unlabelled series (Table 2). Comparing the frequencies brood by brood, reveals also a consistently higher frequency in the labelled than in the unlabelled series.

One particular abnormality that was frequently encountered in the offspring was hemithorax. The overall difference in the frequency of such flies between the two series was statistically significant, the higher incidence occurring in the labelled thymidine series. A brood by brood comparison shows a higher frequency in the labelled series, except for Brood 2 which had the same frequency in both series. Brood 6 in the tritiated thymidine series had the highest incidence of all broods tested.

Presumptive mutants from used-type were sought at the marked loci on the X-chromosome. Thus, in one of our runs 141 presumptive achaete mutants were found. These were all tested, but none could be shown to be true mutants. The extra labor involved in testing presumptive mutants forced us to ignore presumptive scute and achaete phenotypes. In the unlabelled thymidine series one *sn* mutant was found in Brood 4. Several mutants were found in the labelled thymidine series. Thus, two γ mutants, one in Brood 1 and one in Brood 4, and two *pn* mutants, one in Brood 1 and one in Brood 3, were found. Two *sn* mutants, one in Brood 3 and one in Brood 5, were also recovered. Thus, among 345,894 offspring tested in the unlabelled thymidine series, one recessive sex-linked mutant was obtained, while in the labelled series six mutants were found among 342,439 offspring.

DISCUSSION

CHANDLEY and BATEMAN (1962) found that the most heavy labelling of sperm by tritiated thymidine occurred in those available for insemination on the 11th day after injection. From this fact it may be expected that tritium-induced mutation will have its highest frequency on that day, and maybe the next one, in a daily brood pattern system.

STRÖMNÄS (1962) tested the mutagenic effect of carbon-14 and tritium-labelled DNA precursors injected into 6 to 24 hour old *Drosophila* males. With the radioactivity used, there was no detectable increase in the frequency of dominant lethals. However, a significant increase in the frequency of sex-linked recessive lethals was observed. There were, also, indications that the highest frequency of lethals was induced in those sperm which are available for insemination 12 to 13 days after injection. This is compatible with data obtained by CHANDLEY and BATEMAN (1962) and KAPLAN (personal communication) and KAPLAN and SISKEN (1960) on labelling of sperm with tritium, and on the brood pattern. The data presented in this paper indicate, however, that the sixth brood after injection has the highest incidence of such mutations as Minutes, X-chromosome detrimentials, and the phenotype hemithorax. On the basis of the data for sex-linked lethals, they would be expected to appear in the fourth brood, unless we have recovered effects produced in spermatogonia. SÄVHAGEN (1961) has given, however, an illustration of the sensitivity pattern for different types of mutations after X-ray treatment. The highest mutation frequency of translocations is recovered on the sixth day, of recessive lethals on the seventh day, of nondisjunction females on the eighth day and of attached-X0 females on the eighth and ninth days after irradiation. The highest frequencies of dominant lethals occur on the fifth to the tenth days. Her conclusion is that there is a marked difference in sensitivity both with regard to type of aberration and with regard to spermatogonial stage investigated.

GLASS (1955) has pointed out the difficulty in having too crowded cultures whereby the more slow-growing Minutes would have to compete with the vigorous wild-type larvae for food. We reduced this problem by having only four egg-laying females in each half-pint milk bottle, and by enriching the culture medium with live bakers yeast. Although no bottle was considered too crowded, the number of flies per bottle went down from about 130 flies in the first brood to about half that number in the sixth brood.

The data presented in this paper demonstrate that the radioisotope used is effective in producing Minutes and possibly recessive visible mutations. The mutation rate induced was very low with the radioactivity employed in this investigation. Further experiments are needed to demonstrate whether or not radioisotopes incorporated into chromosomes are effective in producing back mutations.

If tritiated thymidine can cause recessive lethal and detrimental mutants, one would expect, following nondisjunction in the mother, that X0 males would have a lower survival rate in the treated than in the control series. This has been found in the present work (Table 2), the greatest reduction occurring in Brood 6, which was the brood with the highest rate of Minutes.

SCHMID (1959) points out that irradiation of embryos and of young larvae may result in somatic mutations and modifications. The formation of visible changes of these types occurred with a quite high frequency. The predominant changes were crippled legs, unilateral and bilateral changes of wings, and hemithorax formation. The author emphasizes that if these types of alterations are used in quantitative tests, it is important that the test organisms be as homogeneous as possible with respect to age at the time of treatment. The hemithorax flies recorded in the present paper are of the same type as those illustrated in Picture 12 in SCHMID's paper. We observed but did not record the two other types of alterations mentioned by SCHMID. In the tritium-labelled thymidine series, the frequency of hemithorax is 0.25×10^{-3} , while in the unlabelled series it is 0.17×10^{-3} . The difference is statistically significant. The highest frequency of hemithorax was found in Brood 6 in the labelled series. The highest frequency of mosaic females was found in Brood 3 in the labelled thymidine series. The frequency of all mosaic females observed in the labelled series is 0.39×10^{-3} and the frequency in the unlabelled series is 0.23×10^{-3} . The difference is statistically significant.

SUMMARY

Drosophila melanogaster adult males were injected with tritium-labelled or unlabelled thymidine when 1 to 24 hours old. One and a half days later they were mated to a series of females, to sample sperm delivered over successive three-day periods, furnishing six three-day broods. The injected males in the labelled series were less viable and fecund than those in the control series. The sixth brood in the labelled series had a significantly higher frequency of Minutes than any other brood in either series and had the lowest frequency of wild-type males. The frequency of mosaic females and of phenotypic hemithorax flies was significantly higher in the labelled series than in the control series.

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