EXPERIMENTS ON MUTATIONS INDUCED BY NEUTRONS IN DROSOPHILA MELANOGASTER SPERMS

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TWO somewhat puzzling phenomena have been reported in the literature on neutron-induced lethals in Drosophila. NAGAI and LOCHER (1938), in the first work on this subject, kept the offspring of different neutron-treated males separate during the tests for sex-linked lethals and reported that the lethals were not distributed at random among the offspring of different males. Two or more lethals occurred within the offspring of a single male with a frequency that was considered significantly in excess of its expected value. This was attributed to a hypothetical "grouping" of lethals within the chromosomes of adjacent sperms. Although the interpretation of this effect was not quite satisfactory, the report did not give rise to any related investigation, until NISHINA and MORIWAKI (1941) attempted to detect grouping of lethals within single chromosomes. These authors proceeded on the assumption that if neutrons are especially likely to produce an accumulation of lethals within the X chromosomes of adjacent sperms, they should also frequently produce more than one lethal within any single chromosome. The detection of a striking effect of grouping was reported from this experiment.

In view of the considerable importance of these findings for the interpretation of radiation effects, and since the technical methods of NISHINA and MORIWAKI were open to some criticism, it appeared worth while to repeat the basic experiments on grouping effects, using modified methods.

Many of the X-ray-induced sex-linked recessive lethals detected by the standard CIB method are known to be associated with chromosomal rearrangements affecting the X chromosome. The usual interpretation of this association attributes such lethals to a secondary ("position") effect of the rearrangements. Attempts to account for all the observed dose-effect relationships on the basis of this interpretation, however, have not been quite successful (cf. FANO 1941). Therefore, it also appeared worth while to obtain some data on the association of lethals and chromosomal rearrangements in neutron-treated material.

Finally, it seemed advisable to gather some new data on the efficiency of neutrons in producing dominant lethals, even though some information on this subject had already been published by DEMPSTER (1941).

MATERIAL AND METHODS

An inbred wild type Canton stock of Drosophila melanogaster was used in this experiment. Young males were neutron-rayed with the COLUMBIA UNIVERSITY

1 Cytological analysis was done principally by Dr. E. SUTTON, now at THE JOHNS HOPKINS UNIVERSITY, Baltimore, Maryland. It is a pleasure to acknowledge her substantial contribution.
cyclotron, which was kindly made available and operated for us by the cyclotron group, under the direction of DR. J. R. DUNNING.

The instrument measuring the neutron intensity was calibrated with a Victoreen dosimeter, using the 25 r chamber. Because of this circumstance, the “neutron unit” corresponding to the reading of 1 r on our dosimeter may be slightly greater than the conventional neutron unit determined using the 100 r chamber. In any case, the conversion of any one of these units into units of energy delivered to a unit volume of tissue is not very accurate. It is usually estimated (AEBERSOLD and LAWRENCE 1942) that one neutron unit is energetically equivalent to a value between 2 and 2.5 roentgens of X-rays.

Two groups of males were treated with about 600 and 1200 neutron units, respectively, and bred as follows:

1. 236 males treated with 1200 units were mated separately, each to four or five CIB/ec ct v g virgin females. The F1 Bar females of each successful culture were mated singly with ec ct v g males. F2 maleless cultures containing at least 25 females were classified as carriers of lethals. F2 wild type females of each of these lethal cultures were further mated with ec ct v g males. The F3 generation thus obtained was subjected to genetic and cytological tests:

   (a) Analysis of the crossover classes of the F3 served to determine the approximate location of the lethal and its possible association with a chromosomal rearrangement. This method was not entirely satisfactory, as shown below, because of the lack of markers to the right of g in the stock employed. (Use of genetic tests with a more complete set of markers had previously met with difficulties on account of low viability.) (b) Cytological analysis was made of the salivary gland chromosomes of at least five non-v female larvae from each F3 culture in order to detect any rearrangements affecting the X chromosome. Although particular attention was devoted to a search for deficiencies of considerable size, no attempt was made to detect deficiencies involving only a few bands.

Tests (a) and (b) were carried one generation further, when necessary for their completion.

2. The other males that had been treated with 1200 units were mated in mass to Canton virgin females. Eggs from this mating were collected on spoons with corn meal agar for a few days, transferred in batches of 25 onto corn meal-agar slants in vials, and incubated at 25°C. The numbers of eggs hatched and of adult flies emerging in each vial were scored.

3. Males treated with 600 units were bred as in (2).

4. Five males treated with 600 units and previously used in experiment (3) were later mated singly with virgin CIB/ec ct v g females (but separated on the 12th day after treatment), and their offspring were tested as in (1).

One control experiment on dominant lethals, using untreated Canton males, was carried out with the technique indicated in (2).

RESULTS

Experiment (1) gave the following results:
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Males yielding successful ClB tests 170
X chromosomes tested 998
  X chromosomes tested per male: minimum 1
  maximum 17
  average 5.9

X chromosomes acting as lethal carriers 60
  X chromosomes carrying one lethal without rearrangement 32
  X chromosomes from which two different lethals without rearrangements were separated 2
  X chromosomes carrying one lethal associated with a rearrangement 24
  X chromosomes carrying independent lethal and rearrangement 2

Total number of lethals separated from one another 62
  Total number of lethals separated from one another and from rearrangements 38
  Number of lethals associated with rearrangements 24

One lethal-carrying chromosome was lost before analysis and was arbitrarily classified as carrying one lethal without rearrangement. The test of one other chromosome yielded only v-g males (with one exception, which was v alone), although crossing over in the v-g region appeared normal, judging from the F3 females. A plausible interpretation is that there were two different lethals in the v-g region; but for practical purposes this chromosome, too, was arbitrarily classified as carrying one lethal without rearrangement.

The observed distribution of lethals among the offspring of different males must be compared with the distribution to be expected according to the hypothesis that lethals are distributed at random (that is, without exhibiting any grouping effect). The "expected" distribution would be a Poisson series, if the same number of tests were carried out in the offspring of each male. This condition is not fulfilled, but the deviation from it can be taken into account in computing a "corrected" expectation. The observed and expected distributions are:

<table>
<thead>
<tr>
<th>Lethals per male</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>125</td>
<td>33</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Expected: (Poisson)</td>
<td>119.5</td>
<td>42.1</td>
<td>7.4</td>
<td>.9</td>
<td>.1</td>
<td>.0</td>
</tr>
<tr>
<td>(Corrected)</td>
<td>120.7</td>
<td>40.1</td>
<td>7.8</td>
<td>1.2</td>
<td>.2</td>
<td>.0</td>
</tr>
</tbody>
</table>

The observed frequency of occurrence of two different lethals in the same X chromosome must be compared with the frequency to be expected according to the hypothesis that lethals are distributed at random among all treated X chromosomes (that is, without exhibiting any grouping effect). The apparently obvious basis for calculating the expected frequency is to assume that all 62 different lethals were distributed at random among the 998 chromosomes tested. The expected frequency of chromosomes with 0, 1, 2, etc., lethals would then constitute a Poisson series. It may be argued, however, that the coincidence of two lethals in the same chromosome may escape detection when one lethal is associated with a chromosomal aberration. This argument may be taken into account in the calculation by assuming that only the 38 different lethals that are not associated with rearrangements are distributed at random. However, the values obtained using either basis of calculation do not differ
greatly from each other or from the observed value, as is shown by the following table.

<table>
<thead>
<tr>
<th>FREQUENCY OF OCCURRENCE OF TWO LETHALS IN THE SAME CHROMOSOME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

The expected frequency of occurrence of three lethals in the same chromosome is less than .05, and no such case was observed.

Experiment (4) yielded three lethals out of 156 chromosomes tested. Two of these lethals were without rearrangement, the other was associated with the intercalary translocation of a section of X to 3L.

A few other findings incidental to the cytogenetical analysis will be reported here, even though they involve small numbers of observations and do not appear to disagree with other information relating to similar questions. Three lethals (all of which were associated with rearrangements) showed phenotypical effects—namely, one Notch and two echinus. Among all the 25 observed associations of lethals and rearrangements, no deficiency was detected. At least six of these rearrangements involved more than two breaks. In five cases, two breaks were observed within one numbered "division" of the X chromosome or within two adjacent "number sections." Among the 19 cases where the lethal appeared to be associated with a simple inversion or reciprocal translocation, 12 involved an inversion and seven a translocation. Since the numbers of X-ray-induced reciprocal translocations and of inversions affecting any given chromosome limb are known to be about equal, and since inversions involve a double chance of producing a lethal (one at each end point), the observed ratio of 12 to seven is not unexpected. Finally, nine cases of cytologically detected rearrangements affecting the proximal region beyond g were not detected by the genetic test.

The dominant lethal experiments yielded the following percentages of eggs undergoing full development:

<table>
<thead>
<tr>
<th>Controls</th>
<th>600 r</th>
<th>1200 r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage</td>
<td>89.6</td>
<td>27.2</td>
</tr>
</tbody>
</table>

Parallel experiments on X-ray-induced dominant lethals, carried out a year later with the same stock and the same technique gave the following results:

<table>
<thead>
<tr>
<th>Controls</th>
<th>1000 r</th>
<th>2000 r</th>
<th>3000 r</th>
<th>4000 r</th>
<th>5000 r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage</td>
<td>76.9</td>
<td>60.8</td>
<td>42.6</td>
<td>28.5</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Further details on these results (including graphs) are given in a separate paper on dominant lethals (DEMEREC and FANO 1944).

DISCUSSION

The interpretation of "grouping effects" is based on the lack of uniformity of the ionization produced by neutrons, which is concentrated along compara-
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Relatively few and narrow paths of recoil protons. Because of this lack of uniformity, any region of tissue so small that it stands only a slight chance of being traversed by even one proton during the entire treatment will be particularly strongly affected if it does happen to be so traversed. Hence the neutron effects are expected to be “grouped” within regions of such size. In the usual treatments of Drosophila sperms, equivalent in order of magnitude to a few thousand roentgens of X-rays, each region whose diameter is of the order of magnitude of $\mu m$ has about an even chance of being traversed by a neutron. Therefore, grouping effects should be expected only when the region taken as a unit in connection with the grouping effect is smaller than $\mu m$.

The grouping reported by Nagai and Locher refers to a cluster of adjacent ripe sperms of a male, occupying a region very much larger than $\mu m$, and therefore is unexpected from the physical standpoint. The results reported in the present paper, on the contrary, agree with the physical considerations, inasmuch as they do not show any significant grouping of that type, even though the number of cases of “one lethal per male” is somewhat smaller than expected, both in our experiment and in that of Nagai and Locher. Actually, their conclusion about the existence of “grouping” seems to derive from a comparison of the results obtained with neutrons and $\gamma$-rays; but it is not supported by any calculation of the probable effect of random distribution of lethals to be expected in each case. Inspection of the tables published by Nagai and Locher shows that the average number of lethals per male (and hence the chance of random coincidence) was much smaller in their $\gamma$-ray series (19/89) than in the neutron series (44/69). A copy of the original records has been made available to us through the courtesy of Miss Nagai and Dr. Altenburg. The information supplied by these records does not permit us to compute the “exact” but only the Poisson expectation, which compares with the observed results as follows:

<table>
<thead>
<tr>
<th>Lethals per male</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>40</td>
<td>16</td>
<td>11</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Expected (Poisson)</td>
<td>36.6</td>
<td>23.3</td>
<td>7.4</td>
<td>1.6</td>
<td>0.2</td>
</tr>
</tbody>
</table>

As in our results, the class with one lethal per male shows here some deficiency, compensated by excess in the classes with no and two lethals (while no excess is found in the class with three lethals). The discrepancy ought to be further reduced if the “exact” expectation were calculated; but it does not appear to be significant even as it is. In the $\gamma$-ray series by Nagai and Locher, about two cases with two lethals per male should have been expected. The fact that none was found can be interpreted as a sampling error.

The “grouping” of lethals in single chromosomes reported by Nishina and Moriwaki appears at first to be fairly plausible, in view of the size of a chromosome and of the data on the physical action of neutrons considered earlier. However, owing to the elongated shape of chromosomes, it might not be very likely for a neutron hitting one point of a chromosome to hit the same chromosome at another point far removed from the first. Nishina and Moriwaki themselves pointed out that their method cannot distinguish the existence of
two different lethals unless they are separated by a considerable distance along a chromosome. Our data, presented in this paper, do not show any significant grouping of the kind reported by NISHINA and MORIWAKI and appear definitely to exclude the possibility that such grouping would occur with the high frequency (over 40 percent) reported by them. To explain the discrepancy between the two experiments, it may be noted that two different lethals were actually isolated in only one out of the eight cases of grouping reported by NISHINA and MORIWAKI. In two other cases, the grouping involved one lethal and one phenotypical (cl) change, but no evidence was offered indicating that the two changes were distinct. In the remaining five cases the existence of two independent lethals was argued on the basis of deviations from the expected sex ratio in the F1. In view of the presence of numerous genetic markers which may reduce the viability of hemizygous males, this criterion does not seem quite reliable. On the one hand, no control data were shown supporting the validity of the method; on the other hand, in our experiments a sex ratio exceeding 3:1 in the F2 appeared commonly in cases where the crossover classes indicated the existence of only one lethal.

The value found by us for the proportion of lethals associated with chromosomal rearrangements (about 40 percent) is one of the highest on record. Comparable information on X-ray-induced lethals may be derived from OLIVER'S (1932) data, which indicate that the corresponding proportion is about 10 percent for a treatment producing about 6 percent lethals. This result may be in error by defect, since it is based on a genetic method using only the three markers sc, v, and f. DEMEREC (1937) has obtained values ranging up to 40 percent with X-ray treatments roughly equivalent to our neutron treatment. It may be argued, however, that DEMEREC'S sample of lethals was selected by means of phenotypical effects, which may themselves be particularly closely associated with rearrangements. At any rate, the value obtained with neutrons is not surprisingly high in view of the evidence indicating that, as a rule, neutron treatments produce fewer gene mutations and more (or at least not fewer) chromosomal rearrangements than energetically equal doses of X-rays. In fact, the rate of production by neutrons of sex-linked lethals as a whole (that is, including the fraction associated with rearrangements) is about 0.7 times the comparable rate of production by X-rays. This result has been obtained by several authors since ZIMMER and TIMOFEEFF-RESSOVSKY (1938) and is confirmed by the data of the present paper. Assuming that one neutron unit is equivalent to 2.5 r, 1200 units are equivalent to 3000 r; but 3000 r X-rays produce about 9 percent lethals in most wild type stocks, instead of the 6 percent found by us. In order to compare the efficiency of X-rays and neutrons in producing lethal gene mutations only, one must subtract from 6 percent the contribution of lethal rearrangements, as pointed out by GILES (1943). Thus 1200 units produce only about 3 to 4 percent (that is, about 60 percent of 6 percent) lethal gene mutations, while 3000 r X-rays produce about 6 to 8 percent of them. The neutron efficiency factor for gene mutations is then about 0.5 instead of 0.7. In the production of chromosomal rearrange-
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terial: Kaufmann's (1941) results set the efficiency ratio of neutrons and X-rays at about one, and Dempster's (1941) at about 1.25. One possible interpretation of the disagreement is offered by Demerec's suggestion that an important percentage of neutron-induced dominant lethals consists of "one-event" deficiencies involving a considerable segment of chromosome (that is, one numbered "division" or more). To adjust this interpretation to the available evidence, specifically to that offered by Kaufmann (1941), one should also assume that there is no important number of these deficiencies short enough to act as recessive instead of dominant lethals in the X chromosome, but that some of them may act as recessives in the autosomes, particularly in 3R. It is realized that the evidence available at present, including that supplied by the dominant-lethal experiment reported here, is not adequate to supply a clear picture of the comparative efficiency of neutrons and X-rays in producing the various types of chromosomal changes in Drosophila sperms.

SUMMARY

New experiments on the phenomena of "grouping" of sex-linked lethals reported by Nagai and Locher (1938) and by Nishina and Moriwaki (1941) seem to disprove their existence. The theoretical difficulties raised by the interpretation of those phenomena are thus removed. A large percentage of neutron-induced lethals is associated with chromosomal rearrangements; certain difficulties that had previously been met in discussing this association are thus considerably increased. A new measurement of the frequency of neutron-induced dominant lethals is reported, but the comparative efficiency of neutrons and X-rays in producing various types of chromosomal change in Drosophila sperms is not satisfactorily elucidated thereby.

LITERATURE CITED


OLIVER, C. P., 1932 An analysis of the effect of varying the duration of X-ray treatment upon the frequency of mutations. Z.A.V. 61: 447–488.