the male pronucleus, the breaks or potential breaks may remain capable of reunion for a limited time during which contacts with other chromosomes may be realized.

For the present the question must remain open concerning the nature of the physical and chemical processes which permit breaks or potential breaks to retain their capacity for reunion over a period of several weeks.

Summary.—Comparison has been made, using salivary gland chromosomes, of the numbers of breaks induced by irradiating sperm of *D. melanogaster* with two equivalent doses of x-radiation, one of which was delivered continuously, the other in a series of fractions. Comparable frequencies obtained with both types of treatment indicate that no appreciable "healing" occurs even when the interval between successive fractions is 16 days. The presence of multiple-break, complex rearrangements following the fractionated treatment strengthens the evidence for the theory that breakage precedes reunion.

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MECHANISM OF THE ORIGIN OF X-RAY INDUCED NOTCH DEFICIENCIES IN DROSOPHILA MELANOGASTER

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Communicated December 12, 1940

It is well known that deficiencies in Drosophila may occur spontaneously and also that they may be readily induced by x-rays. Cytological analysis of spontaneous and induced deficiencies indicates that in both types a section of the chromosome is missing. In the Notch deficiencies the

missing section varies in size from one salivary chromosome band to as many as 36 bands. Cytogenetic analysis shows that some changes which behave biologically as deficiencies have the full complement of bands, that is, they are cytologically normal. If cytological analysis is taken as a true index of the situation, the Notches which have a full complement of bands may be considered as gene mutations, and the cytologically detectable deficiencies as changes in which a section of the chromosome has been eliminated. The latter might conceivably have occurred as a result of a single event or through two independent events each causing one break in the chromosome. In this paper data will be presented showing the lengths of the Notch deficiencies and an attempt will be made to analyze the manner in which these deficiencies originated.

Material.—This analysis will include all changes which show the Notch phenotype and are not associated with chromosomal rearrangements (translocations or inversions). In addition three Notches are included which are associated with chromosomal rearrangements but are at the same time deficiencies for a segment which covers the Notch locus.

Cytogenetic studies indicate[1] that the Notch locus is represented in the salivary gland chromosomes by the band shown on Bridges'[2] map as 3C7. Thus all deficiencies which include this band are classified as Notch deficiencies. It is well known that in chromosomal rearrangements the genes in the proximity of a break are frequently affected. In our collection of 85 x-ray induced Notches there are 34 in which the Notch phenotype arose in conjunction with either a translocation or an inversion. A similar effect on adjacent loci may be expected in deficiencies where the nature of the chromosomal rearrangement is the loss of a segment. Three such cases are available in our material. In each a change in the Notch locus is associated with an adjoining deficiency which does not include the 3C7 band. These three Notches are classified as non-deficiencies. Among 48 induced Notches not associated with chromosomal aberrations, 37 are cytologically detectable deficiencies while 11 are not.

On Bridges'[2] map of the salivary *X*-chromosome a considerable number of bands are indicated as doublets, that is, they are shown as two lines very similar in appearance and placed close together. In the studies conducted in this laboratory no case has been found showing a break between two lines which are represented on the map as a doublet. Therefore in this paper all doublets are counted as single bands so that the total number of bands in the *X*-chromosome is 647.

The induced Notches originated from experiments in which males received an x-ray treatment of between 2500 and 3000 roentgens. All fertile Notches so obtained were kept and analyzed. Of the 13 spontaneous Notches, 5 were found in this laboratory while 3 were found by Doctor O. Mohr, 3 were obtained from the Pasadena Laboratory and 2

from Doctor G. Gottschewski. In table 1 the Notches are classified according to the number of bands involved in the deficiency.

TABLE 1

NOTCHES CLASSIFIED ACCORDING TO THE NUMBER OF SALIVARY CHROMOSOME BANDS INCLUDED IN THE DEFICIENCY

	NUMBER OF BANDS INCLUDED IN DEFICIENCY																		
NOTCHES	0	1	2	3	5	6	8	10	12	13	14	15	20	21	27	30	31	33	36
Induced	11	9	5	2	1	2	..	2	1	..	3	2	3	2	1	1	1	1	1
Spontaneous	2	4	2	1	2	..	1	..	..	1	..	..	..	..	..	..	..	..	..

Discussion.—It has been suggested[3] that the observed deficiencies produced by 2500–3000 roentgens which involve the Notch locus might arise by two different processes. The longer deficiencies would correspond to the usual chromosomal aberrations involving two independent breaks

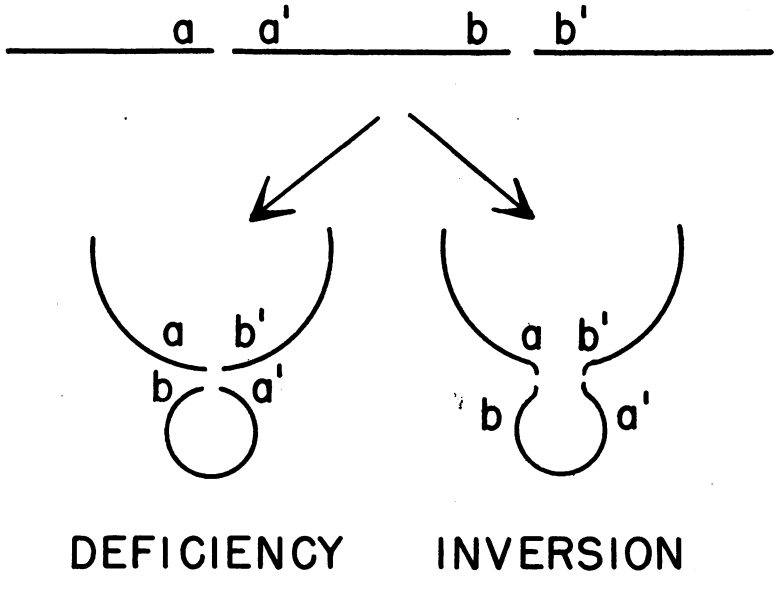

FIGURE 1

Diagram indicating symmetry in the origin of deficiencies and of inversions.

and may be classified as "two-event" processes. The short ones, which are particularly frequent, would arise from some different "single-event" process, and correspond more closely to the mutations affecting the Notch locus which are not cytologically detectable deficiencies. It is of interest to distinguish between the two groups of phenomena in order to ascertain the maximum size of the affected region involved in "single-event" processes. An attempt will be made to do so.

As a first step we shall try to calculate the expected frequency of "two-event" deficiencies involving the Notch locus. A considerable amount of data on cytologically detectable chromosomal rearrangements produced by x-rays has been published by Bauer, Demerec and Kaufmann and by Bauer.[4] Although the process involved in production of chromosomal aberrations is not yet clear, it seems reasonable to assume that any large size deficiency is just as probable as a corresponding inversion since the two phenomena (Fig. 1) arise from alternate analogous rejoining of the same chromosomal fragments. This consideration takes into account the deficiencies arising from simple two-break processes only and excludes those associated with more complicated rearrangements as well as those which are actually not deficiencies but intercalary translocations of chromosomal fragments. Actually all these types are included in the experimental material under consideration but for the sake of simplicity we shall omit multiple-break rearrangements from the following calculations. This should not greatly affect the results, since multiple-break rearrangements are much less frequent than those with two breaks only (94 and 265, respectively, in Bauer's material).

We shall calculate first the frequency of X-chromosome inversions produced by 3000 roentgens involving n bands and including any given locus. According to Bauer,[4] 104 two-break aberrations were produced by 3000 roentgens in 595 tested sperma; 412 out of 671 two-break aberrations had both breaks within the euchromatic regions of long chromosome arms; 29 out of 399 such aberrations were euchromatic X-chromosome inversions. The frequency of euchromatic X-chromosome inversions at 3000 roentgens is then approximately:

$$\frac{104}{595} \times \frac{412}{671} \times \frac{29}{399} = 0.0078.$$

According to Bauer, Demerec and Kaufmann[4] the length of inversions is distributed at random. On this basis the expected frequency of inversions involving a certain number, n, out of a total number of N euchromatic bands within the X-chromosome is:

$$\frac{2(N-1-n)}{(N-1)(N-2)} \approx \frac{2}{N-2} \text{ if } n \ll N.$$

The *a priori* probability that any particular band is included among those which are involved in such an inversion is approximately $n/N-2$ (the first and last euchromatic bands cannot be included). Therefore, the expected frequency of inversions or deficiencies produced by 3000 roentgens within the X-chromosome involving a small number n of bands and including any given locus is:

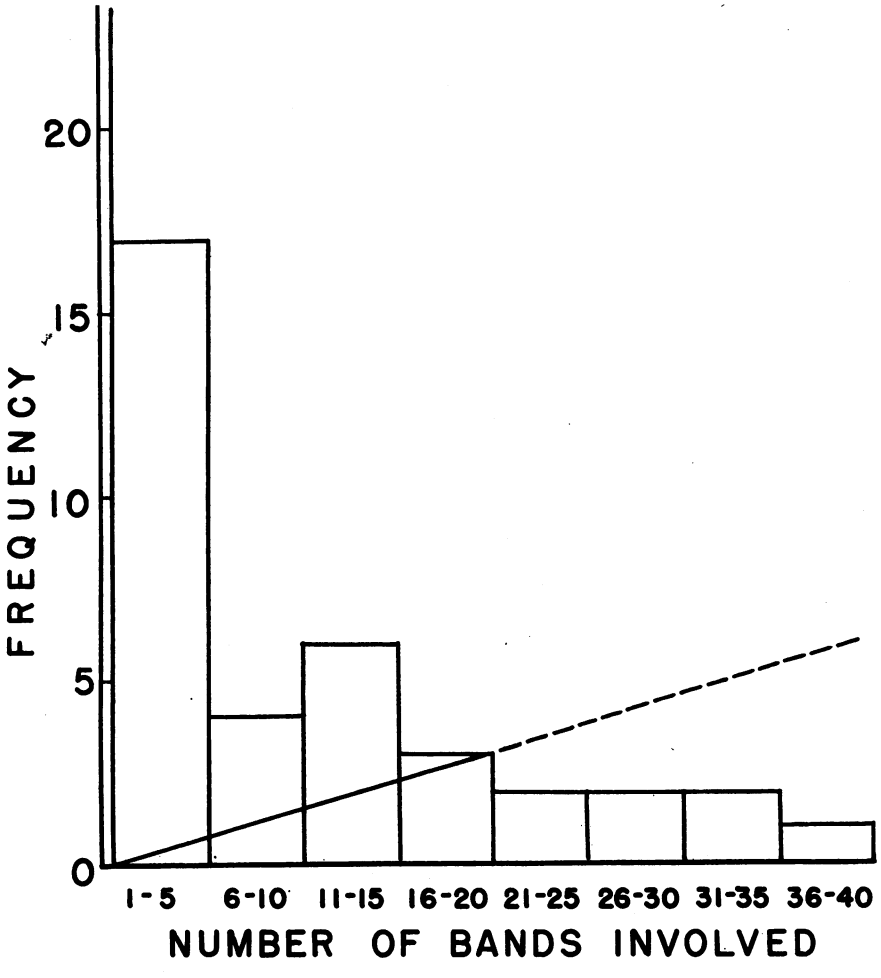

FIGURE 2

Histogram showing the frequency distribution of x-ray induced Notch deficiencies arranged according to the length of the deficient segment (data from table 1). The straight line represents the theoretical frequency distribution of deficiencies arising from two independent breaks according to formula (1). The expected frequency should be lower in the dotted region of the line on account of low viability of long deficiencies.

$$0.0078 \frac{2}{N-2} \times \frac{n}{N-2} = 0.0078 \times 2 \times \frac{n}{645^2} \approx 3.85 \times 10^{-8} \times n.$$

Material considered in this paper involves 85 fertile Notch mutations. Demerec[5] has reported finding 13 such mutations among 112,977 gametes irradiated with approximately the same dose. One can therefore assume

that about: $\frac{85}{13} \times 112{,}997 \approx 740{,}000$ gametes have been investigated in the material reported here. The expected number of deficiencies involving n bands and including the Notch locus is then:

$$3.85 \times 10^{-8} \times 740{,}000\, n \approx 0.03\, n. \qquad (1)$$

This result is expected to hold only if n is so small that no locus affecting vitality in the neighborhood of Notch is affected, that is, for $n < \approx 20$–25.

Comparison of formula (1) with the experimental results (Fig. 2) shows that the formula represents correctly the frequency of deficiencies involving more than about 15 bands. On the other hand, the number of shorter deficiencies is far in excess of the expectation, particularly in view of the fact that according to (1) the shorter deficiencies should be less frequent rather than more frequent than the longer ones. This is taken as an indication that, whereas deficiencies of 15 or more bands may be due to the same process as the large size inversions, the shorter deficiencies must be caused by a different process.

To prove that short deficiencies are due to a "single-event" process, while the larger ones correspond to a "two-event" process, it is necessary to show that the ratio of the frequencies of large to that of short deficiencies is dose-dependent. Another conceivable explanation of the high frequency of short deficiencies could be that the number of large size chromosomal aberrations is reduced by some healing process. That would mean that two breaks would have a better chance of giving rise to an aberration if they occurred within a very short distance, below a limit of approximately 15 bands. In this case the ratio of the frequency of long deficiencies to that of the short ones would not be dose-dependent.

Analysis of deficiencies among spontaneous Notches yields some evidence on this subject. The largest among 11 spontaneous Notch deficiencies involves 13 bands, whereas among 37 Notch deficiencies induced with 3000 roentgens 15 exceeded 13 bands. This suggests that large deficiencies are less frequent among spontaneous than among x-rayed Notches. A statistical test of the homogeneity of the distribution of x-ray induced and spontaneous deficiencies has been made by means of an analysis of the contingency table:

	13 BANDS OR LESS	MORE THAN 13 BANDS	TOTAL
X-ray induced	22	15	37
Spontaneous	11	0	11
Total	33	15	48

The χ^2-test (which gives $P \approx 0.011$) is not reliable in this case because of the small numbers which are involved. The exact treatment involves a consideration of the probability of all possible distributions of deficiencies in the four cells of this contingency table. The particular distribution which has been found had the *a priori* probability $\frac{33!\ 15!\ 37!\ 11!}{48!\ 22!\ 11!\ 15!\ 0!} = 0.0086$. The level of significance is $P = 0.0102$. This evidence suggests that short and long deficiencies are not equally distributed among the x-ray induced and the spontaneous Notches.

More conclusive evidence should, however, be obtained by the analysis of Notches produced by a different dosage of x-rays, because only in this way can the results be directly compared with those obtained with high dosages. Experiments with this aim are in progress.

Therefore, one might assume that the length of 15 bands represents approximately an upper limit for a "single-event" deficiency. This length is about 6 μ in salivary gland chromosomes and should probably be about 100 times smaller in the sperm chromosomes, that is, approximately 600 Å = 0.06 μ. This finding is analogous to the phenomena involved in the "single hit chromatid breaks" described by Sax.[6]

It is possible that short deficiencies have the same origin as the 11 mutations of the Notch locus, which show no detectable cytological effect. The frequency of this group is approximately the same as that of the single-band deficiencies, both in the x-rayed and in the spontaneous material.

It is of interest that the critical length at which formula (1) yields a frequency comparable to that obtained experimentally is about the only one which would have been acceptable (15 to 20 bands), whereas the formula has been derived quite independently of any experiment involving Notch. The critical length should, in fact, not have been much shorter than the largest spontaneous deficiency (13 bands), since "two-event" spontaneous chromosomal aberrations are so rare that they need not be taken into consideration. Neither could it have been much longer than 20 to 25 bands because of the influence of viability factors.

Summary.—Extensive data on the frequency of deficiencies of various lengths involving the Notch locus are reported. Calculations show that deficiencies which include more than about 15 bands can be accounted for as being analogous to the large size chromosomal aberrations, that is, they are caused by two breaks induced by two independent events. It has been shown that it is very likely that most of the short deficiencies, including all the spontaneous ones, arise as a result of a different process, possibly a "single-event" process, having a radius of action of about 600 Å.

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ANOTHER CASE OF UNEQUAL CROSSING-OVER IN DROSOPHILA MELANOGASTER

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Communicated November 18, 1940

Females homozygous for the sex-linked dominant, Bar, occasionally give rise to wild-type reversions and to forms with more extreme eye reduction than Bar. This behavior was shown by Sturtevant[1] to result from unequal crossing-over. The Bar-reverted type was considered to be a deficiency for the Bar gene; while the extreme form, called Ultra-Bar or Double-Bar, was interpreted as a duplication for that gene. Later, Wright[2] suggested that Bar itself had something additional present which when lost by unequal crossing-over would give back a normal chromosome (Bar-reverted).

The cytological nature of Bar was cleared up independently by Bridges[3] and Muller, *et al.*,[4] who investigated the salivary gland chromosomes. They found Bar to be a tandem duplication, in normal order, for an *X*-chromosome section composed, according to Bridges' detailed analysis, of six bands. Bridges further demonstrated that Bar-reverted had the identical banding of a normal chromosome, whereas Double-Bar had a serial triplication for the region present twice in Bar and once in Bar-reverted.

The second case of a tandem duplication being responsible for a dominant "mutation" is that of the sex-linked Hairy wing, which Demerec[5] has shown is a repetition for a single heavy band near the tip of the *X*-chromosome. However, its location in a region of extremely low crossing-over prevented a study of unequal crossing-over.

This paper is a preliminary report on an autosomal tandem repeat which was detected as a suppressor of the dominant mutant, Star (*S*, 2-1.3).

An analysis of the salivary gland chromosomes of this suppressing factor, when homozygous, when closely paired with a normal chromosome and when present as an unpaired haploid strand, consistently showed the presence of a tandem duplication in direct order near the left end of the