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Mutagenic effects of X-rays and formaldehyde food in spermatogenesis of *Drosophila melanogaster*

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1. INTRODUCTION

The addition of formaldehyde to the food of *Drosophila* larvae produces structural chromosome changes which differ from those induced by X-rays by several characteristic features. Some of them, like a high proportion of mosaics and a shortage of large rearrangements, especially interchromosomal ones (translocations), have been established by genetical methods (Auerbach & Moser, 1953*b*). Others, like a very high proportion of deficiencies and duplications of a repeat type, and an apparent shortage of breaks in the heterochromatic regions, were found by the cytological method, which is more suitable for detecting these types of change (Slizynska, 1957). These differences between the cytological effects of X-rays and formaldehyde food (FF) were explained by Auerbach's (1949, 1951) hypothesis of chemically induced potential chromosome breakage.

There remained, however, the possibility, that the stage of spermatogenesis at which the cells were treated might be at least partly responsible for the characteristic spectrum of FF induced cytological changes. FF acts preferentially on auxocytes (Auerbach & Moser, 1953*a*), while the available cytological observations on the action of X-rays in *Drosophila melanogaster* (Bauer, 1939), are concerned mainly with the first few days after irradiation of adult males, which corresponds to mature or nearly mature spermatozoa. It is possible that X-rays, when applied to auxocytes, might give results that, at least to some extent, are similar to those obtained after FF.

In order to investigate this possibility, it was necessary to obtain cytological data on changes induced by X-rays in earlier stages of spermatogenesis. Since irradiation of larvae meets with many difficulties, such as the very high mortality of treated larvae, the next best method was used. Adult males were irradiated, and successive broods were analysed cytologically.

2. METHODS AND MATERIAL

A cytological analysis was carried out on salivary gland chromosomes of larvae whose fathers had been irradiated. The whole complement of the chromosomes was examined, except the Y-chromosome, which is unsuitable for this type of cytological analysis. Consequently only female larvae were used. Permanent preparations were made by the usual method.

A strain carrying the sex-linked genes *y* (yellow) and *w* (white) was used for irradiation. Males 1–2 days old were treated with 2500 r and mated the next day to females obtained by the cross of two strains: M-5 (Muller-5) and Ore-K (wild-type). Only pair matings were used.

The experiment was planned as follows:

P: 1 ♂ *y w* (treated) × 3 ♀♀ *M-5/Ore-K*
F_1: ♀♀ *y w/M-5* were used for lethal tests
♀♀ *y w/Ore-K* were used for cytological analysis (as larvae)

Since the *M-5* X-chromosome contains w^a (white-apricot), larvae of the two types could be distinguished by the colour of their Malpighian tubules. The progeny of each male were recorded separately. Every 4 days, each male was supplied with three fresh females. In this way four broods (*a*, *b*, *c*, *d*) were raised, covering the period of 2–17 days after treatment, during which successively younger treated germ cells—from mature sperm to spermatogonia—are being utilized in fertilization. A male which failed to produce progeny in any one brood was discarded to avoid utilization of stored spermatozoa in the next brood.

To measure the effectiveness of treatment, a test for sex-linked lethals was done on 500–600 treated X-chromosomes in each brood.

For cytological analysis 200 *y w/Ore-K* F_1 female larvae in each of the first three broods (*a*, *b*, *c*) and 400 in the fourth brood (*d*) were used. As in the analysis of changes induced by FF, the following types of change were scored: translocations (T), inversions (In), deficiencies (Df), repeats (Rp, duplications (Dp) and loss of chromosome 4 (loss 4). Later on only the symbols will be used.

Among structural changes induced by FF, exchanges between arms of the same chromosome have been included in the class of T. The same rule is applied here. Two independent changes in one larva are considered as two changes. The class 'T or In' in Table 2 represents structural changes in which one break was in a heterochromatic region so near the centromere that it was impossible to determine the chromosome involved.

3. RESULTS

Out of altogether 160 treated males only 98 produced offspring in all four broods. Among males which produced offspring, broods *b* and *c* seemed to be the least fertile, and brood *d* the most fertile. This agrees with the results of Auerbach (1954). In most of her experiments, a period of low fertility occurred 8–10 days after irradiation.

As will be seen subsequently, only the overall sensitivity, i.e. the total number of changes, varies between broods, whilst in regard to the types of change and their relative frequencies all broods are similar. For this reason the exact determination of the cell stages underlying broods is of no great importance for a comparison of the effects of FF and X-rays.

Taking the brood pattern of lethal mutations and the period of low fertility as the criterion, it may be assumed that broods *a* and *d* derive mainly from treated

spermatozoa and spermatogonia, and broods *b* and *c* from intermediate stages, namely spermatids and spermatocytes.

The main results are summarized in Table 1, together with the data from FF experiment.

The frequencies of different types of change are listed in Table 2 and illustrated in Fig. 1; Table 2 gives the number of changes per 100 spermatozoa, and shows different types of change expressed as the percentage of all changes.

The relations between inter- and intrachromosomal changes are presented in Table 3.

4. ANALYSIS OF RESULTS

(i) *Sensitivity of broods to X-rays*

The sensitivity of successive broods to the mutagenic action of X-rays is measured by the percentage of spermatozoa carrying chromosomal changes (Table 1) and by the number of changes in 100 spermatozoa (Table 2). It agrees well with the sensitivity as estimated from sex-linked lethals: the peak is in brood *b*, and brood *d* shows the lowest values. The differences between broods would probably be more pronounced with shorter broods.

The sensitivity to FF lies between the sensitivities to X-rays of broods *c* and *d*, but the number of breaks in affected cells is lower after FF than in any X-ray brood (Table 1).

Table I. *General results*

			X-rays (dose 2500 r)			
			Broods			
		FF	*a*	*b*	*c*	*d*
Days after treatment			2–5	6–9	10–13	14–17
Sex-linked lethals	%	9·2	8·2	11·2	8·6	5·5
Spermatozoa analysed	*n*	680	200	200	200	400
Spermatozoa with structural changes	*n*	96 (17)	51 (3†)	57	43	27
	%	14·1	25·5	28·5	21·5	6·75
Multiple breaks‡	%	31·6	33·3	57·3	43·9	60·9

( ) spermatozoa carrying mosaic changes.

† One out of three mosaics was loss of fourth chromosome.

‡ Percentage of breaks found in spermatozoa with the minimum of three breaks. For example 31·6% of all FF breaks was found in spermatozoa carrying more than two breaks. This gives a measure of the heterogeneity of the break distribution.

When the four main types of change are considered, the general pattern is the same in all four X-ray broods: T's and In's are considerably more frequent than Rp's and Df's. This is completely the reverse of the FF data, where Rp's and Df's form over 60% of all changes. Table 2 and Fig. 1 illustrate this point very clearly.

FF induced Df's ranged in size from 6 to 76 bands and Rp's from 10 to 434 bands. Up to 76 bands the numbers of Df's and Rp's were equal (31 and 32 respectively).

The analysis of FF-induced Rp's showed that most or all Rp's result from potential breaks (Slizynska, 1963). The presence of some Rp's in all X-ray broods

Table 2. *Frequencies of different types of change*

Types of change	Total numbers					Numbers per 100 spermatozoa					Percent of all changes				
		X-rays					X-rays					X-rays			
	FF	*a*	*b*	*c*	*d*	FF	*a*	*b*	*c*	*d*	FF	*a*	*b*	*c*	*d*
T	19 (6)	28 (1)	33	23	15	2·8	14·0	16·5	11·5	3·75	16·4	45·2	49·3	45·2	48·5
In	19 (6)	19	25	18	6	2·8	9·5	12·5	9·0	1·50	16·4	30·6	37·3	35·3	19·4
T or In	3	2	4	5	3	0·4	3·5	2·6	2·5	0·75	2·6	11·3	6·0	9·8	9·7
Df	31 (4)	4	3	4	4	4·6	2·0	1·5	2·0	1·00	25·0	6·5	4·5	7·8	12·9
Rp	43 (4)	2 (1)	2	1	3†	6·3	1·0	1·0	0·5	0·75‡	35·3	3·2	3·0	2·0	6·5§
Dp	3 (1)	1	—	—	—	0·4	0·5	—	—	—	2·6	1·6	—	—	—
Loss 4	2	1 (1)	—	—	—	0·3	0·5	—	—	—	1·7	1·6	—	—	—
In? Rp?	—	—	—	—	1	—	—	—	—	0·25	—	—	—	—	3·2
Total	120	62	67	51	32	17·6	31·0	33·5	25·5	8·00	100·0	100·0	100·1	100·1	100·1

( ) indicates in brackets number of mosaics (e.g. 19 (6) means that of 19 translocations six were mosaics).

† Two out of three repeats occurred as a cluster.

‡ Cluster of two repeats counted as two cases.

§ Cluster of two repeats counted as one case.

implies that potential breaks can be induced also by X-rays. This conclusion is supported by closer analysis of the X-ray data. In Table 2 and Fig. 1 the structural changes in the four successive broods have been subdivided into the four main types: T, In, Df, Rp. It will be seen that in all broods T and In are considerably more frequent than Df's and Rp's. Moreover, the frequencies of T and In vary considerably between broods, while those of Df's and Rp's remain fairly constant. For rearrangements arising at the same cell stage and from the same type of break, such a difference could not easily be accounted for. It is, however, readily understood if the two classes of rearrangement have a different origin, T and In originating mainly from immediate break, Rp's and Df's from potential ones.

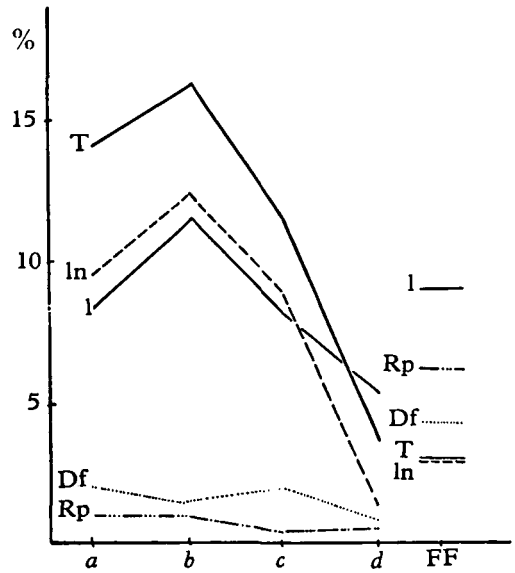

Fig. 1. Proportions of different types of change per 100 spermatozoa (from Table 2).

a, *b*, *c*, *d*	X-rays broods
FF	formaldehyde food experiment
T, In, Df, Rp	translocation, inversion, deficiency, repeat
l	sex-linked lethals

Direct proof that some potential breaks were formed in the present experiment was provided by one F_1 larva in brood *a*. All salivary gland nuclei of this larva carried a two-break change; in addition about one-third of the nuclei (22 out of 65 analysed ones) carried an unreversed Rp, comprising about 267 bands.

The simplest interpretation of this mosaic is as follows (Fig. 2). Initially, two immediate and two potential breaks were produced in the same spermatozoon or spermatid. The immediate breaks gave rise to the ubiquitous structural change in the F_1 larva. The potential breaks opened at one of the first cleavage divisions, but had a different fate in the two sister cells. In one, they restituted, giving rise to the sector of cells not carrying a Rp. In the other, they resulted in an exchange between sister chromatids, giving rise to a Rp in one chromatid and a Df in the other. The

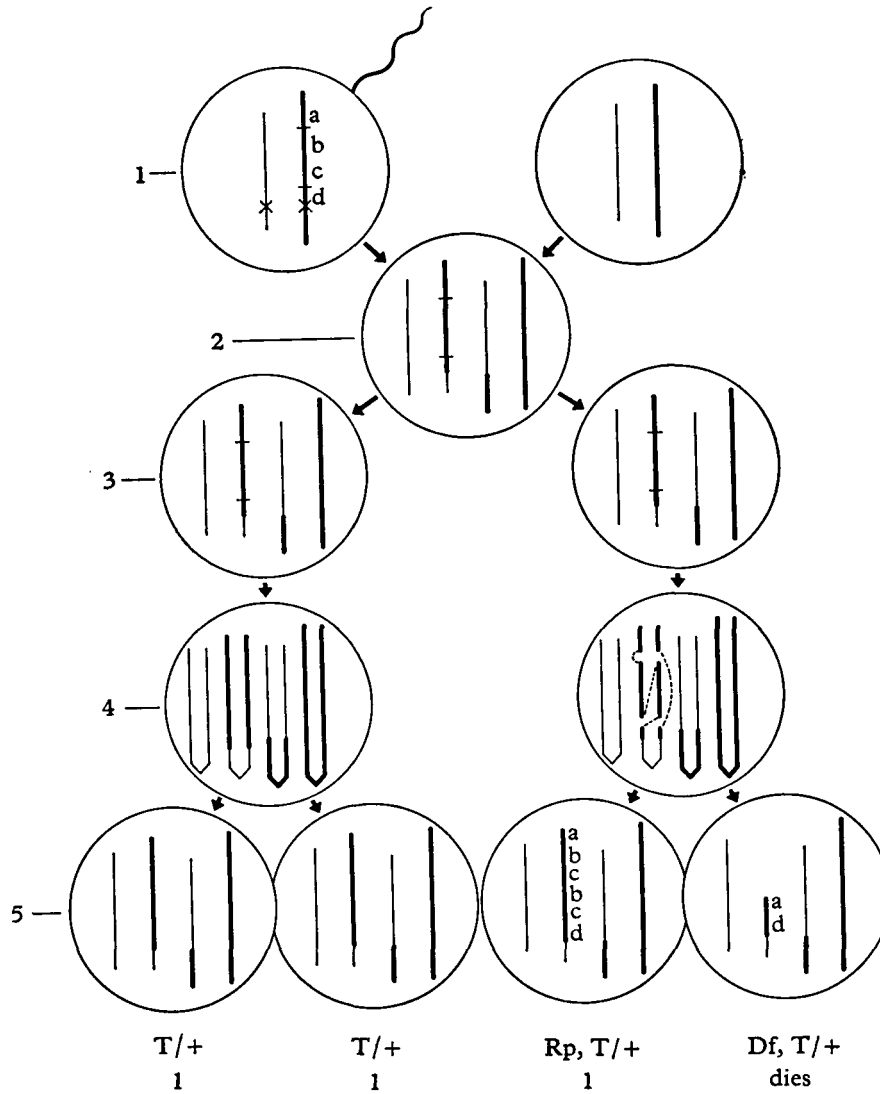

Fig. 2. Diagram showing the presumed origin of a larva carrying a translocation in all cells and a mosaic repeat in some (see text).

(1) Spermatozoon with two immediate breaks (×) and two potential breaks (−), and an ovum from an untreated female; a, b, c, d—the sequence of segments in one of the chromosomes.

(2) Zygote: immediate breaks have formed a translocation; potential breaks did not open.

(3) Two daughter cells, both containing a translocation and unopened potential breaks.

(4) Prophase of the second cleavage division; in the left cell the potential breaks have restituted; in the right one they have opened in two sister chromatids and rejoined in a new way, forming a repeat in one chromatid and a complementary deficiency in the other.

(5) The embryo at four-cell stage; all cells contain a translocation; one cell has died because of a cell-lethal deficiency; one out of three remaining cells contains a repeat.

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Df was of the size which, according to previous analysis (Slizynska, 1957) is cell-lethal; the Rp gave rise to a sector of viable cells. The observed ratio of one cell with Rp to two cells without agrees with expectation. Since rejoining cannot have occurred before, at the earliest, prophase of the second cleavage division, the original breaks must have been potential ones. A mosaic of essentially the same nature had already been found after FF treatment (Slizynska, 1957), which produces mainly potential breaks.

(ii) *Inter- and intrachromosomal changes*

Auerbach & Moser (1953*b*) in their work on the mutagenic effects of FF, compared the frequency of genetically detectable interchromosomal changes (T) with that of intrachromosomal changes (large deletions); they found a more pronounced shortage of the former.

The cytological analysis of FF effects (Slizynska, 1957) gave similar results in regard to T and In: there was a shortage of both types, but especially of T, so that the T/In ratio was lower than is usually found in X-ray experiments. The T/In ratio in the present experiment exceeded that found after FF treatment in all broods, although it was slightly lower in broods *b* and *c*, presumably due to the spatial conditions in spermatocytes.

There was no shortage of other intrachromosomal changes (Rp's and Df's) after FF treatment. Consequently, the most striking difference between the effects of FF and X-rays is in the ratios between inter- and intrachromosomal changes (Table 3). This ratio was approximately 1 in all X-ray broods, but only 0·2 after FF treatment.

Table 3. *Inter- and intrachromosomal changes in FF and X-rays experiments*

		X-rays (broods)			
Ratio	FF	*a*	*b*	*c*	*d*
T/In	1·0	1·5	1·3	1·3	2·5
T/In, Df, Rp	0·21	1·1	1·1	1·0	1·3

(iii) *Mosaics*

Both genetical and cytological analysis revealed a high percentage of mosaics among structural changes induced by FF. In the present X-ray experiment a small number of mosaics was found in brood *a*, while other broods had none but 'complete' changes (Table 1).

(iv) *Breaks in heterochromatic and euchromatic regions*

One of the characteristic effects of FF in *Drosophila melanogaster* is the low frequency of breaks scored in heterochromatic regions (9·7% of all breaks) (Slizynska, 1957). In the present experiment, where X-ray induced breaks were

scored in exactly the same way, the proportion of 'heterochromatic' breaks in all four broods was almost twice as high as in the FF experiment: in brood *a* 19%, in *b* 17·1%, in *c* 22·6%, in *d* 19·5%. The broods do not differ between themselves in the frequency of 'heterochromatic' breaks, but all of them differ in this respect from breaks induced by FF. The differences are statistically significant.

In explanation of the apparent low proportion of 'heterochromatic' breaks in *Drosophila melanogaster* after FF it has been assumed (Slizynska, 1957), that the opening of potential breaks in euchromatic and heterochromatic regions is connected in some way with the reduplication of the chromosomes, which may not be synchronous between euchromatin and heterochromatin. As a result, the rejoining of broken ends in heterochromatin with those in euchromatin will be restricted, while joining of two 'euchromatic' or two 'heterochromatic' ends will be favoured. In salivary gland chromosome analysis, where changes with solely 'heterochromatic' breaks are usually not scorable, this would appear as a shortage of breaks in heterochromatic regions. No such restriction applies to X-ray induced breaks, most of which presumably open simultaneously, immediately after treatment.

5. DISCUSSION

The different stages of spermatogenesis vary in overall sensitivity to X-rays. Yet the proportions of different types of change are fairly similar in all stages and are entirely different from those found after FF treatment (see Table 2).

Since the germ cells samples in brood *c* of the X-ray experiment include the spermatocytes and are therefore most closely related to the larval auxocytes, which are most sensitive to FF, this brood may be taken as the basis for comparison with FF.

The effects of FF when compared with brood *c* are characterized by: (1) an overall shortage of structural changes for a similar percentage of sex-linked lethals; (2) a very high frequency of Rp's and Df's which represent over 60% of all changes as compared with 7–19% in X-ray broods; (3) a much lower ratio of inter- to intrachromosomal changes; (4) a high proportion of mosaic changes; (5) fewer breaks in heterochromatic regions.

The same five points of difference are found when the results of FF are compared with broods *a*, *b* and *d*. This suggests that it is the mutagen and not the sensitive stage which is responsible for the characteristic pattern of the FF effects.

The differences between the effects of X-rays and of FF may be attributed to the different proportions of potential breaks induced by these two mutagens. Evidence has been presented for concluding that most of the X-ray induced breaks are immediate, while most of FF-induced breaks arise as potential ones. Further support for this assumption comes from the fact that in all X-ray broods, Df's are more frequent than Rp's, while FF produces more Rp's than Df's. This is as expected if X-rays induce Df's mainly by immediate breakage of chromosomes,

while the majority of FF-induced Df's arise from potential breaks and are accompanied by complementary Rp's (Slizynska, 1963). Loss of Df's beyond a certain length accounts for a slight excess of FF-induced Rp's over Df's.

The proportion of X-ray-induced potential breaks can be roughly estimated from the Rp's. These represent one-half of all potential breaks, the other half giving rise to Df's. Accordingly, the number of potential breaks can be calculated from the number of breaks involved in the formation of Rp's, multiplied by 2. The following values were obtained: in brood *a* 6·8% of all breaks were potential, in *b* 5·0%, in *c* 3·9%, in *d* 9·6%. The same calculation applied to FF data, gives an estimate of 64% potential breaks. This, however, is an underestimate, which does not take account of the observed mosaics of T's and In's; if these are taken into account, the minimum proportion of potential breaks in FF experiment rises to 73%.

It is likely that a certain fraction of genetical X-ray effects is due to an indirect action via mutagenic radicals or molecules. There is some evidence that the size of this fraction depends on dose-rate, but the data (Clark, 1956; Russel, W. L., Russel, L. B. & Cupp, 1959; Sobels, 1960; Stone, 1956; Wolff, 1957) are conflicting. The intensity of irradiation in the present experiment was well below 1000 r/min., a dose rate below which delayed mutations are rare according to Clark (1956); a higher dose rate might have given more potential breaks. Cytological analysis of the types of change produced by radiation of different intensities would probably give information on this point.

6. SUMMARY

The structural changes induced by X-rays in cells at different stages of spermatogenesis were analysed in salivary gland chromosomes of *Drosophila melanogaster* and compared with the changes induced by formaldehyde added to the food (FF) of the larvae.

The different stages of spermatogenesis vary in sensitivity to X-rays when measured by the percentage of sex-linked lethals, by the percentage of spermatozoa carrying structural changes, and by the number of changes in 100 spermatozoa. The proportions of the different types of change (T, In, Rp, Df), however, are fairly similar in all stages of spermatogenesis, but entirely different from those found after FF treatment. This suggests that it is the mutagen and not the sensitive stage which is responsible for the characteristic pattern of the FF effects.

The differences between the effects of X-rays and of FF are attributed to the different proportions of potential breaks induced by these two mutagens. Evidence has been presented indicating that while most of FF induced breaks are potential (about 73%), most of the X-rays induced breaks are immediate. For the dose rate used in the present experiment (below 1000 r/min.) only a small proportion (4–10%) of breaks induced by X-rays was found to be potential.

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