SOME PARAMETERS OF EMS AND X-RAYS INDUCED MITOTIC RECOMBINATION AND CELL-DEATH IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

In adult Drosophila heterozygous for recessive cutile markers one occasionally finds patches of homozygous tissue in an otherwise phenotypically wild type background. Stern /1936/, in a brilliant series of experiments demonstrated that this phenomenon is due to recombination occurring during mitosis /MR/.

Normally a cell heterozygous for recessive mutation /s/ will appear wild type. Advantage is taken of this fact in MR experiments by making the flies heterozygous for recessive mutations. If recombination does not happen during mitosis, the genotype of the daughter cells remains unaltered and like mother cell remains phenotypically wild type /Fig. 1 A/. However, if recombination occurs, one of the two daughter cells /if only one chromosome carries the marker/ becomes homozygous for the mutation, i.e., becomes genetically marked. The descent of this cell propagated in the surrounding of unmarked cells can be recovered on the adult cuticle as a "spot" or a clone /Fig. 1 B/.

If both homologous chromosomes carry different recessive markers and if recombination occurs proximal to the markers, both the daughter cells

become genetically marked and will express their recessive mutations adjacent to each other called a "twin spot".

According to Stern /1936/ MR leads to mosaic spots only after one of the two possible types of segregation. The first type of segregation in which the 1st and 3rd chromatids go together in one daughter cell and 2nd and 4th in the other, is called "x" segregation /Fig. 1 A and B/. When the 1st and 4th chromatids segregate in one and the 2nd and 3rd in other daughter cell, it is called "z" segregation.

It should be mentioned that mosaic spots can arise only if the MR is followed by x-segregation. However, an exception to this is observed in heterozygotes for mutant alleles within one complex locus, e.g., in the case of $\underline{w}^{65a25}$ and $\underline{w}^{1}$ /Stern, 1969/. Recombination between the two sub loci yields a pigmented mosaic spot both by x-and z-segregation.

Spontaneous MR

Stern's theory of MR based on the explanation that the lack of y sn /sn 1-21.0; singed rather than straight bristles/ in y +/+ sn females and the absence of twin spots in sn/+ + females demands that the target cell must be at four strand stage. This excludes the possibility of 2-strand MR since in both cases neither x - nor z - segregation will lead to the

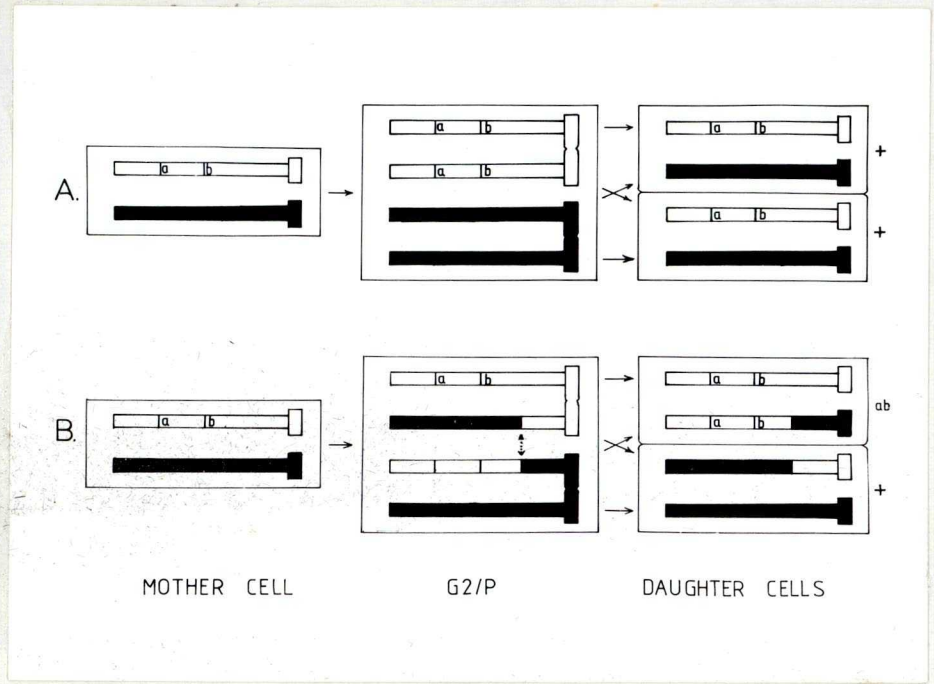

Figure 1. Schematic representation of MR. Without MR one mitosis produces two daughter cells without altering the genotype /A/. If MR happens, the daughter cell becomes homozygous for the mutation /s/, gets genetically marked, and expresses itself as a clone on the phenotypically + background /B/.

spot types at 2-strand MR. Bateman /1968/, however, has shown that only stages in spermatogenesis prior to mitosis undergo recombination upon irradiation. According to Hannah-Alava /1965/ if MR happens at 2-strand stage, it will lead to complimentary clusters of identical size. The same will happen if MR happens at 4 stran stage followed by z-segregation. Hence, there is no way to distinguish between 2- and 4-strand MR in gonial cells.

In experiments with y sn/+ + females, Stern found some sn single spots. This was attributed to double MR event. In his experiment the rates of MR in y sn and sn - centromere yielded a ratio of 1:2.0; whereas the meiotic recombination ratio was 1:1.75. On the basis of these results he concluded that a comparatively high proportion of MR takes place proximal to sn.

The frequency of MR can be increased by temperature, Minute mutants, high oxygen pressure and ring chromosomes /see Postlethwait and Schneiderman, 1974/.

An increased frequency of MR in ring/rod heterozygotes favours the idea that MR can happen after breaks in non-homologous regions of the chromatids, because if the break happens only on homologous sites, then ring/ring combination should also show an increase in MR frequency.

If MR can take place between non-homologous sites, then the daughter cells carrying chromatids with deficiencies and duplications might be delayed in their development and may not lead to visible mosaic spot. It may particularly be true if such an event happens in euchromatin.

The unequal exchange of chromatids especially in euchromatin might account for differences in spot frequencies in eu- and heterochromatin. Another explanation can be that MR happens predominantly in heterochromatin. Unfortunately not enough data are available on the role of eu- and heterochromatin during spontaneous MR. According to Stern /1936/ and Kaplan /1953/ in X chromosome and in autosomes, the frequency of MR was observed in the regions near to centromere, i.e., in the heterochromatin.

Induced MR

The frequency of MR can be increased by radiations such as UV, and X-rays and by chemicals like mustard gas, TEM, MMS and EMS, etc.

Quantitative aspects of X-ray induced MR have been investigated by Haendle /1971 a and b/. When the larvae were irradiated with 100 kV for 30 seconds, a discontinouos slope in dose-effect curve was observed /Fig. 2./. However, treatment for 150 seconds did not show the discontinous slope. When constant doses were applied over varying interval of time, the dose--rate curve indicated that part of induced MR were

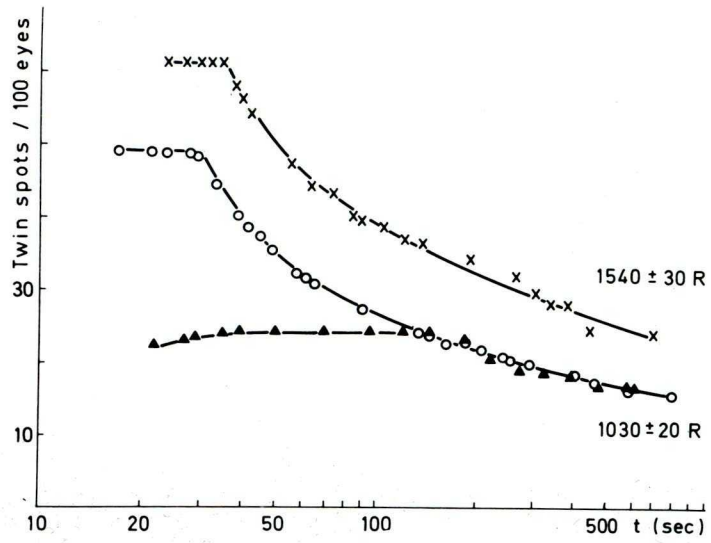

FIG. 7. Dependence of twin spot frequency on length of irradiation time using 100 kV (× and ○) and 55 kV (▲) X-rays. (From Haendle, 1971a.)

Figure 2. The effect of two different parameters of X-rays on the frequency of induced MR. Note that irradiation with 100 kV for about 30 second results in discontinutiy of the slope. This is absent at irradiation with 50 kV. This indicates that there is a type of break which can be induced with 100 kV /and probably higher kVs/ and stays available for some 30 second for undergoing recombination.

due to multiple-hit events. It also indicated that there is a type of break involved which stays available for 30 seconds for undergoing recombinational event with a second break. The discontinuity of the dose response curve and the dependence on time, therefore, indicates that there are at least two events leading to recombination.

In another series of experiment she /Haendle/ treated larvae with 55 kV and 100 kV. It was found that there was no change on the dose effect curve when the larvae were treated by 55 kV; no matter whether 30 or 150 sec. The curve was also identical to 100 kV curve at 150 sec. The results indicate that one of the two reactions triggered by 100 kV spectrum is identical to that of 55 kV. It also became apparent that 100 kV triggers another reaction at application less than 150 seconds, which stimulates a rapid repair system.

The above experiments confirm that the hard portion of 100 kV spectrum induces break and repair simultaneously. They also indicate that the two types of breaks - of which one is available only for 30 seconds - do not lead independently to full amount of MR, rather there exists a third component "X-ray induced chromosome pairing", /Haendle, 1971 b/, which depends on the total dose.

The sensitivity of different regions of chromosomes for undergoing MR change during development. This was demonstrated by Tokunaga and Arnheim /1966/

who concluded that irradiation early in development induce MR predominantly in the distal region of the left arm of 2 chromosome /left to marker Tuft /Tft 2-53.2/. Irradiations later in development induce MR right to the marker, i.e., nearer to the contromere. The left/right ratio of MR increases with the age of larvae. However, it may be mentioned that mainly the spot number right to the marker increases whereas it stays almost unchanged to the left of the marker. This suggests the following interpretation. Histoblast cells are arrested in the early G_2 phase of mitotic cycle. With increasing age, the late replicating heterochromatin proceeds from 2 to 4 strand stage in an increasing number of cells. The left/right MR shift ratio, therefore, is not due to a change in the sensitivity of the two regions - the eu- and heterochromatin as the spot number in euchromatin remains unchanged. What might be considered a change in sensitivity in heterochromatin region in due to a gradual increase in number of 4 strand stages.

There is a discrepancy between these data and those of Garcia-Bellido and Merriam /1971 a/. They found that the primodia of mesothorax divide during the interval investigated by Tokunaga and Arnheim /1966/ to such an extent that the number of spots should have increased about 16 fold. The above mentioned data, therefore give no clear basis for

assuming that the sensitivity of various chromosome sections is dependent upon age.

Becker /1957/ after considering the work of Parker /1948/ Lefevre /1947/ and Fano and Demerec /1941/, concluded that X-rays cause a physiological change in the cells which provide the conditions under which MR can happen. On the basis of his own data he considered it possible that this condition may be maintained for one or more cell divisions. To test this assumption he X-rayed $\underline{w}^{co}/\underline{w}$; $\underline{ru}^{+}$ $\underline{se}/\underline{ru}^{g}$ $\underline{se}^{+}$ and $\underline{z}$ $\underline{w}^{+}/\underline{z}^{+}\underline{w}$; $\underline{ru}^{g}$ $\underline{se}^{+}/\underline{ru}^{+}$ $\underline{se}$ larvae /Becker 1956, 1969/ and scored for X chromosomal and autosomal markers. In all experiments some cases were found where mosaic spots showed the phenotypes of markers of X chromosome and autosomes. This means, here two non-homologous chromosomes had undergone MR in one cell. In some cases the mosaic spots of X chromosome markers were larger than the autosome and vice-versa. This indicates that MR had taken place in consequtive cell division in one cell line. However, this could not be demonstrated precisely.

The frequency of spot generally increases with the age at which larvae are irradiated. This is to be expected since the cells in imaginal analagen increases with age. Garcia-Bellido and Merriam /1971 a/ found that the frequency of $\underline{mwh}$ spots in the wing disc increases steadily throughout the larval development, which indicates a uniform

rate of mitosis. On the basis of these results they calculated the average cell cycle of wing disc cells to be 8.5 hours. Becker /1957/, however, noticed a temporary decrease in eye mosaic spot frequency at the end of both first and second instar larvae. Since the number of presumptive eye cells do not decrease during these stages, it implies that the MR frequency does not depend exclusively on the number of treated cells. The possible explanation of this decrease is that the cells at the end on an instar rest for sometimes in G_1 phase.

The size of the induced mosaic spots depends largely on the age of the larvae at the time of irradiation /Becker, 1966/. The size of the white single spots in the eye $\underline{w}^+/\underline{w}$ females decreases from an average of 20.1 ommatidia/spot to 4.1 ommatidia/spot after treatments at the beginning of first and second instar larvae. This indicates that the eye cells undergo two divisions during the first larval instar.

As mentioned earlier MR can also be induced by UV and chemicals.

Prudhommeau and Proust/1969/ treated pole cells and oogonia with UV light of 2537 °A. Both single and double clusters were found. As in the case of X-rays, the distribution of UV induced

MR seems to be similar in nature, i.e., most MR occurs near to the centromere. In a later experiment Prudhommeau /1972/ tested males derived from eggs whose pole cells had been irradiated. Majority of males yielded recombinant offspring. He interpreted that UV induces chromosomal lesions which at the time of DNA replication, lead to MR.

MR induced by chemicals was first demonstrated by Auerbach /1945/. She treated eggs of different ages with mustard gas. In y w sn/lz there were considerable number of w/lz twin spots and in y w lz/sn, y/sn twin spots were recorded. The overall spot frequency was 60/100 females. Similar results have also been obtained by feeding w^{co}/w; se h/se h larvae with TEM /Becker, 1975/.

In Becker's /1975/ experiments the overall twin spot frequency was 66 %. This comes close to Haendle's /1971 a/ results if about 70 % X-ray induced MR, supporting the assumption of Auerbach /1945/ that the basic mechanism of X-ray and chemically induced MR might be the same.

Becker /1975/ by judging the general appearance of the spot size found that the size of spots were fairly uniform and not scattered over all size classes from very large to very small spots as observed by Auerbach /1946/. He, then concluded that the mutagen /TEM/ might have acted immediately and that the action of mutagen did not

continue during the rest of larval life.

Mollet and Szabad /1978/ induced mosaicism in the eye disc and female germ line by MMS. The sensitivity of eye disc cells to 1.2 mM MMS fed for 4 hours was found to be 10.9×10^{-4} and that of the germ line cells 9.4×10^{-4}. They concluded that the mutagen was equally effective both to eye disc and germ line cells in induction of MR. They also evaluated the sensitivity of 6 mM MMS fed for 4 hours and found it to be 70 % as effective as 1000 R of X-rays with respect to induction of MR.

Mitotic maps

Map distances in mitotic maps are relative distances expressed as the per cent of MR events that occurs within the interval between the markers taking the total number of recombination event within the chromosome as 100 % /Pontecorvo and Käfer, 1958/.

Becker /1974/ compared the meiotic, mitotic and cytological maps of the X chromosome with respect to markers y, sn and f, and the centromere. The difference between the cytological maps of the salivary gland chromosome and of the metaphase chromosome is based on the consideration that in metaphase stage the proximal half of X chromosome is heterochromatic /Cooper, 1950/. Rudkin /1965/ measured the DNA content of In/1/sc^8 and In/1/

sc^4sc^8 chromosomes. The latter chromosome contains about 30 % less DNA than the former one. Since the $In/1/sc^4sc^8$ lacks most of the proximal heterochromatin, it was concluded that at least 30 % of X chromosome is heterochromatic. The actual proportion of X chromosomal heterochromatin probably ranges between 30-50 % as the $In/1/sc^4sc^8$ crossover chromosome still carry a short heterochromatic segment.

Garcia-Bellido /1972/ made mitotic maps for all major chromosome arms for both spontaneous and X-ray induced MR. His results for X chromosome are based on pooled data from four types of females, y f/+ +; y +/+ f; y sn/+ +; and y +/+ sn, i.e., from cis and trans experiments. In all cases y spot is considered to be derived from single MR event between y and f or y and sn respectively. yf or y/f and y sn or y/sn are considered to be derived from single MR event between f and centromere and sn and centromere respectively. Spots f and sn are considered to be derived from double MR.

The spontaneous and induced mitotic maps are more similar to metaphase map than the meiotic map. This is particularly true for spontaneous mitotic map. This means that there is no differential distance for spontaneous MR.

According to the map, the induced MR seems to be more frequent in the proximal region than sponta-

neous MR. This speaks of itself that induced MR takes place preferably in heterochromatin. The problem of unequal exchange also can not be ruled out. If the breaks in the two non-sister chromatids are randomly distributed, duplications and deficiencies would result. Such abbarations might be less harmful in hetero-than in euchromatin.

An independent indication that induced MR is recovered preferentially from events in hetero-chromatin came from comparison of MR frequencies in X and 3rd chromosome /Becker, 1966/. This was done by X-raying z +/+ w; ru^g +/+ se first instar larvae with 1250 R and scoring the mosaic spots for both X and 3 chromosome markers. The twin spot ratio for X and 3 chromosome was 20:1. Thus, it was concluded that the relative lenghts of heterochromatic region of X and 3 chromosome were mainly responsible for the different observed frequencies.

This interpretation was further tested by using chromosomes with different amount of eu- and heterochromatin between the marker and the centromere /Becker, 1969/. For this purpose 9 inversions were used which differed in the amount of eu- and hetero-chromatin between the marker and the centromere. The chromosomes were marked with w and w^a and

the frequency of w and w^a twin and single spot was registered.

When the total distance between the right break point of an inversion and the centromere was shortened by removing euchromatic sections of various lengths, there was little influence on the spot frequency. However, the more the heterochromatic sections was shortened, the spot frequency went down. This is indicative that MR takes place predominantly in heterochromatin. This view has recently been confirmed by Schweizer /1978/ on the basis of cytological analysis of neuroblast cells of Drosophila after treatment with X-rays. It can be summarised from forementioned experiments that the distribution of both induced and spontaneous MR is quite different from that of meiotic recombination. Spontaneous MR seems to be distributed more or less evenly all through the chromosome. Induced MR also takes place both in eu- and heterochromatin, but predominantly in the latter.

Clonal analysis

In recent years one of the most important uses of induced MR has been the study of normal features of development. The major advantage of this technique is that one can label a single cell at random at a given time of development to initiate

a homozygous clone in a heterozygous background. The frequency of spontaneous MR is very low, but can be significantly increased treating a dividing population of cells by X-rays.

The characteristic of clones are informative in various ways /Nöthiger, 1972; 1976/, e.g.

1./ The clone size gives an estimate of the number of cells present in the organ at the time of clone induction. Clone size is dependent on the number of cell divisions that occured between the initiation of the clone and the final differentiation.

2./ The shape of the clone provide information about the morphogenetic process.

3./ The range or extent of the clone reveals developmental capacities and restrictions of the ancestor cell of the clone.

4./ Autonomous mitotic rate of cells can also be studied by clone induction. This was rendered possible by using Minute technique /Morata and Ripoll, 1975/. Using Minute technique a cell can be made homozygous for wild type allele /M^+/M^+/ which divide rapidly and over grow their neighbour. Such a clone contributes much more towards the adult structure, but nevertheless the adult structure is normal in shape and size. Depending upon the

time of induction, the M^+/M^+ clone does not cross certain demarcation lines, but runs along them for great distances in spite of its large size. These findings have lead to the important discovery, called compartments /Garcia-Bellido et al., 1973, 1976/. Each compartment is derived from a group of founder cells called a polyclone /Crick and Lawrence, 1975/.

5./ If descendants of the marked cell are found on two different organs /overlapping clones/ one can say that those organs have common origin. For example, Steiner /1976/ found overlapping clones restricted to one compartment only between the left-right forelegs, wing and second leg and haltere and third leg, when the clones were induced very early in development. This indicates that compartmentalization occurs earlier than disc determination.

Cell-death

Evidence suggests that the technique of MR can only be used at the expense of disrupting normal development. Many authors like Friesen /1936/, Waddington /1942/, Villee /1946/, Postlethwait and Schneiderman /1973/ and Postlethwait /1975/ have noted abnormalities in Drosophila adults irradiated as embryos, larvae or pupae. It was suggested that cell-death caused by X-rays might be the reason for such abnormalities. Spreij /1971/ showed

cytologically that cell-death occurs after irradiation in the imaginal cells of calliphora.

Schweizer /1972/, by clonal analysis of genital disc, detected that irradiation causes severe damage to the disc cells. If irradiation was done early in development the adult organ was normal. This was attributed to extensive cell divisions to compensate for the loss. The proliferation became apparent by the analysis of clone size, which showed a graded increase with increasing X-ray dose. Abnormalities were produced by irradiating older larvae, because there was not enought time for additional proliferation until metamorphosis.

Recently Haynie and Bryant /1977/ studied the effect of several doses of X-rays applied to 72 hour old larvae on the extent of cell-death in the wing disc. Their results indicate that irradiation significantly reduces the number of cells which undergo normal proliferation in the imaginal wing disc. They used dose of 500-4000 R and found a linear increase in the clone size. On these basis it was estimated that a dose of 1000 R reduces 40-60 % the number of cells capable of making a normal contribution to the developing wing. Part of this reduction was due to unability of cells to proliferate, possibly due to radiation-induced

aneuploidy and part was due to cell-death. However, even after treatment with 4000 R, where at least 75 % of the cells were killed or mitotically inhibited, normal pattern of wing was found in adults. Only in rare cases occasional thickening of the wing veins and the appearance of single adventitious bristle on the proximal radius were found.

The restitution of normal pattern of differentiation after extensive cell-death was due to the extra proliferation of the surviving cells as indicated by the clone size. This suggests that a high degree of developmental plasticity is retained by the disc cells. The success of this regulatory process by which the final pattern was restored after extensive cell-death has been explained as a result of intercalary regeneration /Haynie and Bryant, 1976/, because the pattern of cell division during intercalary regeneration was similar with respect to direction of division, i.e., aspect ratio /length/width of the clone/ to that found in the wings induced by all the four doses.

Considering these facts we directed our study to answer two main questions. First, what is the role of eu- and heterochromatin in MR induced by different parameters of X-rays and EMS, and, second, the extent of cell death caused by

different parameters of X-rays and EMS?

To answer these questions separate mitotic maps for imaginal discs and abdominal histoblasts have been constructed for X chromosome with respect to markers y and f and the centromere. This would also tell the response to X-rays and EMS by the cells of different proliferation dynamics, i.e., those of imaginal discs and histoblasts.

Different laboratories traditionally use different parameters to induce MR. Thus, to study the cell-death caused by different parameters of X-rays and EMS, clones on the wing have been analysed and the relationship between the frequency of clone induction and cell-death have been established.

In Drosophila the imaginal discs /the adult cuticular primodia of head, thorax and genitalia/ and histoblast cells /the primodia for abdominal cuticle/ provide a favourable system for analysis of developmental features at cellular level. The other advantage of this system is that imaginal disc cells continuously divide throughout the larval life whereas the histoblast cells do not divide in larval life at all. Hence, studies on such a system not only provide an insight of the mechanism of chromatid exchange and cell-death caused by X-rays and EMS on Drosophila, but also

in other disciplines of Biology, where the cell types are of similar nature like those of malignant cells which are also continuously dividing like imaginal disc cells.

MATERIAL AND METHODS

Mutants

y, f^{36a} and *mwh* were used as markers for detection of clones. The chromosomes also carried *jv* and *se* but were not used for analysis of clones. Their location and phenotype is mentioned in Table 1. Other relevant details of these mutations can be found in Lindsley and Grell /1968/.

Experimental design and egg collection

Two series of experiments were done. The details of crosses made and the genotype of resultant larvae is presented in Table 2.

Virgin females from the experimental series were collected, aged for 3-4 days on standard *Drosophila* medium and mated to corresponding males. One or two days after mating, the adults were transferred to plastic egg-collecting cylinders /height 12 cm; diameter 8 cm/. These cylinders rested on 9 cm diameter petri dish having another smaller petri dish /diameter 6 cm/ mounted in the centre. The smaller petri dishes were filled with *Drosophila* medium which was roughened and smeared with a thick yeast paste. The petri dishes were

Table 1.

Genetic markers used in present study

Marker	Location	Phenotype
yellow /y/	1-0.0	body and bristle yellow in colour
forked /f^{36a}/	1-56.7	bristles shortened, gnarled and bent, with ends split or sharply bent. Hairs similarly affected
multiple wing hair /mwh/	3-0.0	wing cells contain groups of 2-5 hairs instead of one hair per cell as in wild type
javeline* /jv/	3-19.2	all bristles and hairs cylindical instead of tapered, with small enlargement before the tip.
sepia* /se/	3-26.0	eye colour brown at eclosion darkening to sepia and becoming black with age

* not analysed in clones

Table 2.

The experimental design. Chromosome order represents 1 and 3 chromosome respectively

Experiment	Crosses: Virgin females	Males	Genotype of F_1 larvae
	sevelen /wild type/		
cis	$\frac{+}{+}$; $\frac{+\ +}{+\ +}$	$\frac{y, f^{36a}}{Y}$; $\frac{mwh, se}{mwh, se}$	$\frac{y\ f^{36a}}{+\ +}$; $\frac{mwh\ se}{+\ +}$ and $\frac{+\ +}{Y}$; $\frac{mwh\ se}{+\ +}$
trans	$\frac{y}{y}$; $\frac{mwh,\ jv}{mwh,\ jv}$	$\frac{f^{36a}}{Y}$; $\frac{+\ +}{+\ +}$	$\frac{y\ +}{+\ f^{36a}}$; $\frac{mwh\ jv}{+\ +}$ and $\frac{y}{Y}$; $\frac{mwh\ jv}{+\ +}$

changed at 2 hourly interval and two or three initial collections were discarded in order to maintain synchrony. The eggs hatched and larvae developed at 25°C and a RH of 70-80 % in the petri dishes till the treatment.

Treatments

72 ± 1 hour after oveposition the larvae were floated off the food by 50 % glycerine and X-rayed in plastic petri dishes /see Table 3/.As the feeding on EMS was to be done for 4 hours /Table 3/ only 70 ± 1 hour old larvae were used. Hence, the middle of feeding corresponded to 72 ± 1 hour. Care was taken that larvae did not drown in EMS solution. In all cases the X-ray dose rate was 500 R/min and the total dose was 500 R.

After the treatments larvae were transferred to vials /30-40 larvae/vial/ containing Drosophila food and kept in the incubator at 25°C. The time of pupariation and emergence were recorded.

Controls were also done for each series. They were treated identically to their EMS fed and irradiated siblings, but instead were fed on 1% sucrose solution. The frequency of MR in control experiments provide a measure to which the experimental values were compared.

Table 3.

Details of treatments applied

Nature of treatment	Concentration / parameters	Time
Fed on EMS	Freshly prepared 16 mM EMS in 1 % sucrose solution	4 hour
X-rayed*	50 kV; 0.055 mm Al filter	1 min.
	110 kV; 1.5 mm Al filter	1 min.
	150 kV; 2.0 mm Al filter	1 min.

*by a BX-1 /TRAKIS, Hungary/ X-ray machine.

Analysis of MR

Three days after emergence the flies were preserved in a mixture of alcohol and glycerine /3:1 v/v/. Wings were removed from the females and were mounted in Faure's. Females /without wings/ were cooked in 10 % KOH solution for 8-10 min and processed according to the method described by Szabad /1978/. The cooked flies were serially embedded in Faure's on a slide, dried, and mounted in a mixture of Faure's and distilled water and sealed. Mounted preparation were scored by a compound microscope at 300 X or 600 X. The position, nature and number of clones of mutant cells were recorded. Clone size was established by counting the number of mutant bristles or hairs.

Construction of mitotic map

Data on the mitotic recombination between cell marker mutations can be used to construct maps in terms of MR units. Map distances in mitotic maps are relative distances that occurs within the interval of markers considering the total number of recombination as 100 % /Pontecorvo and Käfer, 1958/.

In simpler way it can be expressed as follows:

Map distance between two markers =

$$\frac{\text{Recombination between the two markers}}{\text{Total number of recombinations}} \times 100$$

Total number of recombination is the sum of the spots of one recombination event plus two times that of double MR spots /f clones can be originated only from double crossovers in cis experiments/. For example if there are 7 y; 13 yf and 3 f spots, the total recombination will be:

$$(7 + 13 + /2x3/) = 26$$

Calculation of the observed number of clones induced either by EMS of X-rays from control data

The actual number of clones induced either by EMS or X-rays was calculated in the following way.

Suppose in the control the number of a particular clone type is n_c on N_c organs tested. If N_{exp} is the number of organs tested from the experimental set, the number of clones expected /n_{exp}/ in the experimental set /based on control data and assuming that the frequency of spontaneous events is the same in control and experimental series/ will be

$$n_c : N_c = n_{exp} : N_{exp}$$

or

$$n_{exp} = \frac{n_c \times N_{exp}}{N_c}$$

Then, the number of clones induced in the experimental set will be decreased by n_{exp} and will be:

$$n_{induced} = n_{observed} - \frac{n_c \times N_{exp}}{N_c} \qquad /1/$$

where, $n_{observed}$ is the number of a particular clone type in the experimental set.

y and f correction

In trans experiments, sometimes one of the partners of a twin spot may lie in a "silent" zone and may not materialize into chetae. Thus, we notice high frequency of y and f single spots, most of them are actually partners of the twins. If we know the frequency of y and f in cis experiment, the trans data can be corrected on the basic of cis data. A method of such a correction is presented below:

If we assume that the frequency of breakage of chromatids does not depend on the position of markers used, one can correct trans data based on the cis data.

In ideal case /big clone size/ the frequencies of y and f should be the same in both cis and trans experiments. In experimental cases, however, it is much higher in trans experiments. This is because of disappearance of one of the partners of y/f

twin spots. The additional y and f clones should be, then, considered as twin spots.

Correction can be done in following way:

suppose are n_{yc} yellow clones in cis experiment on N_c organs tested. Then the frequency of y will be $= n_{yc}/N_c$

If N_{tr} is the number of organs tested in the trans experiment, the number of y clones $/n_{y\ exp\ tr}/$ can be predicated on the basis of cis data in the following way:

$$n_{y\ exp\ tr} = \frac{N_{tr}}{N_c} \times n_{yc} \qquad /2/$$

After knowing the number of y clones in trans position $/n_{y\ exp\ tr}/$, the number of twin spots can be calculated as follows:

$$n_{twin} = n_{twin\ obs} + /n_{y\ tr} - n_{y\ exp\ tr}/ \qquad /3/$$

Correction of f can also be done in the same way.

The two above mentioned formulae can be generalized in the following way:

$$n_e = \frac{N_{tr}}{N_c} \times n_c \qquad /2/$$

where n_e is the number of clones expected in *trans* position; N_{tr} and N_c are the number of organs tested in *trans* and *cis* experiments respectively and n_c is the number of clones observed in *cis* position,

and

$$n_t = n_{to} + /n_{tr} - n_e/ \qquad /3/$$

where n_t is the number of twin spots which should be present, n_{to} is the number of twin spots observed; n_{tr} is the number of a particular clone /partner of a twin/ observed in *trans* position and n_e is the number of clones expected.

Statistical analysis

To test the significance whether different maps are different a four-field /χ^2/ test was done by the following formula and the P value were compared by χ^2 table.

$$\chi^2 = \frac{\left[/(a \times d) - (c \times b)/ - \frac{n}{2}\right]^2 \times n}{/a + b/ \times /a + c/ \times /c + d/ \times /b + d/}$$

where a, b, c, and d are the four variants and n is the sum of a+b+c+d.

To determine whether the clone size induced by EMS and different parameters of X-rays are different from each other, the "student's" *t* test was performed by the following formula and the p values were compared by *t* table.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s}\sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

Where $\bar{x}_1$ and $\bar{x}_2$ are the mean of two clone size distributions and n_1 and n_2 are the number of clones in two samples respectively.

RESULTS

To detect the clones in imaginal disc /head and thorax/ and histoblast derivatives /abdominal tergites II-VI/, two marker genes on X chromosome, y and f^{36a} /Table 1/ were used both in cis and trans position /Table 2/. The third chromosome was also marked with mwh, se and mwh, jv but was not used for this purpose. The markers y and f^{36a} are excellent having 94 % and 100 % expressivity respectively /Garcia-Bellido, 1972/. Mitotic recombination in females, proximal to the markers heterozygous for the mutations /y f/+ +/ will result in doubly marked homozygous clone in one of the daughter cells showing the characteristic of both the mutations, i.e., forked bristles of yellow colour /Fig. 3/. If MR takes place between y and f, only a y clone /Fig. 4/ will be produced. Rarely double recombination may also happen, one between y and f and the other between f and centromere. In such cases a f clone would result /Fig. 5/. If the markers are in trans position /y +/+ f/, recombination proximal to the markers will result in a twin spot /Fig. 6/.

These results will be obtained if:

a./ recombination involves non-sister chromatids and

b./ chromatids belonging to centromeres will go to opposite poles, i.e., will follow x-segregation /Fig. 1 B/. Another fraction of mosaic spots can also be attributed to genetic deletions or somatic mutations, but as reported by Garcia-Bellido /1972/, these events are rare in spontaneous and low-dose X-ray induced MR.

Figure 3. A yf clone on the abdomen in yf/++ background. Recombination proximal to the markers results in formation of a yf/yf daughter cell, the descendent of which form yf clone subsequently /see also Fig. 1/.

Figure 4. A y clone in yf/++ or y+/+f background. Recombination between y and f results in y homozygous clone. In case of y+/+f, y clone can be formed by disappearance of f partner of the twin.

Figure 5. A f clone in yf/++ or y+/+f background. Such a clone is the result of two recombination events - one between y and f and the other between f and centromere. In case of y+/+f, f clone can be formed by disappearance of the y partner of the twin.

Figure 6. A y/f twin spot in y+/+f background. Recombination proximal to the markers results in homozygousity for y in one and f in the other daughter cells, thus, both the daughter cells get marked.

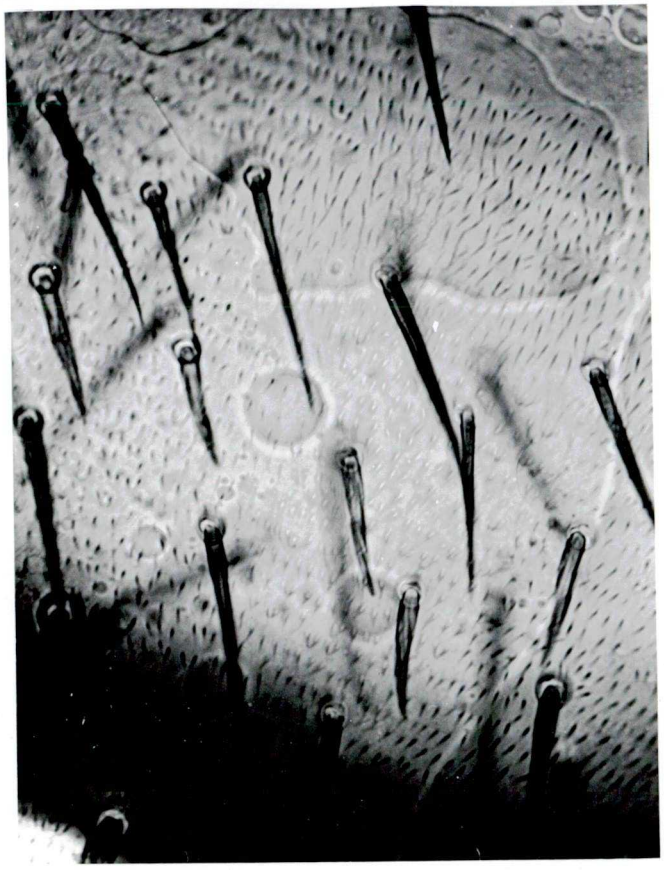

Figure 3.

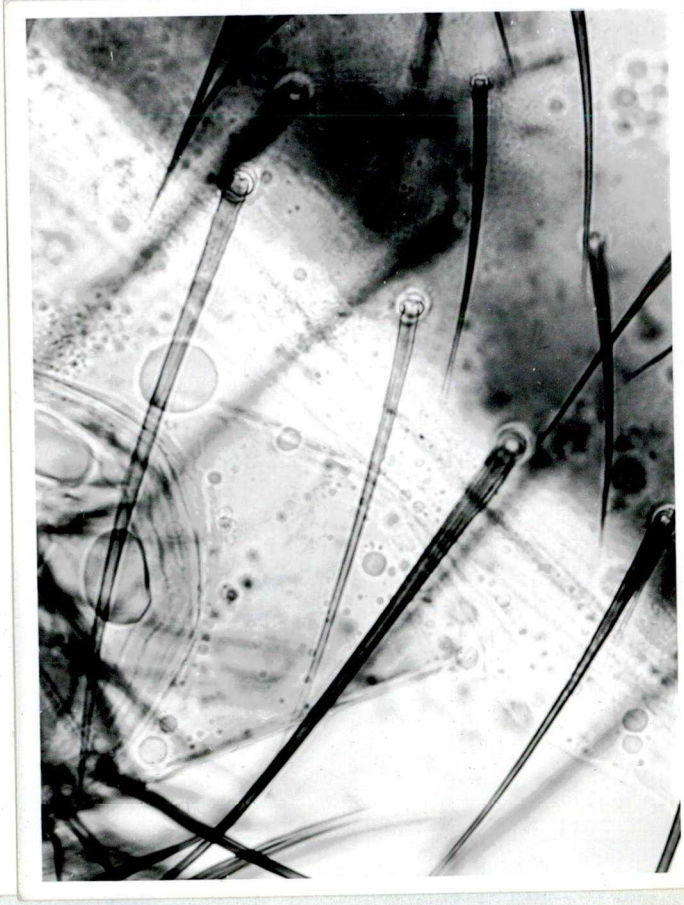

Figure 4.

Figure 5.

Figure 6.

SPONTANEOUS, EMS AND X-RAYS INDUCED MR IN *CIS*

EXPERIMENT /*y f*/+ +/

Frequencies of spontaneous and induced spots resulting from one or two MR event in head and thorax, and abdomen are shown in Tables 4 and 5 respectively. The frequencies of *yf*, *y* or *f* spots relative to total number of spots /frequency b/ in abdomen /Table 5/ either arising spontaneously or induced by different parameters of X-rays are fairly uniform. However, when compared to EMS the frequency of *yf* is fairly low whereas that of *f* is more than double. The frequency of *y* spot does not seem to be affected.

In head and thorax, on the other hand, the frequencies of spots either arising spontaneously or induced by EMS or different parameters of X-rays are different from each other /Table 4/. This might be due to differential sensitivity of the two cell types /imaginal disc and histoblast cells/ to EMS or X-rays and will be discussed later /see Discussion/.

If the frequency of single MR event per cell for the two spots *y* and *yf* is known, the frequency of double recombination event can be predicted /Garcia-Bellido, 1972/. If we consider that each adult hemitergite is derived from 8 cell population throughout the larval period /Garcia-Bellido

and Merriam, 1971 b/, then the total number of cells treated would be 80 /since only tergites II-VI were scored/. The expected frequency of double MR event should correspond to observed frequency of MR proximal to the marker multiplied by observed frequency of MR distal to the marker, divided by 80. Thus for the data of Table 5 the frequency of double MR event /i.e., the frequency of appearance of f should be 0.0002, 0.001, 0.009, 0.004 and 0.004 in control, EMS treated and X-ray induced - 50 kV, 110 kV and 150 kV - respectively. As seen from Table 5 the actual values are much higher than predicted. This is true not only in the experimental cases, but also in the control experiments.

The spot sizes in the experimental series were rather big. In EMS treated larvae 18 % f spots either consisted of 2 or more than two bristles. In X-rays treated larvae on an average 27.5 % f spots consisted of two or more than two bristles. Thus, majority of these spots represent genuine double MR event. Some others, however, might have originated due to somatic mutation or intercalar deletion of normal locus, or by some other unknown mechanism. The possibility of aneuploides or phenocopies also can not be excluded. Unfortunately we can not do such a calculation for head and thorax, because we do not know the actual number of cells

Table 4.

Frequencies of spontaneous and induced spots in head and thorax. Freq /a/=Frequency of spots per head and thorax; Freq /b/=Frequency of spots relative to total number of spots

Treatment	No. of Head and Thorax examined	1 MR event				2 MR events				Total No of spots	Frequency of spot/Head + Thorax
		Spots	Number	Freq /a/	Freq /b/ %	Spots	Number	Freq /a/	Freq /b/ %		
CONTROL	574 /284+ 290/	yf	13	0.022	56.5	f	3	0.005	13.0	23	0.040
		y	7	0.012	30.4						
EMS 16 mM	573 /283+ 290/	yf	161	0.280	55.1	f	79	0.137	27.0	292	0.509
		y	52	0.090	17.8						
X-RAYS 50kV	295 /146+ 149/	yf	213	0.722	84.5	f	4	0.135	1.5	252	0.854
		y	35	0.118	13.8						
X-RAYS 110kV	292 /144+ 148/	yf	114	0.390	70.8	f	10	0.034	6.2	161	0.551
		y	37	0.126	22.9						
X-RAYS 150kV	299 /149+ 150/	yf	207	0.692	80.8	f	5	0.016	1.9	256	0.856
		y	44	0.147	17.1						

Table 5.

Frequencies of spontaneous and induced spots in abdomen. Freq /a/=Frequency of spots per abdomen; Freq /b/=Frequency of spots relative to toal number of spots

TREATMENT	No. of abdomen examined	1 MR event				2 MR events				Total No. of spots	Frequency of spots/ /abdomen
		Spots	Number	Freq /a/	$Freq_{\%}$/b/	Spots	Number	Freq /a/	$Freq_{\%}$/b/		
CONTROL	290	yf	75	0.258	61.4	f	20	0.068	16.3	122	0.420
		y	27	0.093	22.1						
EMS 16 mM	286	yf	155	0.541	42.3	f	133	0.465	36.3	366	1.28
		y	78	0.272	21.3						
X-RAYS 50 kV	146	yf	240	1.643	66.1	f	55	0.376	15.1	363	2.486
		y	68	0.465	18.7						
X-RAYS 110 kV	148	yf	196	1.324	69.0	f	46	0.310	16.1	284	1.919
		y	42	0.283	14.7						
X-RAYS 150 kV	150	yf	163	1.086	65.7	f	40	0.266	16.1	248	1.65
		y	45	0.3	18.1						

treated. This is because that the wings and halteres were not scored for this purpose. Also part of these discs contribute to the notum, which was scored. It may be noted, however, that the frequency of spots/organ tested either in spontaneous or in induced MR /Tables 4 and 5/ are much higher in abdominal histoblasts than in imaginal disc cells.

Spontaneous MR

Tables 6 and 11 and Fig. 7 present the mitotic map occurring spontaneously in the presumptive cells of head and thorax, and abdomen in terms of the per cent of recombinations occurring between the marker mutations.

As mentioned earlier the frequency of MR in head and thorax is very low. Out of 574 heads + thoraces checked only 23 spots were recovered. Inspite of the low figures there is a general agreement between the map distances in abdominal histoblasts and imaginal disc cells, the χ^2 value being only 0.093. This points out that the mechanism of spontaneous MR may be similar in both cell types.

EMS induced MR

EMS induced MR is presented in Table 7. Although the total number of recombination in head and thorax, and abdomen is almost similar, there are

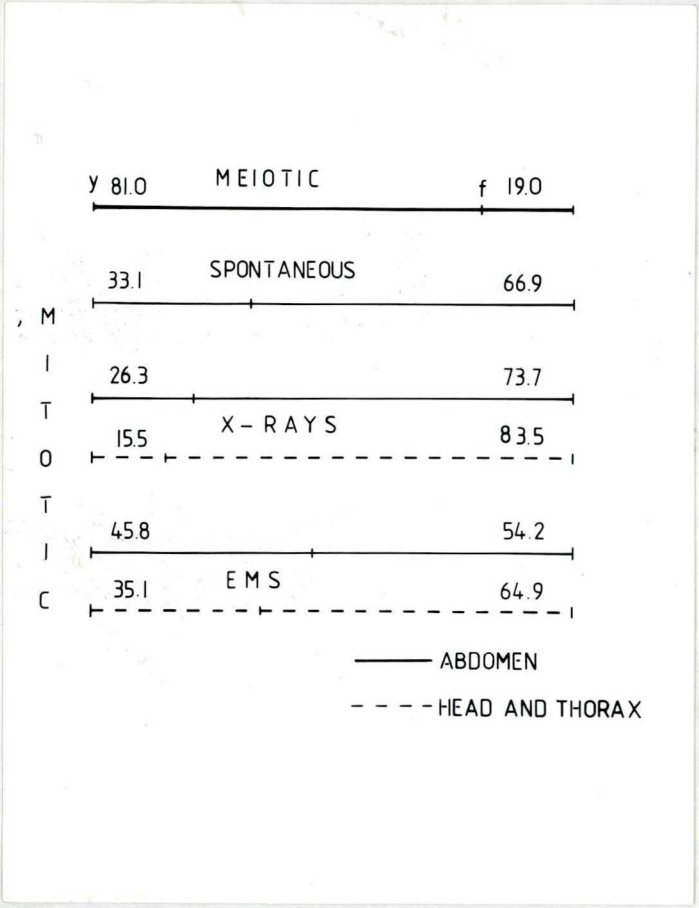

Figure 7. Meiotic and mitotic maps with respect to markers y and f and the centromere. Uppermost thick solid line is the meiotic map. Thin solid lines represent maps with respect to abdominal histoblast and broken lines that of imaginal disc derivatives. Numbers represent map distances between y and f and f and centromere.

Table 6.

Number of different spot types and map positions of y and f induced spontaneously in head and thorax /pooled/ and abdomen

Experiment/Genotype	HEAD + THORAX /POOLED/								ABDOMEN							
	Examined	SPOTS			Total spots	Recombination	MAP		Examined	SPOTS			Total spots	Recombination	MAP	
		y	yf	f			y↔f	f↔c		y	yf	f			y↔f	f↔c
cis/yf/ /++	574 /284+ 290/	7	13	3	23	26	38.4	61.6	290	27	75	20	122	142	33.1	66.9

c = centromere

sharp differences in the map positions. In imaginal discs the map distance between y and f is 35.1 whereas that in the histoblast cells is 45.8. The data are significant at 1 % level of probability /Table 11/. This points out that in imaginal disc cells, apparently more recombinations are occurring between f and centromere as compared to that of histoblast cells. Surprisingly enough the positions of markers induced by EMS in imaginal discs are not significantly different to that occurring spontaneously; χ^2 value being only 0.18 /Table 11/. The behaviour of histoblast cells, on the other hand, seems quite different. The map positions are significantly different in spontaneous and EMS induced map /Table 11/. This indicates a difference in the response by the two cell types to EMS.

X-rays induced MR

For inducing MR by X-rays, three different X-rays parameters were used /see Material and Methods and Tables 8, 9 and 10/.

The response of histoblast cells to different parameters of X-rays is practically the same as the map positions are not significantly different from each other /Table 11/. Basically the imaginal disc cells also do not respond differentially to various X-rays parameters used. The map positions of

Table 7.

Number of different spot types and map positions of y and f induced by EMS in head and thorax /pooled/ and abdomen

Experiment/Genotype	HEAD + THORAX /POOLED/								ABDOMEN							
	Examined	SPOTS			Total spots	Recombination	MAP		Examined	SPOTS			Total spots	Recombination	MAP	
		y	yf	y			y↔f	f↔c		y	yf	f			y↔f	f↔c
cis/ yf/++	573 /283+ 290/	52	161	79	292	-	-	-	286	78	155	133	366	-	-	-
→	573	45	148	76	269	345	35.1	64.9	286	51	81	113	245	358	45.8	54.2

→ spots after subtracting the control /see Material and Methods/

c = centromere

50 and 150 kV are not significantly different from each other /see Table 11, χ^2 = 0.28/. However, data of 110 kV is different from that of 50 and 150 kV, but this may be due to fewer recombination /156 in 110 kV as compared to that of 243 in case of 50 kV X-rays/ /Table 11 /.

If we compare the map positions of imaginal disc and histoblast cells, we immediately notice the differential behaviour exhibited by the two cell types. The maps of imaginal disc and histoblast cell are significantly different /$p > 0.01$/ from each other /Table 11/. The only exception is 110 kV X-rays where the maps are similar, which as stated earlier may be due to low number of recombination in imaginal disc cells at this parameter.

Table 8.

Number of different spot types and map positions of y and f induced by X-rays /50 kV; 0.055 mm Al filter/ in head and thorax /pooled/ and abdomen

Experiment/Genotype	HEAD + THORAX /POOLED/								ABDOMEN							
	Examined	SPOTS			Total spots	Recombination	MAP		Examined	SPOTS			Total spots	Recombination	MAP	
		y	yf	f			y↔f	f↔c		y	yf	f			y↔f	f↔c
cis/	295 /146+ 149/	35	213	4	252	-	-	-	146	68	240	55	363	-	-	-
yf/++ →	295	31	206	3	240	243	14.0	86.0	146	54	202	45	301	346	28.6	71.4

→ spots after subtracting the control /see Material and Methods/

c=centromere

Table 9.

Number of different spot types and map positions of y and f induced by X-rays /110 kV; 1.5 mm Al filter/ in head and thorax /pooled/ and abdomen

Experiment/Genotype	HEAD + THORAX /POOLED/								ABDOMEN							
	Examined	SPOTS			Total spots	Recombination	MAP		Examined	SPOTS			Total spots	Recombination	MAP	
		y	yf	f			y↔f	f↔c		y	yf	f			y↔f	f↔c
cis/ yf/++	292 /144+ 148/	37	114	10	161	-	-	-	148	42	196	46	284	-	-	-
→	292	33	107	8	148	156	26.3	73.7	148	28	158	36	222	258	24.8	75.2

→ spots after subtracting the control /see Material and Methods/

c = centromere

Table 10.

Number of different spot types and map positions of y and f induced by X-rays /150 kV; 2.0 mm Al filter/ in head and thorax /pooled/ and abdomen

Experiment/Genotype	HEAD + THORAX /POOLED/								ABDOMEN							
	Examined	SPOTS			Total spots	Recombination	MAP		Examined	SPOTS			Total spots	Recombination	MAP	
		y	yf	f			y↔f	f↔c		y	yf	f			y↔f	f↔c
cis/ yf/++	299 /149+ 150/	44	207	5	256	-	-	-	150	45	163	40	248	-	-	-
→	299	40	200	3	243	246	17.5	82.5	150	31	125	30	186	216	28.2	71.8

→ spots after subtracting the control /see Material and Methods/

c = centromere

Table 11.

Number of recombination and map distances between y and f and centromere and their χ^2 values

TREATMENT	HEAD + THORAX /POOLED/						ABDOMEN						χ^{2**} HEAD+THORAX ABDOMEN
	No of recombination			MAP		χ^{2**}	No of recombination			MAP		χ^{2**}	
	y↔f	f↔c	Total	y↔f	f↔c		y↔f	f↔c	Total	y↔f	f↔c		
SPONTANEOUS	10	16	26	38.4	61.6	0.18	47	95	142	33.1	66.9	6.1	0.093
EMS	121	224	345	35.1	64.9	1.09 3.40	164	194	358	45.8	54.2	0.90	7.79*
X-RAYS / 50 kV/	34	209	243	14.0	86.0	0.28 8.6	99	247	346	28.6	71.4	0.55 27.2	16.6*
X-RAYS /110 kV/	41	115	156	26.3	73.7	5.32 3.96	64	194	258	24.8	75.2	2.74	0.047
X-RAYS /150 kV/	43	203	246	17.5	82.5		61	155	216	28.2	71.8		7.03*

* significant

** p 5 % - χ^2 = 3.84

p 1 % - χ^2 = 6.62

c = centromere

SPONTANEOUS AND EMS INDUCED MR IN *TRANS*

EXPERIMENT /*y* +/+ *f*/

Frequencies of spontaneous and EMS induced spots resulting from one and two MR events in head and thorax, and abdomen are shown in Tables 12 and 13 respectively. As was also observed in *cis* experiment, the frequency of both spontaneous and EMS induced MR is much higher in abdomen than in head and thorax.

In the present and earlier /*cis*/ experiment the same markers were used and also the same treatments were applied. The only difference was that in present series the markers were in *trans* position and the marked chromosome was brought from the mother side. Thus, the frequency of MR along the different regions of chromosome should be equal both in *cis* and *trans* experiments. However, what we notice is different. In *trans* experiment, the frequency of *y*/*f* twin spots is very low as compared to frequency of *yf* clones in *cis* experiment/which should, in principle, be equal/. This is true for both spontaneous and induced MR either in head and thorax, or in abdomen. On the other hand the frequency of *y* and *f* single spots is much higher in *trans* experiments /for comparison see Tables 4, 5 and 12, 13/.

Higher frequencies of y and f single and low frequency of y/f twin spot is not surprising in trans experiment. It is to be expected due to the fact that one of the partners of a twin spot may lie in a "silent" region where no chaetae or trichome does differentiate. This largely depends on the size of spot. If the spot is large enough there is less chance of one of the partners of a twin spot not to materialize in chaetae. However, if the spot is small there is always a chance for one of the partners of a twin spot to get lost. This is what we actually notice. In control experiments the spots are very small as compared to those induced by EMS.

It may be noticed from Tables 12 and 13 that the frequencies /b/ of f spots are much higher in control than that of EMS induced MR. In ideal case it should be true for y clones also, i.e., their frequencies in control should also be higher. But the result is totaly different. The frequency of y single spot in control is 34.4 % whereas that in EMS induced MR is 41.6 % /Table 12;frequency /b/. The same is true with respect to abdominal histoblast cells /Table 13/.

These observations indicate that the rate of disappearance of one of the partners of a twin spot is different. This is also different for control

ane induced experiment as the "extent" of disappearance largely depends on clone size - it is higher in small clones as compared to those of larger clones.

If we consider these facts, then it is not fair to calculate the map positions in the way done for cis experiment. However, if we assume the frequency of MR in various regions of chromosome is same in trans experiment as in cis, the data of the trans experiment can be corrected on the basis of cis data. The method for y and f correction is described in Material and Methods and examplified for the following case.

In EMS induced MR in abdomen in cis experiment /Table 7/ the number of f clones originated from 2 MR event is 133 out of 286 abdomens checked.

Then, the frequency of f clone in cis experiments $= \nu_{fc} = \frac{133}{286} = 0.465$

The frequency of f clone in trans experiment /Table 15/ $= \nu_{ft} = \frac{140}{208} = 0.673$ /which is due to the result of 2 MR events + lonely twins/.

ν_{ft} is greater than ν_{fc} /$0.673 > 0.465$/ which is obvious because some of the f clones in trans experiment are from "unpaired" twins. This fraction is 0.673 - 0.465 = 0.208. This means that 0.208 fraction of f spot are from "quiet" twin spots. 0.208 corresponds to 43.27 /0.673 : 140 = 0.208 : x/ clones. For this reason the number of f clones

Table 12.

Frequencies of spontaneous and EMS induced spots in head and thorax /pooled/. Freq /a/= =Frequency of spots per head and thorax; Freq /b/= Frequency of spots relative to total number of spots

TREAT-MENT	No. of head + thorax examined	1 MR event				2 MR events				Total No. of spots	Frequency of spots /head + thorax
		Spots	Number	Freq /a/	Freq /b/ %	Spots	Number	Freq /a/	Freq /b/ %		
CONTROL	442 /219+ 223/	y/f	3	0.006	10.3	f	16	0.361	55.1	29	0.065
		y	10	0.022	34.4						
EMS	414 /205+ 209/	y/f	34	0.082	11.4	f	140	0.338	46.9	298	0.719
		y	124	0.299	41.6						

will be reduced by 43 and the number of twin spots will be increased by 43.

Similarly, correction for y has also be done.

The number of EMS induced y clone in abdomen in cis experiment /Table 7/ is 78 out of 286 abdomen checked.

The frequency of y clone in cis experiment $\nu_{yc} = \frac{78}{286} = 0.272$. This frequency of y clone in trans experiment /Table 15/ $\nu_{yt} = \frac{115}{208} = 0.552$ ν_{yt} is greater that ν_{yc} /0.552 > 0.272/, again indicating that some of the y clones in trans experiment are from "unpared" twins. This fraction is 0.552 - 0.272 = 0.28. This means that 0.28 fractions of y spot is from "quiet" twin spots. 0.28 corresponds to 58.3 /0.552 : 115 = 0.28 : x/ clones. For this reason the number of y clones would be reduced by 58 and the number of twin spots will be increased by 58.

Hence, the corrected figure in Table 15 for abdomen will be; y /115-58/ = 57; f /140-43/ = 97 and y/f /48 + 58 + 43/ = 149.

Similar corrections have been done for spontaneous MR and EMS induced MR in head and thorax also /Tables 14 and 15/. The map positions have been calculated in the same way as for cis

Table 13.

Frequencies of spontaneous and EMS induced spots in abdomen. Freq /a/=Frequency of spots per abdomen; Freq /b/=Frequency of spots relative to total number of spots

TREAT-MENT	No. of abdomen examined	1 MR event				2 MR events				Total No. of spots	Frequency of spots /abdomen
		Spots	Number	Freq /a/	Freq /b/ %	Spots	Number	Freq /a/	Freq /b/ %		
CONTROL	238	y/f	37	0.155	20.5	f	100	0.420	55.5	180	0.756
		y	43	0.180	23.8						
EMS	208	y/f	48	0.230	15.8	f	140	0.673	46.2	303	1.456
		y	115	0.552	37.9						

Table 14.

Number of different spot types and the map positions of y and f induced spontaneously in head and thorax /pooled/ and abdomen for original and corrected data

Experiment/Genotype	Treatment	HEAD + THORAX /POOLED/							EBDOMEN						
		Examined	SPOTS			Total No.of spots	MAP		Examined	SPOTS			Total No.of spots	MAP	
			y	y/f	f		y↔f	f↔c		y	y/f	f		y↔f	f↔c
trans y+/+f	original	442 /219+223/	10	3	16	29	57.8	42.2	238	43	37	100	180	51.1	48.9
	corrected for y and f	442	5	22	2	29	23.0	77.0	238	22	142	16	180	19.0	81.0

c = centromere

Table 15.

Number of different spot types and the map positions of y and f induced by EMS in head and thorax /pooled/ and abdomen for original and corrected data

Experiment/Genotype	Treatment	HEAD + THORAX / POOLED /							ABDOMEN						
		Examined	SPOTS			Total No.of spots	MAP		Examined	SPOTS			Total No.of spots	MAP	
			y	y/f	f		y f	f c		y	y/f	f		y f	f c
trans/ y+/+f	original	414 /205+ 209/	124	34	140	298	60.6	39.4	208	115	48	140	303	65.3	34.7
	corrected for y and f	414	37	204	57	298	26.5	73.5	208	57	149	97	303	38.5	61.5

c = centromere

experiments /see Material and Methods/ both for original and corrected data.

The results of EMS induced MR, presented in Table 15 is the outcome of both spontaneous and induced data, unlike to that of Tables 7-10 of cis data where the spontaneous data was subtracted from the experimental, before doing the final calculation for map positions.

In the present case the spontaneous data can not be subtracted from the experimental one. This is because of the reason that: a./ the frequency of dissapearance of y or f from a twin spot is different and b./ frequency of diappearance of any partner of a twin is different in control than in experimental. This is due to the difference in clone size as discussed earlier.

The maps after y and f corrections /Tables 14 and 15/ are drastically different from those constructed on the basis of original data. The map distances have reduced to half or sometimes more between y and f and have increased almost two-fold or more between f and centromere.

Although after y and f corrections the map distances have come closer to those found in the cis experiments, but still the position of markers in the two experimental series are different from each other.

EMS AND X-RAYS INDUCED CELL-DEATH

For this purpose wings of cis experiment flies /see Material and Methods/ were analysed for mwh clones /Fig. 8/. The distribution of clones found after EMS and X-rays treatment /50 kV, 110 kV and 150 kV/ is shown in Fig. 9. The clone sizes, i.e., the actual number of cells observed in a clone, have been grouped according to the minimum number of cell divisions required for production of clones of that particular size /size class/. To ensure that the clones observed in experimental series were induced by different treatments, 101 control wings were also checked. The results indicate that some of the smaller clones in experimental series, might have originated spontaneously, but that a large majority of clones are due to various treatments applied.

As it is evident from Fig. 9 that there is wide variation in the clone size. This might be due to the damage caused by various treatments. This damage might affect the physiology of cell or alter the form of chromosome structure which might lead to a reduction in cell divisions. Such cells are said to have suffered "reproductive death" because their capacity of division is limited

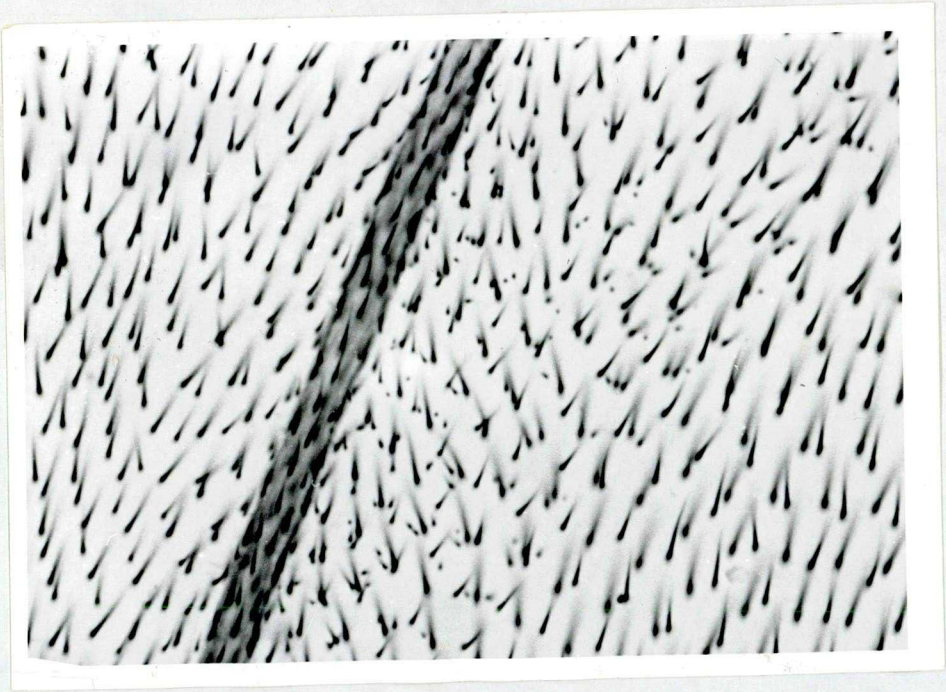

Figure 8. mwh clone on wing blade in + background

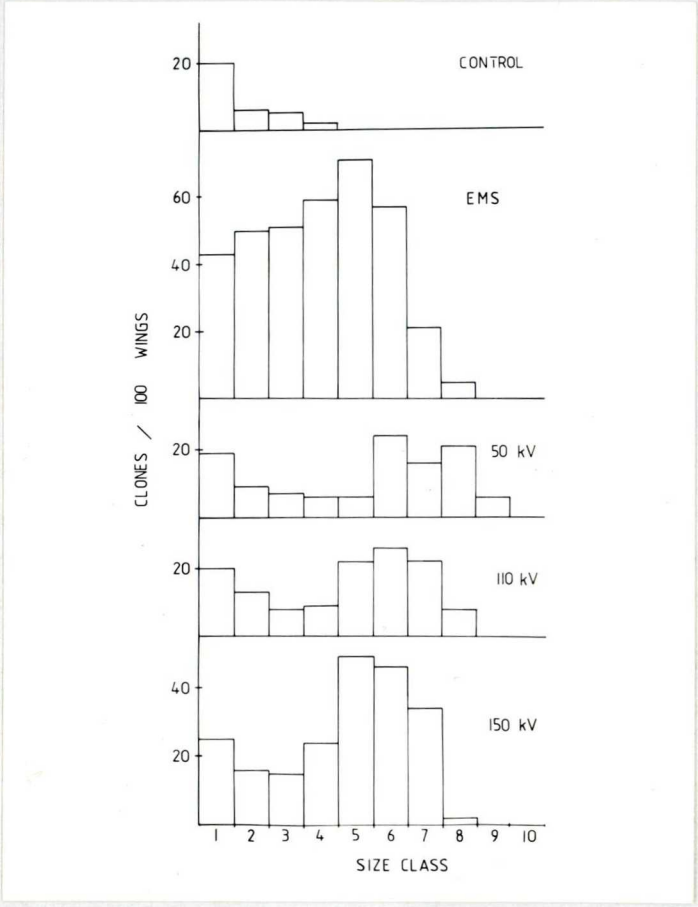

Figure 9. The distribution of clone sizes following feeding on 16 mM EMS and irradiation by different parameters of X-rays /for details see text/ of 72 hour old larvae. Size class is the minimum number of cell division required to produce a single clone of a particular size. N, number of wings examined; n, number of clones observed. Control: N=101, n=33; EMS: N=96, n=344; 50kV: N=98, n=114; 110 kV: N=93, n=116; 150 kV: N=99, n=207

/Morata and Garcia-Bellido, 1973/. Smaller clones - the so called "abortive clones" - have also been found among the progeny of irradiated cells in vitro /Carlson, 1954/.

This means that smaller clones, which have suffered the reproductive death, do not actually represent the proliferation dynamics of normal cells. Hence, for calculating the clone size and further analysis, clones of 4 or less than 4 cells have not been considered. This is not without precedence since the same was done by Haynie and Bryant /1977/.

The mean clone size was calculated by adding the number of cells involved in each clone and dividing by total number of clones. The results are presented in Fig. 10. It may be noted that the differences between all the 4 treatments are statistically significant /t-test/. We find that there is sharp decrease in clone size with increasing "hardening" of X-rays /from 50 kV to 150 kV/: the average clone size by 50 kV X-rays treatment being 54.64 $\pm$ 43.34; that by 110 kV 29.11 $\pm$ 21.3 and by 150 kV 22.73 $\pm$ 14.37. The average clone size in EMS treated wings is smallest being only 18.70 $\pm$ 15.78.

The measurement of average clone size can also be used to estimate the number of surviving cells /Haynie and Bryant, 1977/ after each treatment. If cells which undergo MR do not have

atypical proliferation dynamics, the fraction of adult structure occupied by a clone should be equal to the fraction of the disc occupied by the progenitor cell at the time of clone induction. The reciprocal of this fraction should, therefore, be equal to the cell number in the disc at the time of clone induction. However, as suggested by Haynie and Bryant /1977/, that due allowances must be made of the fact that the cells capable of undergowing MR are not a random sample of cell population, but are at specific stage /G_2/ of cell cycle. Hence, for making estimate of number of cells contributing to adult wing blade, after various treatments, a correction factor of 0.65 has been used /Haynie and Bryant, 1977/.

Thus, if we consider that the total number of cells in the adult wing blade is 30,800 /Garcia-Bellido and Merriam, 1971 a/ and that the average clone size is $\underline{m}$ /after a particular treatment/, then the number of cells contributing the adult wing blade will be:

$$\frac{30800}{m} : 0.65$$

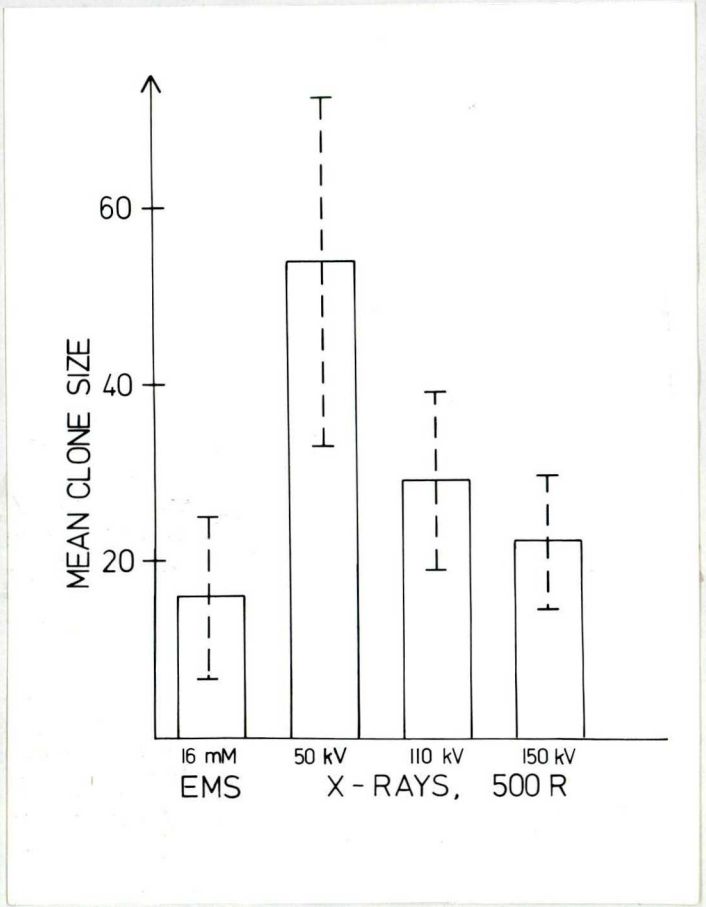

Figure 10. The mean clone sizes after various treatments. The mean clone sizes have been calculated by considering the number of cells of each clone. Clones of 4 or less than 4 cells were not considered, as they do not represent the proliferation dynamics of normal cells and have suffered "reproductive death" /Haynie and Bryant, 1977/. Dotted lines represent standard deviation.

By this calculation the number of cells contributing to adult wing blade after EMS; 50 kV, 110 kV and 150 kV X-rays have been estimated to be 1070, 366, 687 and 880 respectively /Fig. 11/. If we consider that the number of cells in wing disc at 72 hour is about 1000, it means that irradiation by 50 kV X-rays reduces about 60 % cells, whereas 110 kV reduces about 30 % and 150 kV only 10 %. EMS treatment does not seem to affect the number of surviving cells.

An attempt was also made to find out whether there is any direct correlation between the frequency of clone induction and average clone size, which is shown in Fig. 12. As may be noted that the frequency of clone induction decreases with increasing clone size, e.g., in EMS treated wings the frequency is 3.58 and average clone size is 18.70 $\pm$ 15.78, as contrast to 50 kV X-ray irradiated wings where the frequency of clone induction is only 1.6 and the average clone size is 54.64 $\pm$ 43.34. Inspite of this, no direct correlation could be established between the frequency of clone induction and the average clone size.

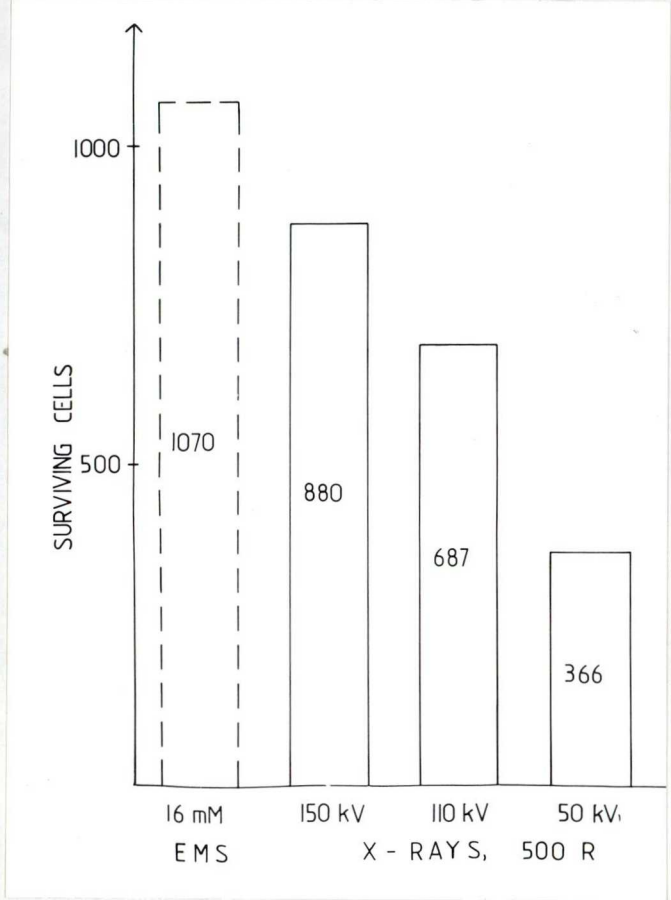

Figure 11. The number of surviving cells after various treatments. For details of calculation see text.

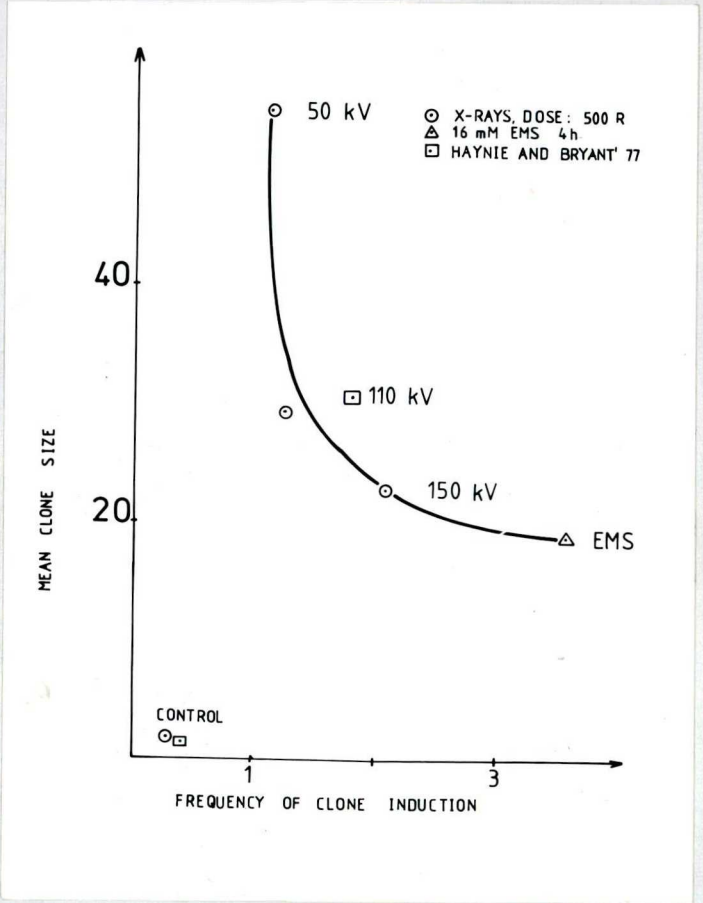

Figure 12. Comparison of the mean clone size and the frequency of clone induction after various treatments. Data of Haynie and Bryant /1977/ are also plotted.

DISCUSSION

In recent years MR has become an indispensable tool for elucidating general problems in cytogenetics, behavioural as well as developmental genetics. This is true not only in Drosophila, but also in the study of fungi, especially in the case of fungi imperfecti where sexual cycle and meiotic recombination is unknown. In Drosophila MR and subsequent clonal analyses have extensively been used for gaining information on the proliferation dynamics, morphogenetic processes and developmental capacities of cells. The technique has also succesfully been used for identification of linkage groups and the order of genes within these linkage groups.

To induce MR, normally a dose of 1000 R of X-rays have been applied. X-irradiation, particularly at that dose causes 40-60% cell-death /Haynie and Bryant, 1977/. Cell-death interferes with cell proliferation and thus, limits the value of clonal analysis. The aim of our investigation was to work on the mechanism of MR, especially the role of eu- and heterochromatin during MR induced by EMS and X-rays. Also we wanted to find out X-ray parameters which kill lesser cells and at the same time induce high frequency of MR.

It is satisfying that X-ray parameters which induce high frequency of clones and kill less cells /150 kV accompanied with 2 mm Al filter/ have been found.

DATA OF CIS EXPERIMENT

Frequencies of spot types

The frequencies /b/ of different spots in abdomen either arising spontaneously or induced by X-rays are fairly similar. The only exception is that of EMS induced MR where the frequency of f clones is much higher and the frequency of yf clones is much lower. The results of spontaneous and X-ray induced spots are in conformity with those of Garcia-Bellido /1972/. However, the frequency of y spots, is much higher in spontaneous recombination than in EMS or X-ray induced MR /Tables 4 and 5/. This points out that MR event in distal region of chromosome is higher in spontaneous recombination than in induced MR and reflects that the mechanism of production of spots is different in spontaneous and induced MR.

In head and thorax, although the frequencies of spots are variable but the relative number of y spots is lower in induced experiments than in control, pointing out a similar mechanism as described for abdominal histoblasts.

The frequency of f spots was always found to be 10-20 times higher than expected. This is true both for spontaneous and induced MR. The same situation was also noted by Garcia-Bellido /1972/.

Although some spots might originate by mutation or intercalar deletion of normal locus, majority of spots represent genuine recombination. According to the prediction based on the frequency of recombination proximal and distal to the marker, the frequency of double MR event should not be as high as observed. However, if we consider that double MR involves 3 of the 4 chromatids, f spots may appear in some cases /Garcia-Bellido, 1972/, but even then the relatively high frequency can not be explained by this mechanism, as breakage of 3 chromatids should be a rare event. Thus, if the way of prediction of double MR event is correct, the mechanism by which high number of double MR event spots appear, remains unexplained. However, it may be possible that because of somatic pairing one ionization event may break both the non-sister chromatids and lead to MR. Also the breakage of one chromatid can induced breakage of adjacent chromatid, which subsequently can lead to MR.

As clear from Tables 4 and 5, relatively high frequency of f spots were recorded in EMS treated individuals, both in head and thorax, and in abdomen. It was thought that this might largely be due to somatic mutation or delation of the normal locus, as EMS is long known as a mutagen inducing such events. For this purpose 100 males

from cis experiment /which carried a wild-type X chromosome/ were checked. It was surprising that only 4 f spots were found. This indicates that high frequency of f spots appearing in EMS induced MR are not due to somatic mutation or deletion. Rather, majority of them represent genuine double MR event.

Mitotic maps

The standard meiotic map and spontaneous and induced mitotic maps are shown in Fig. 7.

The standard meiotic map is very different from any of the mitotic maps -either spontaneous or induced. In this map the distance between y and f is 81 and between f and centromere only 19 map units. In mitotic maps the distance between y and f is much smaller whereas that between f and centromere is much larger. For example the spontaneous map for abdominal histoblast cells shows a map distance, 33.1, between y and f and, 66.9, between f and centromere. This difference in the map position clearly demonstrates that meiotic recombination predominantly takes place in distal region of the chromosome, i.e., in euchromatin whereas MR is concentrated in proximal region, i.e., in the heterochromatin.

Spontaneous mitotic map

The spontaneous mitotic maps of both imaginal disc and histoblast cells are similar /Table 11/. Although spontaneous recombination occurs throughout the larval and early pupal life, but in histoblast cells it takes place preferentially in the prepupal period, when the cells are rapidly dividing. This is due to two reasons:
a./ there are very few histoblast cells /8/hemitergite/ and b./ they do not divide during larval life and are arrested in G_2 phase. This is possibly the reason that the maps are similar, because the behaviour of two cell types is fairly similar with respect to spontaneous recombination.

As indicated earlier, the spontaneous map shows more recombination in the distal part of the chromosome as compared to those induced by X-rays /Table 11 and Fig. 7/. There can be two basic explanation of this observed behaviour: a./ the higher frequency of recombination in the distal region of chromosome is a characteristic feature of spontaneous recombination and the mechanism, thus, is different from that of induced MR, and b./that spontaneous recombination takes place in prepupal period when the cells are fast dividing.

According to Barigozzi et al. /1966/ in rapidly dividing cells the heterochromatin replicates later than euchromatin and could remain unreplicated for a longer fraction of cell-cycle time and, thus, escape MR.

The other possibility is that during spontaneous MR, due to somatic pairing, the chromatids break at homologous sites and that there is no unequal exchange of chromatids, hence, the cells undergoing spontaneous MR do not suffer with deficiency or duplication and can be recovered. Stern /1969/ found 4 pigmented spots in 6137 females heterozygous for two alleles $\underline{w}^{65a25}$ and $\underline{w}^{1}$, both located at different sites of the complex white locus. In this case pigmented spots can only be found if recombination takes place within the w locus. This observation strongly suggests the idea that spontaneous recombination takes place at homologous sites.

X-rays induced mitotic maps

The positions of y and f in the map of abdominal histoblast cells is not significantly different from that of control /Fig. 7/. If we consider only the cis data of Garcia-Bellido /1972/, the same situation is noticed. However, in imaginal disc cells the map

position in spontaneous and X-rays induced MR are significantly different from each other. In this case the marker f is pushed very near to y. This indicates that X-rays induced MR predominantly takes place in proximal region of chromosome, preferably in heterochromatin. This is also true for histoblast cells where comparatively more recombinations have been recorded in the proximal region of chromosomes than in spontaneous.

There can be two main reasons for this difference between induced and spontaneous MR. The work of Haendle /1971 a and b/ has shown that both the soft and hard portions of 100 kV sprectum of X-rays induce MR on the basis of multiple hit events. Considering this fact it seems possible that the breaks in two non-sister strands may occur at non-homologous sites resulting in duplication--deficiency situation. This may easily be tolerated in heterochromatin than in euchromatin. Thus, such an event happening in euchromatin goes undetected mimicking that more recombinations are occurring in heterochromatin.

The other explanation is that the breaks occur at homologous sites. In accordance with this conclusion is the observation that relatively

more spots originate from miotic recombination in heterochromatin than in euchromatin. This means that heterochromatin is more sensitive to X-rays than euchromatin and, therefore, shows high incidence of breakage-reunion events. In other words the chromosomes have "hot" points - possibly located in heterochromatin - which is possibly because of a difference in the state of condensation between the two types of chromatin - the eu- and the hetero-chromatin.

The latter possibility seems more plausible as the recent cytological study of Schweizer /1978/ on the metaphase chromosome of neuroblast cells of Drosophila after X-irradiation, clearly demonstrates that the break happens mostly in distal region of the heterochromatin.

As mentioned earlier that the map of X-ray induced MR is not different from that of sponta-neous in histoblast cells but is significantly different in imaginal disc cells. The reason for this may be the difference in the proliferation dynamics of these cell types. The abdominal his-toblast cells do not divide during larval life /Garcia-Bellido and Merriam, 1971 b/ and are in G_2 phase of cell cycle /Garcia-Bellido, 1972/, whereas the imaginal disc cells continuously

divide throughout the larval life. After X-ray damage the histoblast cells, thus, have enough time for repair, whereas the imaginal disc cells not.

Observations suggest that the mechanisms of action of X-rays is the same and the different parameters do not have important role as for as the site of breakage of chromatids is concerned /Table 11/. This is obvious in abdominal histoblast cells as all the parameters yielded similar results. In imaginal disc cells also the map positions induced by 50 and 150 kV are the same. Only on 110 kV the map position is different, which as pointed earlier, may be due to fewer number of recombinations.

The map positions in histoblast and imaginal disc cells, induced by X-rays, are significantly different /Table 11/. As pointed earlier, the repair mechanism and proliferation dynamics might be the reason for this difference.

EMS induced mitotic map

The map positions induced by EMS both in histoblast and imaginal disc cells are significantly different from those of X-rays /Fig. 7/. This points out that the mechanism of MR induced by EMS is different to that induced by X-rays. In this case more recombina-

tions are noticed in the distal part of chromosome, i.e., in euchromatin, which is in contrast to X-rays induced recombination. This difference may be due to one of the two possibilities:

1./ The mode of action of EMS is the same as that of X-rays, but it does not break chromatids at non-homologous sites. If it is true then the cells will not suffer from deficiency-duplication event and can be recovered.

2./ The other possibility, which seems more plausible is, that the mechanism of action of EMS is totally different. That the chromosomes have "hot" points, predominantly in euchromatin sensitive to EMS as contrast to that shown recently for X-rays /Schweizer, 1978/, which mostly break chromatids at the distal heterochromatic region.

Again here we find significant difference in the map positions in histoblast and imaginal disc cells /Table 11/. This is, as is also true for X-rays, due to the difference in proliferation dynamics.

DATA OF TRANS EXPERIMENT

Frequencies of spot types

As mentioned earlier /see Results/ the frequencies of y and f clones in trans experiment are much higher than in cis experiment. Contrary to this, the frequency of y/f twin spots is much lower compared to that observed for yf clones in cis experiment. This is to be expected in trans experiments, as one of the partners of the twin may lie in a "silent" zone and thus, may not materialize into chaetae.

If we consider only the trans experiment, the frequency of y or f is much higher in spontaneous than in EMS induced MR. This means that the frequency of one of the partners of a twin spot to get lost is higher in control than in EMS induced MR. This may be due to the clone size. If the clone size is small /as in spontaneous recombination/ the chance of one of the partners to get lost is higher. However, if the clone size is big /as in induced MR/, this chance is lower.

Mitotic maps

Maps constructed on the basis of trans data are very different from those of cis data. However, if we consider only the trans data of Garcia-Bellido

/1972/, the spontaneous map constructed on the basis of our data is in conformity with his results.

As the maps constructed on the basis of trans data were very different from those of cis, we corrected the trans data on the basis of cis experiment. Again we find that the "corrected" maps are quite different from the maps based on cis data. This is due to differential recovery of one of the partners of the twin in spontaneous and induced experiments.

EMS induced trans mitotic map also exhibit the same characteristic as spontaneous, i.e., both the original and corrected maps are very different from each other and also from the cis map.

For constructing mitotic maps, Garcia-Bellido /1972/ pooled the data from cis and trans experiments. As we have just seen that maps constructed on the basis of trans data are very different, even after y and f correction. This is due to the reason that trans data do not represent the actual recombination event occurring along the different regions of the chromosome. Moreover, in trans experiment, the spontaneous data can not be subtracted from the experimental one, as the frequency of disappearance of a partner of a twin spot largely depends on the clone size.

Due to these reasons, we have constructed the maps separately for cis and trans experiments. In

our opinion, only *cis* data are reliable for constructing the map, and that *trans* data should not be pooled with *cis* data.

CELL - DEATH

Schweizer /1972/, for the first time, detected the effect of X-ray induced cell-death in the genital disc of Drosophila. However, due to limitations of bristle marking mutations, Schweizer could not quantify the extent of cell-death caused by various doses of X-rays. Haynie and Bryant /1977/, on the other hand, showed that the average clone size in the wing of Drosophila is dependent on the X-ray dose used to induce the clone. They used a dose range of 500-4000 R and found increasing clone sizes with increasing X-ray doses.

We used EMS and three different parameters of X-rays to induce the clones. In all the three X-ray parameters used, the dose rate and total dose was kept constant. The dose rate was 500 R/minute and total dose employed was 500 R. The X-ray parameters used were 50 kV accompanied with 0.055 mm Al filter, 110 kV with 1.5 mm Al filter and 150 kV with 2.0 mm Al filter. All the X-ray parameters yielded different results. The clone size was largest in the 50 kV, smaller in 110 kV and smallest in 150 kV irradiated wing discs /see Fig. 10/.

According to Haynie and Bryant /1977/, clone size is a reflection of the damage caused by treatments - the larger the clone - the extensive

the damage. The clone size can also be used to estimate the number of cells surviving after each treatment. This is shown in Fig. 11. The results indicate that 50 kV treatment kills about 60 % of the cells whereas EMS does not kill the cells at all.

Our results of 110 kV are in conformity with those of Haynie and Bryant /1977/. They also used the same parameters and found a reduction of about 30 % in the number of cells. We have also estimated about 687 surviving cells, which is almost the same found by them. It is interesting to note that 150 kV kills only 10 % cells. The results indicate that 50 kV is as damaging as 1000 R /110 kV/ whereas 150 kV seems to be the safest.

According to Haendle /1971 a and b/ 100 kV X-rays /total dose 1030 R/ trigger two types of reactions. The first type of reaction is the breakage of chromatids which can be induced by all the spectra and the amount of MR depends upon the dose in R, and thus, corresponds to the ionization in air. The second type of reaction is triggered at application times of less than 150 seconds and has a rapidly acting repair mechanism.

This might be the reason that 50 kV kills maximum number of cells because it does not trigger the fast acting repair. 110 and 150 kV trigger the

fast repair mechanism, and thus, the clone size was smaller.

The result obtained by EMS treatment are surprising with respect to clone size, which is very small, suggesting that it does not cause cell-death. However, Haynie and Bryant /1977/ claim that even radiation dose as low as 100 R of X-rays causes limited cell-death and Spreij /1971/ suggested that cell-death occurs as a normal part of disc development.

The smaller clone size induced by EMS may be due to three main reasons: a./ the EMS dose /16 mM/ was too low, b./ EMS did not act immediately and c./ the action of EMS continued during the rest of larval life.

As far as the point /a/ is concern, recent work of Mollet and Szabad /1978/ indicate that 6 mM MMS fed for 4 hour is 70 % as effective as 1000 R of X-rays in induction of MR. We know that MMS is five-fold more effective than EMS in induction of MR /Szabad, personal communication/, then a dose of 16 mM EMS fed for 4 hours should be 70-80 % as effective as 500 R of X-rays. Thus, the smaller clone size can not be attributed to low concentration of EMS used.

The late action of EMS may be the plausible explanation of smaller clone size. It seems that

before EMS could act the cells already had completed one more cell-division and that MR happened later. This is reflected by the frequency of clone induction, which is very high, being 3.58/wing. If the cells really completed one more division, then it is not surprising to get such high frequency of clone induction because the target cells in this case will be almost double as compared to those treated by X-rays. This is what we actually notice. If we take the average of the frequencies of clone induced by the X-ray parameters used, it comes to about 1.5/wing, which is a little less than half to that of EMS.

As regards the third possibility, Auerbach /1946/, after treating the eggs of different ages with mustard gas found clones scattered over all size classes from very large to very small clones. On this basis she suggested that the mutagen might have been active even later in development. On the contrary, Becker /1975/ fed larvae with TEM and by judging the size of spots in the eye found that they were fairly uniform. On the basis of these results he concluded that TEM must have acted immediately and that its action did not continue for the rest of larval life.

In our experiment the distribution of clones induced by EMS and X-rays are quite different from

each other. MR induced by EMS show high percentage of smaller clones, i.e., clones of 2-8 cells, which is not exhibited by X-ray induced clones. Moreover, in EMS treated larvae the mean clone size was smaller as compared to that of X-irradiated. Thus, it may be concluded that; 1./ the action of EMS was delayed, 2./ it was active, if not during the rest of the larval life, but at least to a major part and 3./ that EMS induced high frequency of aneuploidy. If this statement is true, it means that EMS did cause cell-death, but as the action was delayed, it could not be noticed by judging the clone size.

In treated larvae /both EMS fed and X-rayed/ very many smaller clones were found all of which can not be explained on the basis of spontaneous recombination. This is true specially in EMS fed larvae. These very small clones can best be explained as resulting from aneuploidy coincident with point mutation or small deficiency at mwh locus. Merriam and Garcia-Bellido /1972/ compared the clones induced by X-rays in the wing discs of normal genetic constitution and of a genetic constitution that would result in a high frequency of aneuploidy coincident with MR. They found larger proportion of smaller clones in the wings when aneuploidy was induced and concluded that smaller clones were aneuploid and were

unable to proliferate normally, although, still maintaining the capacity to differentiate the trichomes. Morata and Garcia-Bellido /1973/ irradiated the disc before dissociation and reaggregation and found that cells apparently unable to divide during culture were still maintaining the capacity to differentiate. Haynie and Bryant /1977/ also found high frequency of clones of smaller size classes /reproductive death/ and concluded that they resulted from radiation induced aneuploidy.

It is interesting to note that even after extensive cell-death, the pattern of wing was found to be normal. Postlethwait and Schneiderman /1973/ and Postlethwait /1975/ found pattern abnormalities in legs, wings and halteres following irradiation of embryos and early larvae. Santamaria and Garcia-Bellido /1975/ reported partial wing deficiencies from irradiated third instar larvae. Although, these authors used very high doses of X-rays /1000-1500 R/ the abnormalities were surprisingly low - if we estimate the cell damage which might have been caused by the dosage used by them.

Haynie and Bryant /1977/ have suggested that the accomplishment of normal pattern after scatterred and extensive cell-death may be explained as a

result of intercalary regeneration /Haynie and Bryant, 1976/. The pattern to a great extent is regulated by extra proliferation of surviving cells.

As mentioned earlier /see Results/ the frequency of clone induction decreases with increasing clone size but no direct correlation was found between the frequency of clone induction and the extent of cell death /Fig. 12/. Thus, if there is a need to kill cells, 50 kV X-rays should be used. However, if one would like to induce MR without much damage to cells, 150 kV is advisable.

SUMMARY AND CONCLUSIONS

Stern /1936/ demonstrated that recombination can happen during mitosis. This type of recombination is called mitotic recombination /MR/. During MR the exchange of chromatids of homologous chromosomes leads to the formation of daughter cells the composition of which is different from that of the mother cells. Using appropriate mutations /genetic markers/ the daughter cells become homozygous for the mutation /will genetically get marked/ and show the mutant phenotype. The descent of such a marked cell propagated in the imaginal primodia /imaginal disc and histoblast cells/ can be recovered as a mosaic spot or clone on the adult cuticle.

MR can happen spontaneously at a very low rate. However, the frequency can be increased by X- and -rays. Chemicals like mustard gas, TEM, MMS and EMS are also known to induce MR /Becker, 1975; Mollet and Szabad, 1978/.

Recent experiments with MR have been done mostly on Drosophila melanogaster because of its easy way of maintenance and enormous wealth of mutations.

By analysis of clones induced by X-rays, the following information can be gained:

- the number of cells of a developing organ at any time of development
- the length of a cell cycle
- developmental capacities of single cells
- genetic regulation of metamorphosis
- autonomy of mutations
- functions of different organs, etc.

It was realised some six years ago /Garcia--Bellido, 1972/ that the mechanism of MR is different from that happening during meiosis. In the former, more recombinations were noticed in the proximal heterochromatin whereas in the latter in the euchromatin /Becker, 1974/. This observation raised several questions like unequal exchange of chromatids and the role of eu- and heterochromatin during MR.

For induction of MR a dose of 1000 R of X-rays have commonly been used. It was realized recently /Haynie and Bryant, 1977/ that 1000 R of X-rays kill 40-60 % cells. Cell-death interferes with normal proliferation and thus limits the value of clonal analysis. A study of the cell killing action of X-rays and chemicals /associated with the breakage of chromatids/ may be useful not only in the field of Drosophila developmental

genetics but also in other disciplines of Biology.

The aim of present study was to analyse the proporties of X-rays and EMS induced MR and cell--death. In particular we wanted to answer the following:

1./ Is the frequency of MR in eu- and heterochromatin different after treatments with different parameters of X-rays and EMS?

2./ Do cells with different proliferation dynamics exhibit different response to X-rays and EMS?

3./ Do different treatments cause the same extent of cell-death?

4./ Is there a direct correlation between the extent of cell-death and frequency of clone induction?

To answer the above questions larvae heterozygous for different markers were either X-rayed or fed on EMS. There were three parameters of X-rays applied:

"soft", 50 kV, 0.055 mm Al filter 500 R/min,
total dosage of 500 R

"mild",110 kV, 1.5 mm Al filter 500 R/min,
total dosage of 500 R

"hard",150 kV, 2.0 mm Al filter 500 R/min,
total dosage of 500 R

16 mM EMS fed for 4 hours.

The treatments were applied 72 ± 1 hours after oviposition.

Emerging flies were mounted and analysed for clones. The number, the size and the kind of the clones were recorded.

The results are summarized as under:

The frequency of MR in eu- and heterochromatin is reflected by the maps /Fig. 7/ based on MR. The points established on the maps were y /yellow body/, f /forked bristles/ and the centromere. The following observations were made:

1./ In all the mitotic maps the position of f is nearer to y as compared to the meiotic map. This is indicative of the fact that in MR, more recombinations occur in heterochromatin. The reason for this difference may be due to the possibility of unequal exchange of chromatids during mitosis, which leads to deficiencies and duplications. The cells which have an unequal exchange in euchromatin die, whereas those having in heterochromatin survive.

2./ If we compare MR maps based on spontaneous and X-ray induced MR there is a significant shift of f towards y in case of X-rays induced MR. This indicates that X-rays induce more MR in heterochromatin. Also there can be hot points, sensitive to X-rays between f and centromere, which are probably located in heterochromatin. X-rays induced MR can be unequal most of the time. Such unequal exchange in euchromatin leads to the formation of abortive

cells, consequently much recombination goes undetected, bringing f near to y.

3./ Surprisingly enough, MR induced by EMS shows the position of f proximal to centromere, than in the control. A plausible explanation of this behaviour can be that EMS also has hot points on the chromosome which are different from those of X-rays. This may imply that EMS and X-rays induced MR are different in nature.

4./ Spontaneous recombination in imaginal discs and histoblasts is not significantly different. It is due to the fact that clones originated by spontaneous MR are induced mostly late in development so the behaviour of both cell types are similar. Another possible explanation can be, that there is no unequal exchange of chromatids during spontaneous MR, hence, the cells do not suffer from reproductive death and survive.

5./ The maps constructed on the basis of clones detected on imaginal discs or histoblasts derivatives induced by X-rays and EMS show significant differences. There were more recombinations detected in heterochromatin in imaginal disc cells. This observation has to be associated with the difference in the proliferation dynamics of the two cell types. Possible role of the repair system could also be considered.

6./ Clones induced by different parameters of X-rays and EMS show significant difference in clone size. Soft X-rays induced the largest clones. The clone sizes were decreasing by hardening of the X-rays. According to Haynie and Bryant /1977/ clone size is an indicative of cell-death; the larger the clone the massive the cell-death. Clones induced by EMS were even smaller than induced by hard X-rays. This means that soft X-rays killed more cells /about 60 %/ as compared to mild /about 30 %/ or hard /about 10 %/ X-rays and EMS.

7./ The frequency of clone induction was increasing with hardening of the X-rays. EMS induced a high frequency of clones. Although harder X-rays kill fewer cells and induce high frequency of clones, there is no direct correlation between the extent of cell-death and the frequency of clone induction.

Summarizing the above observations it could be shown that:

1./ There are differences in EMS and X-ray induced MR. The possible differences may be in X-rays and EMS sensitive hot points on the chromosome.

2./ Induced MR is significantly different in imaginal discs as compared to abdominal histoblasts. This is attributed to different proliferation dynamics and repair system.

3./ X-rays, in general, cause more cell-death than EMS. Hard X-rays cause less death than soft.

4./ There is no direct correlation between the frequency of clone induction and extent of cell-death.

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