MUTATION FREQUENCY AS CONDITIONED BY THE MANNER OF APPLICATION OF RADIATION.

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Being the thesis presented to the University of Edinburgh for the degree of Ph.D.

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THE INEFFECTIVENESS OF TEMPERATURE IN INFLUENCING THE PRODUCTION OF MUTATIONS BY X-RAYS.

(With 2 Tables and 2 Diagrams).

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CONTENTS

INTRODUCTION.

Modern genetics has already collected a large body of data which clearly demonstrate the importance of radiation in causing genetic variations of organisms. Soon after the discovery of the physical properties of X-rays and radium, biologists and physicians had shown that cells and tissues are strongly influenced by these agents. Proof of the production of abnormalities in the development of the next generation as a result of the irradiation of the paternal germ cells was first brought forward by Bardeen (1906) in frogs, by Regaud and Dubreuil (1908)in rabbits, by O. Hertwig and his associates (1911, 1913) in various amphibia and echinoderms and by both Gager (1908) and Guilleminot (1908) in plants. Since then a great deal of work has been done in this direction and it has also been shown by direct observation that different structures in cells, but more especially the chromosomes, can be affected by X-rays and radium rays.

Before the carrying out of successful breeding tests which proved the inheritance of induced changes in later generations, experiments were performed with Drosophila melanogaster which showed the influence of X-rays and radium on crossing over and on non-disjunction of chromosomes.

Mavor (1921, 1922, 1923, 1924) and Mavor and Svenson (1924) showed that X-rays raise the percentage of non-disjunc tion in the X-chromosome and also increase the percentage of crossing over both in the X and the second chromosomes. Plough (1924)found that radium produces the same effect as X-rays on non-disjunction and crossing over. Muller (1925, 1926) found that there is a differential effect of X-rays, as of other agents, on crossing over in different parts of the X, II, and III chromosomes, and Muller and Dippel (1926) succeeded in producing genetically detectable "breakages" of attached X-chromosomes which, as we now know, really involved a kind of crossing over between the X's and the Y.

While the above experiments had given enough evidence to show that the behaviour of the germ plasm is affected by short wave radiations, the problem of whether actual mutations can be artificially induced remained unsolved until the discoveries by Muller (1927 , 1928) of the induction of both gene mutations and chromosome rearrangements by X-rays in Drosophila melanogaster. Following these findings the work on the genetic effects of radiation entered a new phase, and it became extended to many special problems relating to the processes of gene mutation and of chromosome rearrangement.

Thus there has arisen the field sometimes known as 'Radiation-genetics', which already embraces a large volume of literature dealing, among other things, with the nature of the gene, the process of its mutation and the properties of the chromosomes. Among the more important conclusions which have been arrived at through the studies made in this field during the past twelve years, the following may be noted.

I) The X-ray induced mutations are allelomorphic to or identical with spontaneous mutations.

2) Different kinds of mutations can be induced affecting morphological and physiological characters of the organism and its viability. They may be invisible, visible, or lethal.

3) X-rays induce mutations in both sexes, in all kinds of tissues in which tests for them could be made, and under different physiological conditions.

4) The rate of X-ray induced mutation is very high as compared to that of natural mutation.

5) There is a different apparent mutation rate for different genes in the same chromosome.

6) The induced mutation rate is in linear proportion to the amount of ionisation.

7) There is no differential effect of the various wave lengths in the X-ray to gamma ray range on gene mutations.

8) X-rays produce reverse mutations, thereby

showing that induced gene mutations are not mere losses of previously present genes.

9) X-rays cause chromosomal breakages and re-attachments such as translocations, deletions, duplications, inversions, etc.; the percentage of such breaks and re-attachments increases with the increased dose and is found to affect longer chromosomes more than shorter ones.

10) Ultra violet rays have also been shown to produce a significantly high rate of gene mutation.

As gene mutations and chromosomal re-arrangements are the basis of all genetic differentiation, the problems of 'Radiation-genetics' have principally centred around the study of the action of radiation in the production of these changes. Now the types of changes obtained, and also their frequency, may, conceivably, depend upon the external factors accompanying the irradiation and the physiological state of the cell at the time. Bearing on these questions are some experiments that were carried out to determine in what stage of its development the germ plasm is most sensitive to the influence of radiation, and other experiments which attempted to test the influence of external factors upon the production of mutations by X-rays.

Among the first experiments of the former

type were those of Harris (1929) and of Hanson & Heys (1929), which proved that more gene mutations are produced in mature male germ cells (spermatozoa) than in immature germ cells (spermatogonia) of the adults, and those of Muller (1927, 1930), which showed that more mutations are produced in spermatozoa than in larval male germ cells (spermatogonia) or in female germ cells (oogonia or oocytes). In the latter work some evidence was obtained that the same principle holds for chromosome rearrangements also. N.I. Šapiro (1931) clearly demonstrated the high frequency of translocations in mature male germ cells as opposed to immature. The investigations of Patterson, Brewster and Winchester (1932) showed that the frequency of breaks of the X chromosome in female Drosophila melanogaster is higher if adult cells are irradiated. Moore (1934), investigating the comparative frequency of visible mutations induced by X-rays at different stages of development in Drosophila, demonstrated that more of these changes are produced by the irradiation of adult males and females than by the irradiation of larvae.

Among the studies of the influence of external factors accompanying irradiation we may mention in the first place the experiments of Stadler (1928), in which barley seeds were impregnated with salts of

heavy metals (barium nitrate, lead nitrate and uranium nitrate). In these experiments it was found that the frequency of the X-ray induced mutations was raised by the treatment. This was to be expected because of the greater amount of secondary radiation given off by heavier than by lighter elements when irradiated. Medvedev (1933) made a similar study of the effect of lead acetate during irradiation of Drosophila melanogaster, and arrived at the same conclusion.

Among the "external" factors which might influence the action of radiation in a somewhat less direct way, temperature is one of the most important to test. Stadler (1928, 1930), experimenting on barley, X-rayed seeds at temperatures of 10°, 20°, 30°, 40°, and 50°C, and found no difference in the rate of mutation. Muller (1930) X-rayed Drosophila males at 8° and 34°C. There was a suggestion of a higher frequency of mutations at the lower temperature but, as Muller pointed out, the apparent effect may have been caused by more secondary radiation being received by the colder flies, since, being immobilized, they lay nearer to their metal container. Timofeeff-Ressovsky (1934) X-rayed Drosophila at 10° and 35°C, and obtained no

significant effect of temperature on the rate of induced gene mutations. Karl Sax (1938), investigating chromosomal aberrations in Tradescantia, X-rayed microspores at different temperatures. His observations were made in two series, one with a dose of 100 r units at temperatures of 6° and 40°C, and the other with a dose of 200 r units at temperatures of 7°, 25°, and 37°C. In both the series he found a temperature coefficient of 1 for X-ray induced chromosome aberrations.

Contrasting with the above results on temperature are those of Medvedev (1935), which seem to show a highly significant contributory effect of cold with irradiation, in the production of lethal mutations in Drosophila. The combined results of his four series of experiments, each involving two series at 0° and 20°C, respectively, show a difference in favour of the cold series which is 4.6 times as high as its standard error. The frequency for the cold is approximately 3/2 that for the room temperature series. It is possible, however, that the flies of the cold series received more secondary radiation from the water by which their container was cooled, for no water was used for the uncooled series. Medvedev has more recently (1938) published the results of another series of experiments

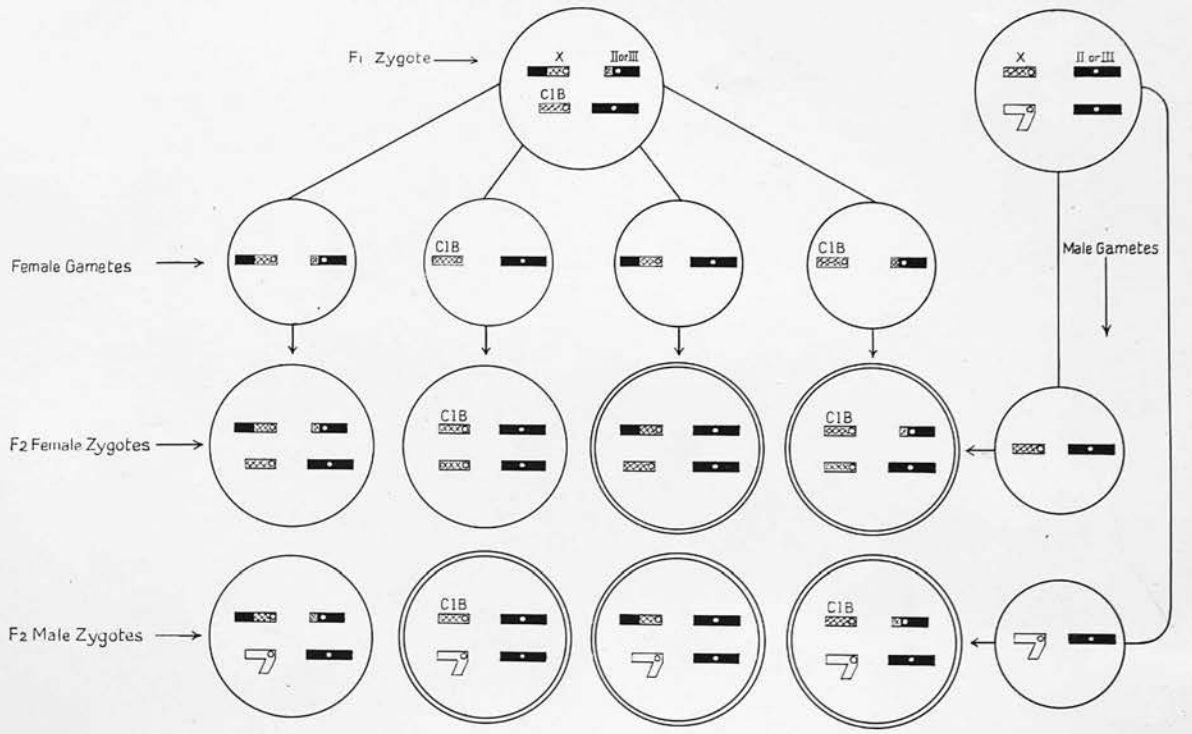
of the same type, wherein the temperatures used were room temperature (19°-20°C.) and a higher temperature (36°-39°C.). The results of his 9 series again show a difference in favour of the lower temperature (in this case room temperature); here the difference is 3.74 times as high as its standard error, and the frequency of mutations in the cooler series is again about 3/2 that in the warmer series. Agreeing with Medvedev's results on gene mutations are those of Papalašvili (1935), on chromosome rearrangements. The latter author studied the combined action of X-rays and low temperature (0°C & "lower") on the frequency of translocations, as compared with the effect of X-rays at room temperature . His results indicate a tendency for an increased frequency of translocations under the combined influence of X-rays and low temperature, but the numbers obtained are rather small and the experimental technique is not given in detail.

THE PROBLEM: Temperature is the most important and easily adjustable factor which might, in whatever manner, be supposed to alter the effectiveness of X-rays. Moreover, the results just referred to are, from the physical standpoint, quite unexpected and would, if confirmed, have very important bearings on the mechanism of mutations. In view of the

conflicting results of previous workers, it was therefore considered desirable to plan a large-scale experiment with a view to finding out definitely the effect of temperature combined with X-rays, both on the frequency of gene mutations and of demonstrable chromosome rearrangements (translocations being used as the most convenient). As the mutations most readily and objectively detected in Drosophila melanogaster are sex-linked lethals (Muller & Altenburg, 1919) and as most of the translocations readily detected involve chromosomes II and III (Altenburg & Muller, 1930), our observations were restricted to detecting sex-linked lethals, and translocations between the chromosomes X, II, & III.

DIAGRAM I.

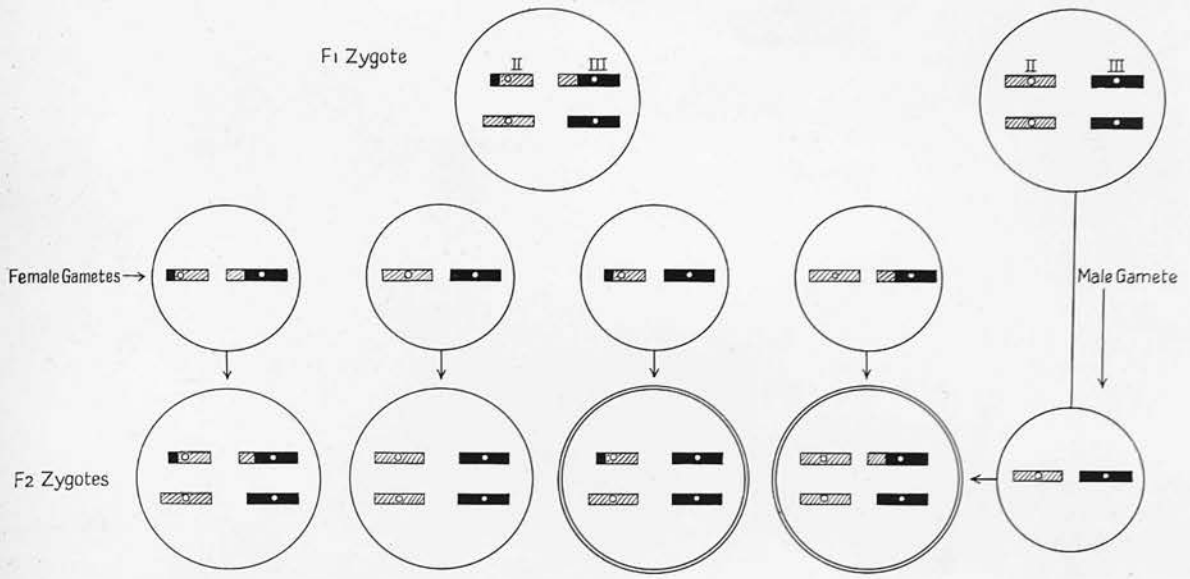
Scheme for detecting translocation between X Chromosome and one of the Autosomes.



1. Translocated chromosomes are indicated by mosaic pattern. The dominant marker Curly or Dichaete is in one of the portions of the translocated chromosome marked in black.
2. Double lined circles indicate inviable individuals.

DIAGRAM II

Scheme for detecting translocation between two Autosomes.



1. Chromosomes not uniformly shown represent those having translocations.
2. The dominant marker Curly is present in some portion of the translocated chromosomes marked by cross hatching, and the dominant marker Dichaete is present in some portion of the translocated chromosomes marked in black.
3. Double lined circles indicate individuals which are not viable.

Material and Method of Experiment.

MATERIAL. The material for the experiment consisted of $\frac{Cy}{}$ $\frac{D}{}$ males and $\frac{ClB}{scar}$ females. The Cy D males have on their II and III chromosomes the dominant mutant genes Curly (Cy) and Dichaete (D), respectively, which served as markers for detecting translocations. Associated with these marker genes, both chromosomes II and III carry inversions that greatly reduce the number of crossover offspring formed. The homologue of the second chromosome marked by Cy carries a group of recessive mutant genes familiarly called 'apl', while the homologue of the third chromosome marked by D carries a recessive group of mutant genes familiarly called 'rucuca'. The complete genetic constitution of the male may thus be represented as:

$$\frac{+}{\quad} \qquad \frac{Cy}{\text{'apl'}} \qquad \frac{D}{\text{'rucuca'}} \qquad \frac{+}{+}$$

The ClB females have on one of their X chromosomes the inversion 'C', which greatly reduces the production of crossovers throughout the X, accompanied by a recessive lethal gene (l) and the dominant Bar eye (B); the homologous X chromosome carries in it the recessive genes scute, vermilion,

forked, and carnation, abbreviated as 'scar'. The female may therefore be shown as having the following genetic constitution,

$$\frac{ClB}{scar} \qquad \frac{+}{+} \qquad \frac{+}{+} \qquad \frac{+}{+}$$

Cy D males of the same stock and of nearly the same age were collected from a large number of fresh bottle cultures and X-rayed in two different lots. The division into these two lots was effected by first mixing together the flies collected from different bottles and then dividing them at random into two parts. X-raying of both the lots was carried out simultaneously, one lot being irradiated at a low temperature which varied from 4° to 6°C (to be called the "cold series"), and the other lot at a high temperature which varied from 36° to 38°C (to be called the "warm series").

The technique of X-raying at the above temperatures may be described as follows. The males to be X-rayed were moderately anesthetised and then placed in gelatin capsules provided with needle holes for ventilation, 200 males in a capsule. Small bits of cotton wool were inserted inside all the capsules so as to crowd the flies together into a space at one end of the capsules. This space

remained approximately constant since the number of males in each capsule and the size of the capsule was the same in every case. This crowding was done for two reasons: firstly, to give the flies of the warm series a living space equal to that occupied by the immobile flies of the cold series, and secondly, to eliminate as far as possible any systematic variations of target distance and of distance from secondary sources of radiation caused by the greater mobility of the flies at the higher temperature. The capsules thus prepared, which were equal in number for the two groups, were placed symmetrically at the bottoms of two special glass vials, one for each group. These vials had first been carefully lined on their inner faces, both at the sides and bottom, with cotton wool. The idea of this lining was to prevent moisture from being deposited on the walls of the vials, and to increase the distance of the flies from the sources of secondary radiation. The capsules were so placed at the bottoms of the vials that they could stand in an upright position, with their cotton-containing ends upwards.

The top of each vial was then closed completely with a cotton plug, through which was passed before and after but not during radiation a centigrade thermometer whose base lay between and touched the capsules containing the flies. The vials so arranged

were then placed in two thermoflasks of the same kind and equal dimensions, one containing iced water (with crushed ice in it) and the other warm water at the required temperature. The tops of the vials were fitted with rubber rings so that when the vials were placed inside the thermoflasks they were held with their mouths up at the mouths of the thermoflasks and at the same time had their remainder completely immersed in water. The mouths of the thermoflasks were covered with a sufficient amount of cotton so as to keep the temperature inside constant.

The two thermometers fixed in the vials read the temperature of the zones at the bottom of vials where the flies were deposited. The temperature of the water in the thermoflasks was first recorded before fitting in the vials. By experience it was found necessary to maintain the temperature of the water, to start with, about 2° higher in the warm series, and about 4° lower in the cold series, than the required temperature at which the flies themselves were to be irradiated. It took approximately half an hour before the thermometers read temperatures which remained nearly constant for another twenty minutes to half an hour. During this latter period the temperature was observed

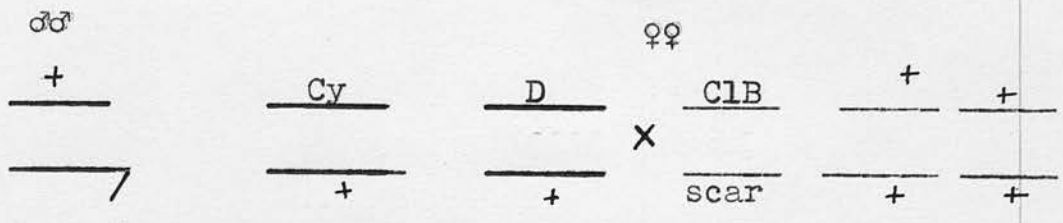
every five minutes. At the end of this period, that is, about one hour after the two vials had been immersed in warm and cold water, respectively, the thermometers were removed and the two thermoflasks placed under the X-ray machine.

The X-raying was carried out with the following dosage factors:- 50 KV peak; 10 M.A.; I mm aluminium filter; 17 cm distance of flies from the target; time of exposure 20 minutes. The dosage amounted to about 2000r, as was later shown by dosimeter readings. The two thermoflasks were held tilted against each other in order to allow the rays a straight passage to the bottom of the vials, and they were interchanged with one another in position after half the irradiation time had elapsed. This was done with a view to eliminating any differences due to the positions in which they rested. On completion of the exposure, the vials were removed from the thermoflasks and the temperature of the water was immediately recorded. These temperature readings always showed that the temperature during the period of X-raying had remained very nearly constant.

The X-rayed males were then removed from the capsules into fresh bottle cultures, those of the 'warm' and 'cold' series being distinctively marked. Virgin ClB females were placed with the

males of both groups in these bottles. In this way the P_1 cross was made up, its constitution being as follows:-

(in the diagrams that follow, heavy lines indicate X-rayed chromosomes).



The flies were left in these bottles for three days, being kept at 23°±.5C during this period. After these three days, the P_1 males were discarded and the P_1 females transferred so as to give rise to a second brood of F_1.

It may be seen that half of the males will not appear in F_1 because of the lethal gene present in one of the maternal X chromosomes, and that the remaining males, those that appear, will all be scar, except as a result of occasional non-disjunction. Furthermore, it may be observed that the B Cy D females, which were required in F_1 for our test both for translocations and lethals, appear in the ratio of only I/12th of the total viable progeny expected. Added to this cause of small numbers of the desired type was the fact that these F_1 females were found to

be less viable, both before and after emergence, than most of the others. Nearly 30 to 40 percent of the B Cy D F_1 females collected died without bearing offspring, because of their low viability. Our task of getting the required number of this type of females in F_1 therefore became a rather difficult one. In all about 100 bottles of each group had to be handled daily for the collection of the virgin F_1 flies, so as to get the required number of B Cy D females.

These B Cy D F_1 virgin females were then mated with scar males in pairs in individual vials, one male to one female, as follows:-

♀♀				♂♂		
+	Cy	D		scar	+	+
			X			
ClB	+	+		/	+	+

For the detection of lethals, all types of F_1 Bar females, viz: Cy non-D, non-Cy D, non-Cy non-D, and Cy D, could be used, whereas for translocations only B Cy D females were useful.

In the examination of the F_2 cultures, derived from the ClB F_1 females, for lethals in the

X chromosome, cases of complete absence of males in a vial (neglecting exceptional 'scar' males, produced by non-disjunction) were taken to indicate a lethal. For one chromosome of the F_1 female (the ClB one) already carried a lethal gene, so that if the other chromosome (that derived from the X-rayed male) also carried a lethal, no male at all could appear. Vials which showed only one or two males, or too few flies in general for a certain determination, were kept as 'semi' or doubtful lethals and carried to the third generation for confirmation.

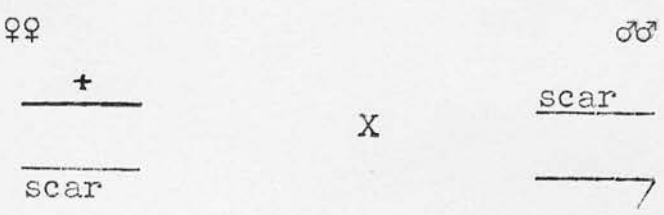
As for translocations, when no lethal and no translocation occur, we get in F_2 four types of males, all scar, but having, respectively, the autosomal characters Cy D, Cy non-D, non-Cy D, and non-Cy non-D. In the same cases there are eight types of females, viz: Bar and non-Bar, each of which may be Cy D, Cy non-D, non-Cy D, and non-Cy non-D. Absence of certain of the above classes indicates the existence of a translocation, since the absent flies have had their development interfered with by the upset in the genic balance caused by their having deficiencies and/or duplications.

It has already been mentioned that the types of translocations studied in this experiment concerned chromosomes X, II and III. If translocation occurs between the X chromosome and the second chromosome, which bears the marker Cy, then (considering

only these chromosomes)we should ordinarily find that all males which appear are Cy. Non-Cy males will ordinarily die before emergence, due to their deficiency and/ or duplication. As for the females, the B Cy and non-B non-Cy classes should be absent because of the similar genic disturbance; this would leave only B non-Cy, and non-Bar Cy females present. Similarly, when translocation takes place between the X and the third chromosome, the latter bearing the "marker" gene Dichaete, all emerging males are D, and among the females there is complete absence of B D and non-B non-D and presence only of B non-D, and non-B D. When there is translocation between the second and third chromosomes-the case which is the most frequent of all - then, among both males and females, there should be no individuals carrying only one of the marker genes, i.e., individuals of type Cy non-D, and non-Cy D, should be completely absent. In other words, all the individuals present (whether male or female) should be either Cy D, or non-Cy non-D. The structural changes of the translocated chromosomes are further depicted in diagrams I and II.

Both lethals and translocations were carried to the third generation for confirmation. In the case of suspected lethals, F_2 non-Bar females

were back -crossed to scar males in mass cultures, as follows:-



The lethals here were confirmed by the complete absence of normal males in F_3, as these cannot appear if the X-rayed + X chromosome bears a lethal gene. Lethals which gave intermediate results in the confirmatory tests, showing only a few wild type males in F_3, were classed as semi-lethals.

No case of a doubtful translocation was observed, however, although there were some cases rejected, especially those that had appeared to involve the X chromosome. Translocations were confirmed by backcrossing the F_2 males carrying the supposedly translocated chromosomes with normal females. Confirmations of a translocation between the X and any one of the autosomes show in F_3 all females Cy or all D, according to which chromosome is translocated, and all males, complementarily, non-Cy or non-D. Translocations between chromosomes II and III give the same results in F_3 as in F_2.

TABLE I

Frequency of sex-linked lethals in relation to temperature during irradiation (ca.2000r)

(Identical series numbers in tables I and II indicate flies irradiated simultaneously with same dose).

Series No.	Cold Series (4°C - 6°C)				Warm Series (36°C - 38°C)				$(p_1 - p_2) \pm$ E diff. (in %)	$\frac{d}{E_d}$	Geometric mean number of cultures $\sqrt{n_1 x\ n_2}$ (n_h)	$n_h p_1$ / P_{1h}	$n_h p_2$ / P_{2h}	$(P_1+P_2)q$
	F_1- F_2 Cultures examined (n_1)	Complete Lethals (P_1)	Semi-Lethals	Percent of complete Lethals (P_1)	F_1 - F_2 Cultures examined (n_2)	Complete Lethals (P_2)	Semi-Lethals	Percent of complete Lethals (P_2)	(d)					
I	1018	63	9	6.19%	748	44	6	5.88%	+0.31±1.14	+0.27	872.61	54.00	51.33	96.0
II	848	40	4	4.83%	678	38	3	5.60%	-0.70±1.15	-0.61	758.25	36.65	42.49	58.0
III	592	31	2	5.23%	610	48	3	7.86%	-2.63±1.42	-1.18	600.93	31.46	47.28	58.0
IV	174	7	1	4.02%	152	4	-	2.63%	+1.39±1.98	+0.70	162.62	6.541	4.28	10.6
V	764	54	4	7.06%	640	44	4	6.87%	+0.19±1.38	+0.14	699.25	49.42	48.07	91.0
Total	3396	195	20		2828	178	16				3093.66	178.07	193.45	313.6

Weighted Mean Results

$\frac{\Sigma P_{1h}}{\Sigma n_h}$ Weighted Mean of cold series (in %) p_{1a}	$\frac{\Sigma P_{2h}}{\Sigma n_h}$ Weighted Mean of warm series (in %) p_{2a}	$p_{1a} - p_{2a}$ (in %) d_a	$\sqrt{\frac{\Sigma(P_1+P_2)q}{\Sigma n_h}}$ E_{da}	$\frac{d_a}{E_{da}}$
5.76 ± 0.42	6.25 ± 0.43	- 0.50	0.60	0.82

TABLE II

Translocation frequency in relation to temperature during irradiation (ca. 2000r)

(Identical series numbers in tables I and II indicate flies irradiated simultaneously with same dose.)

Series No.	COLD SERIES (4°c - 6°c)						WARM SERIES (36° c - 38°c)						$p_1 - p_2 \pm E_d$ (in %) (d)	$\frac{d}{E_d}$	Geometric mean number of cultures $\sqrt{n_1 \times n_2}$ (n_h)	$p_1 n_h$ (P_{1h})	$p_2 n_h$ (P_{2h})	$(P_1+P_2)q$
	F_1-F_2 Cultures Examined (n_1)	Types of translocations I & II	I & III	II & III	Total No. of translocations (P_1)	Percent of total translocations (p_1)	F_1 -F_2 Cultures Examined (n_2)	Types of translocations I & II	I & III	II & III	Total No. of translocations (P_2)	Percent of total translocations (p_2)						
I	384	1	3	11	15	3.91%	398	-	2	12	14	3.517	+0.38 ± 1.35	0.28	390.93	15.27	13.75	28
II	848	2	2	24	28	3.30%	678	1	2	19	22	3.244	+ 0.05 ± 0.91	0.06	758.24	25.03	24.60	48.4
III	592	-	2	17	19	3.21%	610	-	-	13	13	2.131	+ 1.07 ± 0.93	1.15	600.93	19.28	12.81	31
IV	174	-	-	1	1	0.57%	152	-	-	1	1	0.657	-0.83 ± 0.87	0.95	162.63	.9335	1.07	2
Total	1998	3	7	53	63		1838	1	4	45	50				1913	60.51	52.23	109.4

WEIGHTED MEAN RESULTS.

$\frac{\Sigma P_{1h}}{\Sigma n_h}$ Weighted mean of cold series (in %) p_{1a}	$\frac{\Sigma P_{2h}}{\Sigma n_h}$ Weighted mean of warm series (in %) p_{2a}	$p_{1a} - p_{2a}$ (in %) d_a	$\frac{\Sigma(P_1+P_2)q}{\Sigma n_h}$ (in %) E_{da}	$\frac{d_a}{E_{da}}$
3.16 ± 0.40	2.73 ± 0.37	0.43	0.55	.79

RESULTS.

The results of the experiment are summarised in tables I and II. Table I relates to sex-linked lethal mutations while table II deals with translocations between chromosomes X, II, and III. Four of the series were tested for translocations while all of these four, and a fifth series in addition, were tested for lethals. The negativity of the results was so clear at the end of the 4th series of the experiment that it was not considered worthwhile to continue the work further. But at this stage there came to hand the second published paper of Medvedev (1938), on "the contributory effect of heat with irradiation on the frequency of mutations". In view of these results, which were, in essence, a confirmation of his previous (1935) work, and contrary to our own findings, it was decided to carry out another series, this time restricting our observations to lethal mutations only. The results of this fifth series on mutations confirm, as may be seen, the results of our previous four series.

Considering now the data obtained for lethal mutations, it may be observed in table I that series I, IV and V show some increase in the percentage

of mutations of the 'cold' lot as compared to the 'warm' lot. It may also be noted that the increase in series I and V is quite negligible, whereas in series IV, where the increase is as much as 1.39 percent, the number of F_2 vials examined in the cold series is only 174, the standard error being 1.98. Series II and III show a slight decrease in the percentage of mutations in the cold lot as compared to that in the warm lot, but, as is evident, the differences here too are negligible.

The combined results of the five series show that, in all, 3396 chromosomes were examined in the cold lots, which gave 195 complete lethals, and 2828 chromosomes in the warm lots, which gave 178 complete lethals. The difference in frequency shown by these totals is not significant, if calculated in the ordinary way. This is not a correct method, however, in view of the fact that the different series, which probably received somewhat different dosages of radiation, were not represented in precisely the same relative numbers in the totals of the two groups compared (warm and cold). Thus the total for the warm group might, for example, include a higher proportion of cultures derived from a particularly heavy dose than that for the cold group.

For this reason it is more accurate, in comparing the whole set of results of the two groups (warm and cold) with one another, first to obtain weighted averages of the frequencies obtained in the different series of each of the two groups, using the same weights for the frequencies of the two lots (warm and cold) of any given series. The weight used for any given series should of course correspond to the reliability of that series as a whole, for the comparison of the frequencies, p_1 and p_2, of its two lots with one another. The weight to be used turns out to be the so-called "harmonic" or "geometric" mean, n_h, of the numbers of cultures, n_1 and n_2, respectively, in the two lots of each series; $n_h = \sqrt{n_1 n_2}$. When this system of weighting is followed, the difference between the weighted average frequency of all the warm lots taken together and that of all the cold lots taken together has a minimal standard error, relative to its own value. The formula for this error is, as shown below:

$$\sqrt{\frac{\Sigma (P_1 + P_2)q}{n_h}},$$ where P_1 and P_2 are the observed absolute frequencies of individuals characterised by the events in question - here, mutations or translocations - in the warm and cold

lots, respectively, of any given series, and q is the observed proportion of individuals in that series, as a whole, not characterised by the "event" in question. This is a method which (although not laying claim to novelty) has been worked out for the author by Muller, who has furnished the mathematical proof of it given in the paragraph below.

The standard error, E_d, of the difference, d, between the frequencies, p_1 and p_2, respectively, found in the two lots of a given series, assuming the same true value, is, according to the usual formula $\sqrt{\frac{pq}{n_1} + \frac{pq}{n_2}}$. Substituting $\frac{p_1 n_1 + p_2 n_2}{n_1 + n_2}$ for p, and expanding, we have $E_d = \sqrt{\frac{(p_1 n_1 + p_2 n_2)q}{n_1 n_2}} = \sqrt{\frac{(P_1 + P_2)q}{n_h^2}}$. If now we use the respective values of n_h as weights for the frequencies, and frequency-differences, of each series, and thus obtain the weighted average frequencies, p_{1a} and p_{2a}, and their difference, d_a, we have for $p_{1a} - p_{2a} = d_a$ the following:

$$\frac{\Sigma(n_h p_1)}{\Sigma n_h} - \frac{\Sigma(n_h p_2)}{\Sigma n_h} = \frac{\Sigma(n_h d)}{\Sigma n_h} .$$ Now E_{da}, the

standard error of d_a, must be related to E_d as follows: $E_{da} = \frac{\sqrt{\Sigma(n_h^2 E_d^2)}}{\Sigma n_h}$. Substituting in this formula the above value for E_d we have $E_{da} = \frac{\sqrt{\Sigma(P_1 + P_2)q}}{\Sigma n_h}$.

It may be added that a sufficiently close approximation to this value of E_{da} may, in most cases like this, be obtained by the expression $\frac{\sqrt{\left[\Sigma(P_{1h} + P_{2h})\right] q_a}}{\Sigma n_h}$.

Here P_{1h} and P_{2h} represent the artificial "absolute" numbers of events, on the basis of n_h as an artificial whole number of cultures, i.e., $P_{1h} = p_1 n_h$, and $q_a = \left(1 - \frac{p_{1a}\, p_{2a}}{2}\right)$. Thus the values n_h, P_{1h} and P_{2h} may, when desired, be treated as though they represented obtained numbers, in the calculation of the errors of the two weighted averages and of the difference between them.

Making these calculations, it is found that the weighted average percentage of lethal mutations in cold lots was 5.76 ($\pm$ .42) and in the warm lots 6.25 ($\pm$ .43). The difference between them, and its error, then turns out to be 0.50 $\pm$ 0.6. Thus it is evident that the difference between the two groups, taken as a whole,

is far from significant.

Turning now to table 2, relating to translocations, and considering all types of translocations put together, we find that series I, II and III show a slight increase of the percentage of translocations in the cold lot as compared to the warm lot. In series IV, however, where only 174 chromosomes were examined in the cold lot, the latter shows a decrease of 0.83 percent, which again is negligible.

The combined results of the four series show that in all 1998 chromosomes were examined for translocations in the cold lots, which gave a total of 63 translocations. In the warm series, 1838 chromosomes were examined, and a total of 50 translocations was obtained. When averages weighted as before are calculated, it is found in this case that the weighted average percentage of translocations in the cold lots is $3.16 \pm .4$ and in the warm lots $2.73 \pm .37$. The difference between the two groups is $0.43 \pm .55$, which is without significance.

In other words, we see that, as in the case of lethal mutations, so too in the case of translocations, temperature can have little if any effect during irradiation, and certainly no such effect as has been claimed.

Another fact worth noting is that out of a total of 63 translocations of all types in the cold lots, only 10 were between the X and an autosome, that is, ca. 15 percent of the translocations, while 53 were between the two autosomes studied, that is, ca. 85 percent of the translocations. In the warm lots, of the total number of translocations (50), only 5, that is, 10 percent, were between the X chromosome and autosomes, while 45, that is, 90 percent, were between the two autosomes. The difference between cold and warm lots in this respect is not significant. The combined results of cold and warm lots show 113 total translocations, of which 15 involve the X chromosome, that is, 13 percent, and 98 are autosomal, that is, ca. 87 percent. These results may be of interest in the light of the data obtained by Altenburg and Muller (1930), and by Patterson, Stone, Suche and Bedechek (1934), on the comparative frequencies of different translocations affecting different chromosomes. All these authors found a somewhat higher proportion of translocations affecting the X chromosome (ca. 30%). The difference is probably due to our having missed some of the translocations involving the X, owing to the occurrence

of non-disjunction, which is favoured in our experiments by the presence of ClB. Cases of translocations of X were probably also missed when they were accompanied by sex-linked lethal effects, being recorded only as cases of the latter. None of these objections apply, however, to our determinations of translocations between II and III, which, forming the bulk of our data, afford in themselves a sufficient basis for our conclusions.

The frequency of translocations may now be compared with that of lethal mutations. Considering series I, II, III and IV, both cold and warm lots combined, the weighted average percentage of translocations involving chromosomes II and III works out at 2.70 ±0.26. As against this, the weighted average percentage of complete lethal mutations for series I, II, III and IV is 5.65 ± .26. (The same system of weighting is used here as when cold and warm were compared). The relation between the frequency of major autosomal translocations and that of sex-linked lethal mutations, under the present dose of X-rays, may therefore be expressed as ca. 1.: 2. A higher ratio than this would be found at lower doses, and a lower one at higher doses than this, owing to the fact that while the lethal mutation frequency varies nearly in proportion to the dosage, the translocation

frequency varies exponentially - a matter to be discussed in our paper on the translocation frequency in relation to the timing of irradiation.

DISCUSSION AND CONCLUSION

From the results given above, it is clear that a temperature difference between 4°C and 36°C, existing during irradiation, does not significantly affect the rate of production either of lethal mutations or of translocations. Our results therefore support those of Timofeeff-Ressovsky (1934) on lethal mutations in Drosophila, of Stadler (1928, 1930) on gene mutations in barley and of Karl Sax (1938) on chromosome rearrangements in Tradescantia microspores, and disagree with those of Papalašvili (1935) on translocations and of Medvedev (1935, 1938) on lethal mutations in Drosophila.

In order to judge of the bearing which our results may have on the general problem of the mechanism of mutation, it is desirable first to consider in what ways temperature, applied with X-rays, might conceivably have affected the processes whereby gene mutations and translocations are produced. This will involve also a consideration of the way in which the X-rays themselves act to produce these changes.

The fact that the gene mutation rate is in

linear proportion to the X-ray dose proves that a given gene mutation is caused by a single ionisation process. For any definite mutational step, however, there seem to be many alternative ionisations which can initiate the process, since calculation shows any given kind of mutation (as judged by the production of an apparently identical mutant gene) to occur with hundreds of times the frequency that the ionisation of any one particular atom can occur (calculations of Muller & Mott-Smith, 1930; Blackwood, 1932 - see also Muller 1932, 1938; Timofeeff- Ressovsky, Zimmer & Delbrück, 1935). Hence an ionisation, in order to produce a mutational effect, must be followed by some further chemical process or processes, involving a change in the configuration of some otherwise stably arranged atoms. These intermediate processes between the initial ionisation and the mutation itself might conceivably be of various kinds. At any rate, for what seems to be the same mutational step, there must, on this view, be many alternative chemical processes, depending on which one of hundreds of atoms had become ionised - all leading to the same final mutation.

Spontaneous mutations also are to be pictured as initiated by a chemical process or processes

similar to ionisation, i.e. activation of an atom. The energy required for this activation is thought to be supplied by the fluctuation of thermal energy existing in the material in nature at the given temperature. (Muller & Altenburg, 1919; Muller, 1920, 1928; Timofeeff-Ressovsky, Zimmer & Delbrück, 1935). The given activation would then be followed, as in the case of X-rays, by a secondary chemical change or series of changes, leading to the mutation itself.

From what has been said above, one may see that two possibly separable factors concerned in the production of mutations are the process of initial ionisation or activation and the subsequent chemical change leading to the mutation. It is certain that temperature cannot influence, except to a minute degree, the initial process of ionisation by X-radiation. It might however influence the secondary chemical process or processes. If it altered not merely their speed but the probability that they would complete themselves (i.e., the fraction of cases in which the initiating process was turned into a mutation by the subsequent processes), then, and then only, would temperature be able to affect the frequency of mutations produced by radiation.

Lack of such an effect would then mean that the completion of the secondary processes was not affected by temperature.

That the secondary processes are probably little affected in this sense is indicated by the fact that natural mutations show only such dependence upon temperature as would be calculated from the supposed action of the latter upon the primary process (activation of atoms). Hence we should expect little or no detectable effect of temperature on the production of mutations by radiation. Our results are in complete agreement with this expectation.

As it might be imagined that the secondary processes referred to were related to physiological processes occurring in the cell, it may be of interest to mention some investigations dealing with the way in which the response of the latter to radiation may be affected by temperature. In studying non-genetic, physiological effects of temperature during X-raying on the eggs of Drosophila, Packard (1930) has reported that the sensitivity of the cell or embryo to being killed by radiation increases with temperature. Since the division rate of the cells also increases with the increase of temperature and since more rapidly dividing cells are, in general,

more sensitive to radiation, we might attempt in this way to explain the first effect by the second. But this seems very doubtful, for the divisions take a shorter time at higher temperatures. Holthusen (1921) and Dognon (1926) discuss the fact that the sensitivity of a cell to such physiological effects of radiation must depend on two factors. One is, as in the production of mutations, the photochemical effect (ionisation), which is independent of temperature, and the other is concerned with secondary processes, which are affected by the activity of the cell and by temperature. Holthusen thinks that the latter processes are associated with the condition of the nucleus at the time of division, while Dognon holds that they are related to cell metabolism. In the case of mutations, however, it may be noted that Hanson (1935) found no influence of metabolism (varied through starvation or anesthesia) on the induction of mutations by X-rays in Drosophila.

Scott (1937), in a study of the influence of temperature on injuries produced by X-rays, concludes that the radio-sensitivity of the tissues involved is independent of the rate of division and of the metabolic rate, and is very little dependent on temperature. He finds that the average Q10 for all

the experiments in his review is only 1.1. Similarly, Redfield and Bright (1919), while exposing unfertilised eggs of the marine worm Nereis to beta rays of radium at 0° and 24°C, found the temperature coefficient only 1.14 for a rise of 10°C. Since the eggs in the above experiment were unfertilised, the factor of metabolic activity in this case was nearly completely eliminated.

We may, finally, consider what factors might give rise to results which appeared to bespeak an effect of temperature on the process of mutation, when in reality there was no such effect. A departure from the expected results might conceivably occur in some experiments as a result of the presence of secondary radiation of different amounts in the two groups under comparison, namely, the cold and warm series. In an experiment of this nature, therefore, the actual technique of X-raying the flies of the two groups so as to eliminate all possible differences due to secondary radiation of any kind is a matter of utmost importance. Unfortunately, the works published by Papalašvili and Medvedev, whose results are contrary to ours, do not give full details of the actual technique followed by them while X-raying. In an attempt to maintain the different temperature

ranges in the two groups, small differences in the conditions of X-raying are likely to exist. Among these we may note: different material, size and position of containers; different living space for flies in the capsules, thereby causing differences in target distance or in distance from source of secondary radiation; presence in one and absence in the other group of water or other substances surrounding the containers, etc. All these different conditions are likely to give rise to different kinds of secondary radiations and to different intensities of these radiations. These are bound to produce unexpected results. In the technique followed by us and described above, we have tried, as far as possible, to overcome all these difficulties so as to maintain sensibly identical conditions for the two groups while X-raying, keeping only the temperature as variant. In so doing, care was taken to meet most of the points, raised by Muller in 1930 with regard to the interfering effects of secondary radiations, which have already been referred to in this paper.

CONCLUSION. Considering all the points mentioned in the foregoing paragraphs and also considering the number of chromosomes examined in this experiment

and its clearcut results, we conclude, in spite of the contrary results by some workers, that the lack of any marked action of temperature on the frequency of X-ray induced gene mutations and translocations has now been sufficiently demonstrated.

SUMMARY

A study has been made of the effect of temperature during irradiation on the frequency of X-ray induced lethal mutations, and of translocations between chromosomes X, II and III. The flies were X-rayed simultaneously and under otherwise identical conditions, at the temperatures 4° to 6°C and 36° to 38°C, respectively. No significant difference was obtained in the two groups. This is regarded as proving that the temperature coefficient for lethal mutations and translocations during X-raying is very nearly 1. Hence the completion of the secondary chemical processes, leading from the initial ionisations to the observed mutations, must be very little affected by temperature.

ACKNOWLEDGMENT

The author is greatly indebted to Prof. F. A. E. Crew, F.R.S. for his academic hospitality and encouragement. To Prof. H. J. Muller the author wishes to express his deep gratitude for suggesting the problem and for guidance in the course of this work.

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(II)

TRANSLOCATION FREQUENCY IN RELATION TO TIMING OF IRRADIATION.

(With 5 TABLES).

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CONTENTS.

I N T R O D U C T I O N.

Among the problems that have arisen in the course of the numerous investigations on the artificial production of gene mutations and chromosome abnormalities is that of the relative genetic effectiveness of high-energy radiation when applied under different conditions of timing and of intensity.

In this connection, a number of experiments have been performed in Drosophila melanogaster to test the effects of fractionated and diluted dosages of radiation on the induced gene mutation rate. Equal dosages of varying intensities have been tried, both in the case of radium and of X-rays, without any differences in the frequency of lethal mutations associated with either intensity or wave length being found (Hanson & Heys, 1932; Hanson, 1933; Timofeeff-Ressovsky, 1934 et seq, on intensity; Serebrovsky & Dubinin, 1930; Schechtman, 1930; Hanson, Heys & Stanton, 1931; Efroimson, 1931; Gowen & Gay, 1933; Fricke & Demerec, 1937, etc. on wave length). In the case of X-rays, the lowest intensity used was 1r per minute and the highest about 300r; in the case

of radium (see below) the range was slightly less, and included within the former range.

Hanson & Heys (1932) studied the effects on the gene mutation rate of equal doses of radium radiation applied in different concentrations to Drosophila males. In one experiment "6315 r" were applied, spread out over 2.5, 37.5 and 75 hours, respectively, and in the other experiment "12630 r" spread out over 75 and 150 hours, respectively. In these experiments likewise, no significant differences were obtained between the results, although the results they report seem anomolous when compared with those of other experiments, if the frequency of sex-linked lethal mutations per total of r units applied is considered.

Patterson (1931) was the first who studied the effect of continuous versus interrupted irradiation administered in fractions at certain specified time-intervals, on the gene mutation rate of Drosophila melanogaster. He found no significant difference in the gene mutation rate, when the same total dose was administered to males in the following time distributions: in experiment A, (1) single continuous treatment of 14 minutes, (2) 16 one-minute treatments every 12 hours, (3) 32 half-minute treatments every 6 hours; in the experiment B,(1)

single continuous 8-minute treatment, (2) 16 half-minute treatments every 12 hours; in experiment C, (1) single continuous 10-minute treatment, (2) 8 one-and-one-quarter minute treatments at intervals of 24 hours, 12 hours, 8 hours, 1 hour, and half hour, respectively.

Timofeeff-Ressovsky (1934) performed similar experiments with X-rays and found that the gene mutation rate induced by irradiation was not affected either by intensity or by interruption of exposure provided there was the same total amount of ionisation. In his first experiments 3,600 r units were given to mature Drosophila males; (1) in a single 15-minute treatment (of 240 r per minute), (2) in a single 6-hour treatment (of 10 r per minute) and (3) in 6 five-minute treatments at 24 hour intervals (120 r per minute during treatment). In later experiments Timofeeff-Ressovsky and Zimmer (1935) exposed Drosophila males to an X-ray dose of 3000 r. In the first series, the exposure was 10 minutes, that is, at the rate of 300 r per minute; in the second series it was given in 10 five-hour periods on successive days, that is, at 1 r per minute during treatment. The percentage of lethal

mutations was again sensibly the same, showing that the intensity factor, even over such a wide range, has no effect on the rate of lethal mutations.

To the experimental data we have so far reviewed on the effect of the intensity factor, there may now be added the further evidence derived from the results of a very extensive experiment carried out with radium by our colleague S.P. Raychaudhuri, who has been good enough to permit us to refer to his unpublished data. In this investigation, which was carried out simultaneously with our own, an intensity of gamma radiation as low as 1/100 r per minute was used, which was applied continuously to mature Drosophila spermatozoa (contained in the receptacles of females) over a period of one month. In addition, another series of 1/20 r per minute was run in parallel with it, and also untreated controls. The results of this work show clearly that even an intensity of 1/100 r per minute is quite as effective, in the production of both gene mutations and chromosome rearrangements, as 1/20 r or more per minute, so long as the total ionisation remains the same. It was further found that with a change in the total ionisation from 2000 r to 400 r, there

is a directly proportionate change in the total frequency of the gene mutations produced, in the case of these low intensities extended over a month, just as had been found for the far higher intensities previously used. The frequency-dosage relation found for the rearrangements will be discussed later, in more direct connection with our own results.

Another point of interest on which work has been done is the effect of aging treated sperms on the mutation rate. Muller (1927) found that aging the sperm after treatment, before fertilisation, causes no noticeable alteration in the frequency of detectable gene mutations. Harris (1929) has observed that the percentages of gene mutations are sensibly identical when the treated mature spermatozoa are aged and compared in three groups, namely, zero to 4 days, 7 to 8 days, and 16 to 20 days old, respectively. Hanson & Heys (1929) likewise have concluded that when irradiated sperms are aged in the male and no copulation allowed until the time of testing, no decrease in the mutation rate occurs.

Of equal importance, for the consideration of the meaning of our own results, is the work done on the question of a possible delayed or after-effect

of X-rays on the production of gene mutations and chromosome breakages, for here also the time factor had seemed to play its part, though in a different way. Woskressensky early in 1928 had reported at the Russian Genetics Congress, held at Leningrad, the possibility of an after-effect of X-ray treatment, of a type which he termed "enduring modifications". Results of Muller (1927, 1928) and of Timofeeff-Ressovsky (1930) on gene mutations, and of Patterson (1935) on breakages of chromosomes, however, completely contradict the idea of any prolonged after-effect of X-rays. This question had in itself a considerable theoretical significance, for it was considered that if the action of X-rays were delayed, it could possibly have explained the present rate of natural mutations, as being the result of a slowly cumulative effect of radiation from generation to generation, since, according to Muller and Mott-Smith (1930), the natural mutation frequency is at least 1300 times as high as it would be if it were an immediate effect of natural radiation.

It might seem from the foregoing review that a sufficient amount of work had already been done on the effect of the time and intensity factors

in the artificial production of gene mutations. Except for the parallel work of Raychaudhuri with radium, however, there was no work done along these lines to test the effect of these radiation factors on chromosome rearrangements, such as translocations. A study of this problem might be expected to throw some light on the question of the mechanism whereby these are produced. On basis of the "breakage-first" conception (see page 74), it might give evidence concerning the time when the fusions of the broken ends of the chromosomes occur.

It was therefore considered of interest to initiate an experiment with a view to finding out whether or to what extent a division of the irradiation into fractions separated by long intervals, or a protracted aging of the germ cells after treatment, would affect the frequency of gross rearrangements (translocations). While the main purpose of our investigation was to test the effect on rearrangements only, a series for recording sex-linked lethal mutations was run in parallel, chiefly as a check to get a correct measure of the X-ray dose, but also to obtain what further evidence might accrue concerning the effect of these time factors upon the frequency of the gene mutations themselves.

MATERIAL AND METHODS.

Three series of the experiment were carried out. In each of the three, both lethals and translocations were looked for, but different stocks of flies were used for the two tests. In series I and III, for the translocation tests, wild-type males of a stock from Samarkand were mated with females homozygous for the recessive genes 'brown' eye colour (bw), 'ebony' body colour (e) and 'eyeless', eyes small or absent (ey), borne on chromosomes II, III and IV, respectively. In the second series only, females homozygous for the recessive gene dp (dumpy wings) in chromoseme II, and for ebony (e), were used instead of the bw e ey. The reason this kind of female was used in this series was because it was considered that the replacement of the character bw by the more conspicuous character dp would facilitate detection of the marker (and so of any translocation involving it) without the removal of the flies from the F_2 cultures. But although this purpose was served, the dp e females proved to be much less fertile when outbred, so that the obtaining of the necessary number of F_2 individuals became too uncertain and laborious.

We had therefore to revert to the bw e ey stock for females for our third series.

In the description that follows the character bw, wherever it occurs, should be read as "bw or dp".

The wild type males of Samarkand stock and the homozygous bw e ey females were placed in half-pint bottles with yeast food (Offermann's formula), about 100 males to 100 females, for impregnation of the females. The bottles were kept in the constant temperature room at 23° ± .5°C, for 3-4 days. At the end of this period the males were discarded and the impregnated females X-rayed.

For irradiation, the impregnated females from a mixed lot obtained from the different stock bottles were divided at random but in specified numbers into the different groups. These were put in separate gelatin capsules and given X-radiation treatments characterised by the following factors: 50 KV. peak; 10 m.amp.; ½ mm. aluminium filter; 10 cm. distance; time of exposure approximately 6 minutes in all for the heavier dose and exactly one-quarter of the total time for the lighter dose. Except in the first series a dosimeter was used in order to determine the dosage. Dosage for the

first series was approximately controlled by observation of the effect of the radiation on a photoelectric cell (method of K. Mackenzie) which had previously been calibrated against lethal mutation frequency. The "heavy" treatment was of ca. 1500 r and the "light" treatment, being exactly one-fourth of the former, was therefore of ca. 375 r.

The flies for the heavy treatment were divided into three separate groups, for three different forms of the heavy treatment. The first of these was termed the "immediate" - in which the treatment was nearly continuous (see below), and the flies were bred at once after treatment. The second was termed the "postpened" - wherein the flies were given the same nearly continuous treatment but immediately thereafter put into a special incubator at 8°C and kept there on syrup food for one month (or, in the first series, three weeks), after which they were bred at 23° ± .5°C. The third was termed the "divided" treatment - in which the treatment was given in four parts, with interruptions of a week between the parts, the dosage each time being one-fourth the total high dose. Between these treatments and (except in the first series) for a final week after the last treatment, the flies of the "divided" lot were kept

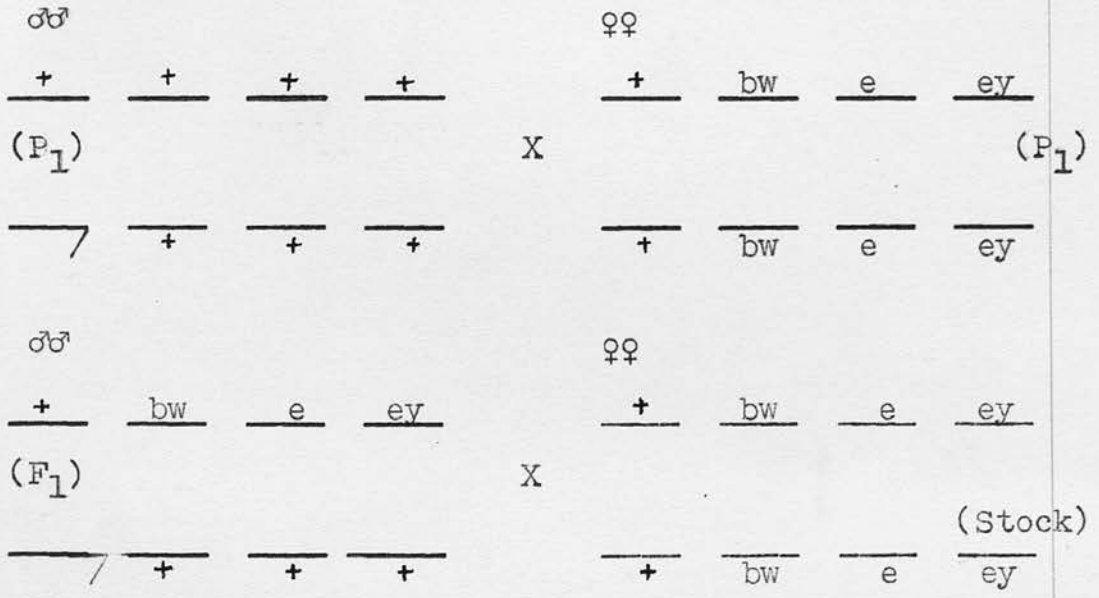
on syrup food at 8°C. Thus they, like the "postponed" lot, were not bred until 30 days after the treatment had begun.

The light treatment was a continuous treatment in which flies were bred immediately after the raying. With a view to eliminating any possible effects due to differences in the efficiency of the X-ray machine at different stages of the total period during which the machine was worked for the heavy treatment, the administration of the light dose was effected, according to the method of Offermann, in four separate and equal periods, each of which coincided with a different one of the fourths of the period during which the "continuous" heavy dose was given. That is, between the starting and the closing of the working of the machine, in giving the heavy dose, it was stopped and restarted three times, at equal intervals, and during the few seconds' intervals, when each quarter of the heavy dose was finished, one lot of light dose flies was removed from the field of irradiation and another lot put in its place.

To get a sufficient number of progeny (F_1) a second "brood" was obtained by transferring the impregnated P_1 females to new culture bottles on about the eighth or ninth day after they had been

put into the first bottles for breeding. The breeding of the flies was always carried out in the constant temperature room (23° ± .5°C).

The P_1 cross, together with the F_1 selected from it, may be represented as follows. (In this and the later diagrams, heavy lines indicate X-rayed chromosomes).



The F_1 males were collected and mated in pairs with the same type of females as the mothers, viz., with bw e ey females of the homozygous stock. The latter females were collected as virgins twice a day from culture bottles especially made up for the purpose.

Only translocations between chromosomes II and III were studied since these are the commonest

types, being found with a frequency of at least 65% of the total number of translocations detectable by the given method (Muller & Altenburg, 1930, as well as unpublished tests of the author, 1939). A quantitative study of translocations involving chromosomes II and III may, therefore, reasonably be expected to be representative of translocations in general.

The principle whereby these translocations were detected, in the examination of the groups of F_2 flies, was the same as that followed by the author in his previous work on the "Ineffectiveness of temperature in influencing the production of mutations by X-rays", the only difference in method being the use of stocks with different marker-genes. As this principle was explained in the paper on the earlier work, a detailed exposition of it will not be necessary here.

Suffice it to say that, where a translocation between II and III has occurred, individuals showing just one of the second or third chromosomal mutant markers would not be found in the F_2 generation. That is, individuals showing bw but not e, and e but not bw, would be completely absent

in such cultures of these translocations, and the individuals present, whether male or female, will consist entirely of bw e and non-bw non-e (i.e. wild type in these respects). These results would be obtained no matter whether the translocation had occurred in the sperm or in the egg.

Cases of the above type obtained in F_2 were carried to the third generation by backcrossing the wild type (heterozygous) F_2 males with bw e ey virgin females. These males necessarily carried the translocated chromosomes in case the translocation had occurred in a spermatozoon(rather than in an egg) of P_1. Hence, in that case, the results of F_2 will repeat themselves in F_3, and it can be inferred that a translocation had occurred in a P_1 male germ cell. If, however, the results of F_2 were not repeated in F_3, re-combinations of bw and e being found, the conclusion could be drawn either that the translocation had occurred in the P_1 female germ cell or that no translocation had occurred at all (the F_2 result in that case having been too small to be representative).

For a parallel determination of the frequency of sex-linked lethals, the P_1 flies used were males, having on their X-chromosomes the recessive genes yellow, scute 4, white, and scute 8, shown thus,

y sc4 w sc8, and females of the constitution
———————/

sc d149 v f
———————— . The X-chromosome of the males contains
sc d149 v f
a very long inversion, or rather a combination consisting of the left part of the well-known scute 4 inversion (with its left breakage point just to the right of scute, the left distal region of the X now being attached to a point that had been just to the left of bobbed and block A) and the right part of the scute 8 inversion (in which the right break had been to the right of bobbed and block A and the left break just left of scute). This chromosome is deficient for bobbed and block A and has two scute loci, but the important thing for our purpose is the existence of the very long inversion and the presence of the markers yellow and white. In the X-chromosomes of the females the symbol "d149" refers to the well known inversion of moderate size which is commonly so designated; sc, v, and f are the markers scute, vermilion, and forked.

The impregnation of the females, their X-raying and the after-treatment, were carried out

similarly and simultaneously with those of the flies for the translocation study. Only one dose was administered; this was the previously described heavy dose, namely, ca. 1500 r. These flies were X-rayed in only two groups: 1) "immediate", which were given the full dose of ca. 1500 r in the nearly continuous period of about 6 minutes, and bred immediately afterwards, and 2) "divided", in which the flies were X-rayed in 4 periods of 1.5 minutes at intervals of a week and (except in the first series) held for an additional week after the fourth treatment, before being bred. During the course of this month in which breeding was prevented, they were kept on syrup food at 8°C.

The P_1 cross in this case, considering only the sex chromosomes, and the F_1 flies derived from it, may be represented thus:-

$$\frac{\text{y sc4 w sc8}}{\text{Y}} \quad (\male\male) \quad \times \quad \frac{\text{sc dl49 v f}}{\text{sc dl49 v f}} \quad (\female\female) \qquad (P_1)$$

$$\frac{\text{sc dl49 v f}}{\text{Y}} \quad (\male\male) \quad \times \quad \frac{\text{sc dl49 v f}}{\text{y sc4 w sc8}} \quad (\female\female) \qquad (F_1)$$

For the detection of the sex-linked lethals, the normal appearing (except that they were somewhat "scute") F_1 females were collected and mated in pairs to their sc dl49 v f brothers or to similar males from the stock bottles.

For consideration of the results to be expected from this cross, it must be pointed out that the presence of the two different inversions, one very long and one of moderate size, in heterozygous condition in the X-chromosomes of the F_1 females, practically prevents the production of any crossover offspring (F_2). Thus we may consider these chromosomes, with their groups of markers, as units. This being the case, it will be seen that only two types of males are expected in F_2, namely:

(1) $\frac{\text{y sc4 w sc8}}{\quad}$ and (2) $\frac{\text{sc dl49 v f}}{\quad}$

These can so readily be differentiated by their body colour and eye colour that their presence may be determined by examination of the flies within the vials without etherisation. Absence of (1) indicates a lethal which arose in a germ cell of the P_1 male, while absence of (2) indicates a lethal that arose in a germ cell of the P_1 female. Absence of (1) and (2), that is, no male at all, would show lethals from both P_1 male and P_1 female. Since males of type

sc dl49 v f can also arise by non-disjunction or elimination of maternal X's (which occurs oftener in the presence of the inversions), some lethals that arose in the P_1 female would fail of detection.

Confirmation of lethals that arose in the P_1 male was carried out by selecting the non-v non-f (nearly wild-type looking) females (of the same constitution as those of F_1) from the F_2 cultures and as before crossing them with sc dl49 v f males from stock bottles. The result in the case of a real lethal in the X derived from the P_1 male was a repetition of the F_2 finding, that is, the absence of y sc4 w sc8 males.

For confirming the existence of lethals derived from P_1 females, the F_1 females homozygous for sc dl49 v f (but heterozygous for the lethal if it were present) were collected from among the F_2 and crossed to y sc4 w sc8 males. The result, in the case of a lethal, would show a sex-ratio of approximately two females to one male.

TABLE I (a)

Ø Translocation frequency in relation to timing of irradiation (ca.1500 r)

Series No.	Treatment condensed, bred immediately.			Treatment condensed, breeding postponed.			$(p_1-p_2) \pm$ E diff. (in %) (d)	$\frac{d}{E_d}$	Geometric mean number of cultures $\sqrt{n_1 \times h_2}$ (= n_h)	$n_h p_1$ (P_{1h})	$n_h p_2$ (P_{2h})	$(P_1+P_2)q$
	F_1 - F_2 cultures examined (n_1)	Translocations. (P_1)	Percent (p_1)	F_1 - F_2 cultures examined (n_2)	Translocations. (P_2)	Percent (p_2)						
I	240	6	2.50%	269	4	1.49%	+ 1.014 ± 1.26	0.8	254.1	6.35	3.78	9.9
II	255	6	2.31%	242	7	2.89%	- 0.58 ± 1.42	0.4	248.4	5.74	7.18	12.7
III	275	7	2.55%	267	8	3.0 %	- 0.45 ± 1.41	0.3	271.0	6.90	8.12	14.6
Total	770	19		778	19				773.5	18.99	19.08	37.2

Ø Only Translocations between chromosomes II and III occurring in spermatozoa were noted.

Weighted Mean Results.

$\frac{\Sigma P_{1h}}{\Sigma n_h}$ Weighted mean. (in %) p_{1a}	$\frac{\Sigma P_{2h}}{\Sigma n_h}$ Weighted mean. (in %) p_{2a}	$p_{1a} - p_{2a}$ (in %) d_a	$\frac{\sqrt{\Sigma(P_1+P_2)\,q}}{\Sigma n_h}$ (in %) E_{da}	$\frac{d_a}{E_{da}}$
2.45 ±0.55	2.46± 0.56	-0.01	0.79	.013

TABLE I (b)

Ø Translocation frequency in relation to timing of irradiation (ca.1500r)

Series No.	Treatment condensed, bred immediately.			Treatment in 4 parts at weekly intervals.			$p_1 - p_2 \pm$ E diff. (in %)	$\frac{d}{E_d}$	Geometric mean number of cultures $= \sqrt{n_1 \times h_2}$ ($= n_h$)	$n_h p_1$ (P_{1h})	$n_h p_2$ (P_{2h})	$(P_1 + P_2)\,q$
	$F_1 - F_2$ cultures examined (n_1)	Translocations. (P_1)	Percent (p_1)	$F_1 - F_2$ cultures examined (n_2)	Translocations (P_2)	Percent (p_2)	(d)					
I	240	6	2.50%	257	5	1.95%	+0.55 ± 1.33	0.4	248.2	6.21	4.83	10.7
II	255	6	2.31%	219	7	3.20%	-0.89 ± 1.51	0.6	236.3	5.47	7.54	12.6
III	275	7	2.55%	199	6	3.02%	-0.47 ± 1.53	0.3	233.9	5.95	7.05	12.6
Total	770	19		675	18				718.4	17.62	19.42	35.9

Ø Only translocations between chromosomes II and III occurring in spermatozoa were recorded.

Weighted Mean Results

$\frac{\Sigma P_{1h}}{\Sigma n_h}$ Weighted Mean (in %) p_{1a}	$\frac{\Sigma P_{2h}}{\Sigma n_h}$ Weighted Mean (in %) p_{2a}	$p_{1a} - p_{2a}$ (in %) d_a	$\sqrt{\frac{\Sigma (P_1 + P_2) q}{\Sigma n_h}}$ (in %) E_{da}	$\frac{d_a}{E_{da}}$
2.45 ± 0.58	2.70 ± 0.60	- 0.25	.83	.30

TABLE I (c).

Ø Translocation frequency in relation to timing of irradiation (ca.1500 r)

Series No.	Treatment condensed, breeding postponed.			Treatment in 4 parts at weekly intervals.			$p_1 - p_2 \pm E_d$ (in %)	$\frac{d}{E_d}$	Geometric Mean number of cultures. $\sqrt{n_1 n_2}$ (= n_h)	$p_1 n_h$ (P_{1h})	$p_2 n_h$ (P_{2h})	$(P_1 + P_2)\,q$
	$F_1 - F_2$ cultures examined (n_1)	Translocations (P_1)	Percent (p_1)	$F_1 - F_2$ cultures examined (n_2)	Translocations (P_2)	Percent (p_2)	(d)					
I	269	4	1.49	257	5	1.95	-0.46 ± 1.11	0.41	261.9	3.89	5.09	8.8
II	242	7	2.89	219	7	3.20	-0.31 ± 1.60	0.18	230.2	6.65	7.35	13.6
III	267	8	3.00	199	6	3.02	-0.02 ± 1.59	0.01	230.5	6.90	6.94	13.6
Total	778	19		675	18				722.6	17.64	19.48	36.0

Ø Only Translocations between chromosomes II and III occurring in spermatozoa were noted.

Weighted Mean Results.

$\frac{\Sigma P_{1h}}{\Sigma n_h}$ Weighted mean (in %) p_{1a}	$\frac{\Sigma P_{2h}}{\Sigma n_h}$ Weighted mean (in %) p_{2a}	$p_{1a} - p_{2a}$ (in %) d_a	$\frac{\sqrt{\Sigma (P_1 + P_2) q}}{\Sigma n_h}$ (in %) E_{da}	$\frac{d_a}{E_{da}}$
2.41 ± 0.57	2.69±0.60	-0.28	.83	.34

TABLE II

Ø Translocation frequency in relation to dosage

(lighter dose = ¼ of heavier dose)

Series No.	Lighter Dose (ca. 375r) F_1 - F_2 Cultures Examined							Heavier dose (ca.1500r) (Combined figures of the three groups of Table 1, (a,b,c.)		
	1st. quarter of treatment	2md. quarter of treatment	3rd. quarter of treatment	4th quarter of treatment	Total	No. of Translocations	% of translocations	Total no. of Cultures	No. of Translocations	% of Translocations
I	135	92	76	153	456	2	0.44	766	15	1.95
II	97	45	48	0	190	0	0	716	20	2.79
III	359	259	82	217	917	3	0.32	741	21	2.83
Sum of above Results					1563	5	0.32	2223	56	2.52 ± ca.0.34 ⁂ 2.57 ± ca.0.34
Ø Results of 400r from radium (Raychaudhri)					1803	2	0.11			
Both Results combined					3366	7	0.21± ca.08			

Ø Only translocations between chromosomes II and III were noted.

Ø Reported in parallel paper

⁂ Mean weighted according to numbers in lighter series.

TABLE III

Ø Frequency of sex linked lethals in relation to timing of irradiation (ca. 1500r)

Series No.	Treatment condensed; bred immediately				Treatment in 4 parts at weekly intervals						Geometric mean number of cultures			
	$F_1 - F_2$ Cultures Examined (n_1)	Lethals: Complete Lethals (P_1)	Lethals: Semi-Lethals	Percent of Complete Lethal (p_1)	$F_1 - F_2$ Cultures Examined (n_2)	Lethals: Complete Lethals (P_2)	Lethals: Semi-Lethals	Percent of Complete Lethals (p_2)	$(p_1 - p_2) \pm E_d$ (in %) (d)	$\frac{d}{E_d}$	$\sqrt{n_1 x n_2}$ (n_h)	$n_h P_1$ (P_{1h})	$n_h P_2$ (P_{2h})	$(P_1+P_2)q$
I	256	17	-	6.64%	241	11	-	4.56%	+2.08± 2.06	1.01	248.4	16.49	11.34	26.5
II	194	13	1	6.70%	169	12	-	7.10%	-0.4 ± 2.67	0.14	181.1	12.13	12.86	23.3
III	218	11	1	5.04%	184	11	2	5.97%	- .93± 2.29	0.40	200.3	10.10	11.97	20.8
Total	668	41	2		594	34	2				629.73	38.73	36.16	70.6

Ø Only lethals in males are here noted.

WEIGHTED MEAN RESULTS

$\frac{\Sigma P_{1h}}{\Sigma n_h}$ Weighted mean of continuous group (in %) p_{1a}	$\frac{\Sigma P_{2h}}{\Sigma n_h}$ Weighted mean of interrupted group (in %) p_{2a}	$p_{1a} - p_{2a}$ (in %) d_a	$\sqrt{\frac{\Sigma(P_1+P_2)q}{\Sigma n_h}} = E_{da}$ (in %)	$\frac{d_a}{E_{da}}$
6.15 ± 0.95	5.74 ± 0.92	-0.41	1.33	0.36

RESULTS.

Table 1 (a, b, c) shows all the data obtained for translocations with the high dose (ca. 1500 r), administered in the three ways already noted, viz.:- (1) condensed treatment followed by immediate breeding, (2) condensed treatment followed by postponed breeding and (3) interrupted treatment in four instalments at weekly intervals. With regard to the latter two treatments, the qualification should be made that in the first series of experiments the period allowed between the first X-raying and the time of breeding was only three weeks, while in the latter two series it was four weeks(as described in the previous section).

It may be seen that in series I the percentage of translocations in the "condensed-immediate" group is[Ø] 1.01 ± 1.26% higher than in the "condensed-postponed" group, and .55 ± 1.33% higher than in the "interrupted" group. In the subsequent two series, on the other hand, where percentage differences between the three groups under comparison are likewise insignificant, what differences in the percentages of

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Ø Standard errors, not probable errors, are used in this article, and figures following the symbol ± always denote the former.

translocations there are give the "condensed-immediate" group a lower apparent value than either the "condensed-postponed" or the "interrupted" group.

In order that the significance of any differences between the results in different groups might be evaluated, the following method of comparison was used, in which each possible combination of two groups was subjected to study, apart from the third group. The percent of translocations found for a given group in a given experiment (series) was weighted by a number - the geometric mean of the total number of cultures obtained in that series in the two groups being compared - representative of the significance of this percent for the purpose of this comparison (see discussion of this method in our preceding paper); this weight was necessarily the same for the two groups being compared. The weighted percents of the different series were then summed, for each group, and a weighted average percent thus obtained for the group, for comparison with that of the other group. For the determination of the standard errors of these averages, for their comparison with one another, the formula $\frac{(p_1+p_2)q}{n_h}$, discussed on page 23 of our preceding

paper was used. In this way it was found that the "immediate-postponed" pair of groups shows a difference of .01 ± 0.79%, the "immediate-interrupted" pair shows 0.25 ± 0.83%, and the "postponed-interrupted" pair 0.28 ± 0.83%. These figures indicate clearly the insignificance of the differences between the three groups. Hence the time factor, as varied in the present experiments, does not seem to have any marked influence on the frequency of translocations.

It may be noticed that, in all the three groups together, 56 translocations between chromosomes II and III were found to have occurred in the germ cells derived from the P_1 males. In addition, there was one apparent translocation observed in the "condensed-immediate' group which was not confirmed in the third generation and which therefore was either no real translocation at all or had occurred in a germ cell of a P_1 female. As this was the only case of its kind it is evident that the rate of induction of re-arrangements in females must be far lower than that in males (a conclusion agreeing with some previous evidence of Muller, 1928 and 1930, and others), although the actual ratio of the effects in germ cells of the two kinds cannot be

known until further work has been done in this direction.

Turning now to table II, relating to the light dose (ca. 375 r) administered continuously(with the flies divided into four lots, for raying simultaneously with the four quarters of the heavy treatment, as shown by the numbers given to them in the table), the combined results of the three series show that, in all 1563 F_1-F_2 cultures which were examined, 5 translocations were observed. This gives a percentage of 0.32 ± .14. Adding to this the results (2 translocations in 1803 cultures) obtained in a parallel experiment with radium conducted by S.P. Raychaudhuri (reported in his parallel paper), the combined results of both the experiments give 7 translocations between II and III (derived from germ cells of males given a dose of 375 r to 400 r), in a total of 3366 chromosomes. This gives a percentage of 0.21 ± .08. As against this, we have a total of 2223 chromosomes from our heavy dose, with 56 translocations of corresponding type, giving a percentage of 2.52 ± 0.34.

A comparison of these figures indicates that the frequency of translocations varies with a power of the dosage lying somewhere between 1.5 and 2. If we use our value for the heavy dose, with its relatively small error, as a basis for calculating the expected value for the light dose, we find that, if the exponent in question were taken as 1.5, the value for the light dose would have been .31 ±.04%. If the exponent were taken as 2.0, the value would have been .16 ± .02%. The difference between the observed and expected values for the light dose in the first case (for exponent of 1.5) is .10 ± .09 and in the second case (for exponent of 2.0) .05 ± .09. On the other hand, if the exponent were taken as 1 (a relation of simple proportionality), the expected light dose value would have been .63 ± .06% and the difference from the observed value would be .42 ± .10%. Hence the evidence is convincing that the exponent is above 1, and it is likely that it is above 1.5.

It should be mentioned that, in addition to the recorded number of translocations at the light dose, there was one more which is not recorded

here as it was not confirmed in the third generation. If a translocation, then, this must have occurred in a germ cell of the treated female.

Table III shows the results for sex-linked lethals obtained by the use of the same heavy dose as applied in the case of the translocations: viz.: ca. 1500 r. As in the case of the translocations, so here too, the period between the first X-raying and the breeding of the P_1 flies was 3 weeks for the first series and 4 weeks for the two subsequent series. Two groups were under comparison for these sex-linked lethals. One was given the condensed treatment followed by immediate breeding, while in the other case treatment was given in four instalments at weekly intervals. Here again, in the first series, the "condensed-immediate" group shows a high percentage of lethals, the difference between the two groups being 2.08 ± 2.06; although the difference here is not really significant, this high result, agreeing with that from the translocations, may be taken as evidence that in this series - the only one in which a dosimeter was not used - the "immediate" group really did receive a higher dose than the "divided" treatment group, owing to some fluctuation in the X-ray machine output or the conditions of irradiation.

This means that we should not regard the higher translocation frequency which we have already noted to have been obtained in the corresponding translocational group of this series as indicating a real effect of the kind of treatment chosen for this series. Thus the value of our having carried out lethal tests as a check on our translocation tests is confirmed. The second and the third series, on the other hand, show the insignificant differences of 0.4 ± 2.67%, and 0.93 ± 2.29%, respectively, in favour of the interrupted treatment.

The results of the three series of lethal tests were combined in the same way as were those of the translocations, as explained above. The weighted mean percentages, as calculated by this method, for the two groups, are 6.15 ± 0.95 and 5.74 ± .92, respectively; these show a difference of 0.41 ± 1.33, which is obviously not significant.

The total number of sex-linked lethals in the germ cells derived from the P_1 males in the two groups taken together was 71. There were besides 3 more lethals observed which were derived from the germ cells of the P_1 females. As before explained, it is likely that some of the lethals in females were missed, due to the appearance of non-disjunctional

males in the F_2 generation. Owing to this unreliability of the number obtained for the germ cells of the females, they are not included in our tables. It may also be stated that there was no case of lethals having occurred in both the male and the female germ cells from which a given F_1 individual was derived.

DISCUSSION AND CONCLUSIONS.

In the case of most of the biological effects of radiation other than the obviously genetic ones it has been found that, at least below a certain intensity, a lowering of the intensity is associated with a disproportionately great reduction of the effect. This has been interpreted as meaning that two or more ionisations must co-operate, through certain intermediate processes, in the production of the observed biological effect, but that if the ionisations are too far removed from one another in their time or place of occurrence certain other changes, of the nature of restitution processes, have a chance to ensue, which nullify their effectiveness before they or their products can co-operate in the causation of the biological effect being studied.

The repair processes sometimes occur visibly, as in the case of irradiated skin which begins to recover immediately after the irradiation and the rate of repair of which becomes slower and slower as time goes on (MacComb & Quimby, 1936). In the latter case it has also been shown that the recovery rate from a second or third successive dose is slower than from the first dose.

Even in the case of most of the biological reactions affected by radiation intensity, however, there is usually a level of intensity, when the period of radiation is short, above which the biological result seems to be dependent only on the total dosage regardless of the time-intensity factor. Here, then, we have the operation of the Bunsen-Roscoe law, according to which the effect of a given total dose of radiation is always the same, regardless of the time or intensity of radiation. The precise significance of this principle, however, is still a matter of dispute in the case of most biological reactions.

There is as yet no evidence of the existence of any repair process of such a kind in the case of gene-mutations. Neither do the results of our own work or that of Raychaudhuri's indicate their existence in the case of chromosome re-arrangements while Drosophila spermatozoa are being irradiated (But for other material see remarks below about the recent work of Sax).

In the earlier days of radiation-genetics, however, there was thought to be a possibility that some sort of process of reversion might exist even in the case of genetic changes such as gene-

mutations and chromosome re-arrangements in spermatozoa. While very short period reactions of this kind could not have been detected, the experimental data so far available on gene-mutations are opposed to the idea of the existence of 'back-reactions' of comparatively long period. Some evidence for this comes from the work of Muller (1927), extended and confirmed by Harris (1929), showing the lack of effect of aging sperm after X-raying on the frequency of lethal mutations in them. Our more extensive results on mutations in spermatozoa aged after X-raying are in agreement with these. Further evidence of the same general nature lies in the results showing the stability of mutant genes both in the cell generations immediately following treatment, in the case of somatic mosaics; and in succeeding generations of individuals, in the case of mutations in germ cells (Muller, 1927, 1928; Patterson & Muller, 1930).

In regard to translocations, it was conceivable that the long periods between the different exposures in the case of the fractionated treatments, and the long period after exposure, both here and in the case of the series with postponed breeding, might

allow reunion of the fragments of some of the broken chromosomes (if the breakage theory of rearrangements were valid). It seems reasonable to suppose that in the treated spermatozoa large movements of the chromosomes, favouring the formation of new chromosome combinations, cannot take place, owing to the chromosomes being closely packed together. On the other hand, the Brownian movements, and other small movements, some of them perhaps due to the activity of the sperm, should give enough chance for the two broken ends of the same chromosome, which at first cannot be far apart from each other, to come into contact in their original positions. Thus, if these contacts could result in the fusion of the broken ends of the chromosome, one would expect, as a result of fractionation of the dose or merely of delayed fertilisation, the formation of a greater number of original combinations ("restitutions"), and, therefore, a smaller number of translocations.

Our results, as may be seen, do not support the above hypothesis, since they show no difference in the frequency of translocations in sperms which were treated and used immediately for fertilisation, those

given a fractionated dose and those treated and used after a month. Our results are, in part, also supported by those obtained by Raychaudhuri, in his parallel work on the effect of the time-intensity-relationship on translocations produced by the gamma rays of radium.

If, then, we regard the chromosome breakages as occurring independently of one another and before the fusions, it follows that, after the breakages have been produced in the sperm by the X-ray treatment, the broken ends cannot fuse together - giving either the original combinations (restitution) or viable or lethal re-arrangements - until the time of fertilisation. This is probably due to the existence of some special condition in the "resting" nucleus of the spermatozoon. It is evident that at some stage during the period of fertilisation this special condition, whatever it may be, is removed, and that the actual fusion of the broken ends then takes place within the fertilised egg. Theoretically, this fusion may occur in a number of possible ways, such as by the formation of the original combinations, of acentric and dicentric re-arrangements, and of viable monocentric re-arrangements. According to the topography of the breaks and unions, deletions,

duplications, inversions, or translocations of various types are formed.

An alternative explanation of the lack of influence of the timing of the treatment could, however, be given on the basis of the so-called "contact" hypothesis of the origin of X-ray-induced rearrangements. If this hypothesis held good, with the breakage and fusion occurring at almost the same momemt, as a result of the same ionisation, no time effect would be expected. Accordingly, before deciding whether fusion of chromosomes can occur in the spermatozoa, we must first decide whether the "breakage" or the "contact" theory of gross re-arrangements is true. Evidence concerning this may be obtained from data on the relation of the frequency of such re-arrangements to the total dosage used (frequency of ionisation).

Our own results on translocations for the light dose (ca. 375 r) as compared to the heavy dose (ca. 1500 r) indicate that there is no direct proportionality between their frequency and the amount of ionisation, but that the former value changes more rapidly than the latter. Although the number of translocations obtained at the low dose is very small (even when the numbers obtained in the parallel

experiment of Raychaudhuri are added to them) and, therefore, affected by a large error of sampling, it can be inferred (both from our own and from the combined results) that, for this range of dosage, the frequency of translocations is proportional to some power of the dosage certainly greater than 1 and probably less than 2 and perhaps about 1.5, as found in work of Muller and his collaborators (1936, 1937, 1938) in Drosophila for higher dosages, and by Sax (1938) in plant material. Considering the results of Raychaudhuri's experiments, however, it becomes probable that, in the low range of dosage in question, the power is nearer to 2 than that which applies at a higher range of dosage.

Although the relation between the dosage of radiation and the frequency of abberations to be expected on the breakage hypothesis is not yet entirely clear, it has become possible with the data now at hand to make certain deductions as to the mechanism with which the gross re-arrangements are formed.

The fact that the data show the translocation frequency to vary at a rate higher than the first power of the dosage definitely excludes the

possibility that the two breaks of a translocation are always produced by only one ionisation. For in the latter case the translocations, like gene-mutations, could not be more than proportional to the dose. But even if not all but some appreciable fraction of the re-arrangements resulted from single ionisations, the frequency-dosage relation should approach proportionality at lower doses, and there is no evidence of this with our very low doses. In fact, as above mentioned, the evidence rather indicates that at these low doses the exponent in question rises above the 1.5 power found in the earlier experiments, dealing with a range of higher dosages, and approaches nearer to 2 than in them.

Now the above results are just what is to be expected on the breakage theory. For at low doses, when more than two breaks would seldom concur in the formation of a re-arrangement, the chance of coincidence of these two breaks would vary nearly as the product as the chances of each, i.e., as the square of the dose. While some of the translocations formed would be inviable combinations (acentric and dicentric), and while other breakages would result in restitution, still the proportion of two-break

cases giving viable re-arrangements would not alter much and so the detected re-arrangements, like the total ones, would at these low doses vary nearly as the square of the dose.

But as shown first in calculations of Haldane (in personal communication to Muller 1935), and as later pointed out also by Stadler (1936) and by Catcheside (1938), at higher doses there would necessarily be more triple and still higher rank re-arrangements at the expense of a larger number of doubles ("saturation effect"). This would, in itself, tend to make the frequency of detectable cases of re-arrangement increase less rapidly than before, with increase of dose. But greatly re-inforcing the latter tendency would be the fact that these cases of more multiple breakage can be shown to result in a much smaller proportion of detectable viable re-arrangements, as opposed to those involving acentric and di- or polycentric chromosomes. Thus the 1.5 power is explained, for higher doses, and for still higher the power must become still smaller.

On the basis of our dosage results, then, reinforcing and extending those obtained by Muller and other collaborators, and those of Sax, we are inclined to the position that the breakage theory

of production of gross re-arrangements may be regarded as proved. This being the case, we may also conclude from the lack of effect of the differences in timing of the irradiation and breeding, that the broken ends are unable to rejoin until, the sperm having entered the egg, the processes of fertilisation and cell division begin to take place.

In this connection it should be mentioned that the results obtained by us which show complete ineffectiveness of the time factor are in sharp contrast with those recently obtained by Karl Sax (1939) in microspores of Tradescantia. This author gives cytological evidence that in his case the broken ends rejoin very quickly (in about an hour), and that their rejoining is independent of the act of fertilisation. It is not unlikely that this different behaviour of the chromosomes of Drosophila spermatozoa and of Tradescantia microspores is associated with their different degree of condensation, which, in turn, is due to some difference in the material surrounding them.

In the light of the above, it might be expected that the results to be obtained in young

female germ cells of Drosophila (oogonia and oocytes) would be more like those found in Tradescantia microspores. But the data obtained by us on the relative frequencies of translocations in male and female Drosophila are too meagre to have a bearing on this question. So far as effects in the female are concerned, our work can only be regarded as confirming previous evidence of Muller (1930) and later workers that many more re-arrangements are to be found in the (viable) offspring derived from treated fathers than in those from treated mothers. Further work in this connection is now most desirable.

SUMMARY.

Experiments on Drosophila melanogaster planned with a view to determining the possible effect of differences in the time factor on the production of translocations by X-rays in spermatozoa clearly show that even long intervals between and after the exposures do not alter the frequency of chromosome re-arrangements, provided the total dose of X-rays, as measured either by ionisation or by the production of sex-linked lethal mutations, remains the same.

Three groups were compared in the tests of the frequency of translocations. In one, the exposure of the inseminated females was continuous and the flies, after treatment, were allowed to deposit their eggs at once. The second group was irradiated similarly and simultaneously, but the deposition of eggs was postponed for one month, by keeping the flies at 8°C on syrup food. In the third series the dosage was divided into four equal fractions, administered at weekly intervals, and the flies were bred a week after the last irradiation, i.e., one month after the first irradiation. The amount of dosage applied in the three cases was the same, viz., ca. 1500 r. The results showed no significant

differences in the frequencies of translocations.

Series to test translocations at a very low dose (ca. 375 r), which was one-fourth of the above, were run simultaneously with those mentioned above. The results of these tests, combined with those of a parallel experiment by Raychaudhuri, showed that, for the range of dosage between 375 r and 1500 r, the number of translocations is proportional to a power of the dosage which is certainly well above 1, and is probably nearer to 2 than to 1.

Sex-linked lethals also were tested simultaneously in all these cases, and the results obtained showed that the differences in timing of the treatment and breeding caused no significant differences in their frequencies. As this was expected on the basis of previous work, these results also served as a check on the dosage given for translocations.

On the basis of the frequency-dosage relations here found, especially when these are taken in connection with the results of other experiments, it is concluded that radiation produces gross rearrangements by first breaking the chromosomes in two or more places by means of separate ionisations, and that the broken ends later undergo recombination.

On the basis of the finding that the results are independent of timing it is further

concluded that the broken ends cannot rejoin so long as they are included within the free spermatozoon, but that after fertilisation some change in conditions make union of the pieces possible.

ACKNOWLEDGMENT.

The author wishes to acknowledge his deep gratitude to Professor F.A.E. Crew, F.R.S. for encouragement and affording facilities for the work and to Professor H.J. Muller for guidance and criticism.

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