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ADVANCES IN RADIATION MUTAGENESIS THROUGH STUDIES ON DROSOPHILA

by H. J. Muller

Of major importance in assessing the amount of genetic effect produced by ionizing radiation is, first, knowledge of the relation between the mutation frequency, especially the "point mutation" frequency, and the radiation dose and, second, knowledge of the differences in frequency produced by given doses on cells of different types or under different conditions.

1. FREQUENCY-DOSE RELATION AT LOW AND MODERATE DOSES

In the case of recessive lethal mutations induced by irradiation of Drosophila spermatozoa it has been demonstrated by a large amount of work carried out during the first two decades of such studies, 1928-1948, that the relation of frequency to dose is substantially linear over a very wide range[1-4] (see the table, Series VI). The range was extended down to 50r or even 25r by Stern and his co-workers[5-7], thus attaining a more than 100-fold amplitude. This being the case, it is likely on theoretical grounds that the linear relation holds all the way down to zero dose[8].

At doses lower than 3000r more than two-thirds of the recessive lethals are associated with no cytologically visible structural change, and this fraction increases with decrease of the dose, as shown by Valencia[9] working at the Indiana laboratory. Hence the linearity principle found for recessive lethals may be inferred to apply primarily to point mutations. Since, however, structural changes induced in the spermatozoa have at moderate and high doses a frequency approximately proportional to the 1.5 power of the dose[3,9] and constitute a considerable fraction of the recessive lethals arising at high doses, it has been somewhat of a paradox that even at high doses the total frequency of lethals has seemed to remain linearly proportional to the dose. We shall revert to this question in a subsequent section.

*Zoology Dept., Indiana University, Bloomington, Ind., U.S.A. Contributors, with present addresses: I. I. Oster, Zoology Dept., Indiana University; I. H. Herskowitz, Biology Dept., St. Louis University; Helen U. Meyer, Zoology Dept., Indiana University; A. Schalet, Biological Laboratory, Cold Spring Harbor, Long Island, N.Y.; S. Abrahamson, Biology Dept., Rutgers University, Newark, N.J.; Sara H. Frye, Zoology Dept., Indiana University; and E. A. Carlson, Biology Dept., Queens University, Kingston, Ont. The address of all these persons was Zoology Dept., Indiana University at the time that their contributions were made.

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Ser.	Ref.	Stage of germ cells & indiv. at irradiat'n	Type of mut'n	Dose in r	Mut'ns found/ chrom. tested	Frequency per chrom. or locus (X 10^{-4})	Ind. freq. per chrom. or locus (X 10^{-4})	Ind. freq. per chr. or locus for 1r, & error (X 10^{-7})	"Doubling dose," inducing freq. = observ. spont.	stand. ♂ spont.
I	11 & this art. Oster	oogonia, larvae " " ♀ controls	leth. in X-chrom.	2400 600 0	33/2,056 117/16,064 49/11,630	161. 73. 42.	119 31 spont.l.=2.1 X ♂-stand.	50±12 51±15	830r	400±75r
II	this art. Meyer	gonia, adult ♂ sp'zoa, adult ♂ ♂ controls	leth. in II-chrom.	3000 3000 0	7/373 248/960 6/1,274	190. 2580. 47.	143 2533 spont.l.= ♂-stand. for II	48±25 844±55	1022r 56r	1000±500r 60±4r
III	12, 48 & unpub. Muller & Valencias	gonia, adult ♀ cytes, adult ♀ ♀ controls ♀ controls	nonleth. visible, 8 loci, X leth., X	4600 4600 0 0	42/94,380 6/8,865 9/62,000 19/2,615	0.56 0.84 0.18 79.0	0.38 0.66 (per-locus spont. = 45±15X10^{-7} at stand. rate) spont.l.=4.0 X ♂-stand.	0.083±.023 0.143±.075	2170r 1260r	550±230r 320±190r
IV	45, 46 Schalet	♂ controls " "	nonl-vis, 10 loci X leth., X	0 0	18/490,000 32/8,706	0.037 37.	(per-locus spont. = 21±9X10^{-7} at stand. rate) spont.l.=1.75 X ♂-stand.			
V	49, Oster & Muller	cytes, adult ♀ ♀ controls sp'zoa in ♀ ♂ controls	back-mut'n of forked√	3200 0 3200 0	6/118,642 3/975,108 8/75,110 4/315,102	0.51 0.03 1.06 0.13	0.48 relation to stand. undet. 0.93 relation to stand. undet.	0.15±.065 0.03±.013	200r 430r	
VI	many, e.g. 2, 44	sp'zoa, adult ♂ ♂ cont., 1st wk	leth. in X	many doses, many experiments, consensus: most usual spont.:		20.		275		73r
VII	17, 18 & this art. Frye	sp'zoa, adult ♂ " " ♂ controls	defic. etc. of y^+ in sc^8	4000 1000 0	126/41,310 148/180,942 3/263,694	30.50 8.17 0.11	30.39 8.06 relation to stand. undet.	7.64±0.69 8.06±0.67	14.5r	
VIII	11 & this art., Oster	sp'tids, pupae " " ♂ controls	leth. in X	1000 250 0	130/1,859 195/8,978 19/2,392	699. 217. 79.	620 138 spont.l.=4.0 X ♂-stand.	620±64 552±96	130r	32±3r

√The set-up made partial back-mutations of less extreme type visible when derived from ♂ than from ♀.

Reliable studies on the frequency-dose relation for other material than Drosophila spermatozoa have not been available until, at the 1955 Conference on the Peaceful Uses of Atomic Energy, Russell[10] made the statement that he had obtained an approximately linear relation for the visible mutations at specific loci induced in his irradiations of mouse spermatogonia, when results from doses of 0, 300 and 600r were compared. During the past 12 months Oster[11] has extended the study of the frequency-dose relation to comparable cells in Drosophila, namely, oogonia, irradiated in third-instar larvae. Possibly included here also was a small minority of early oocytes, but these are known to have induced-mutation frequencies, and a distribution of mutational types, similar to those of oogonia[12]. In both these types of cells, the great majority of induced mutations, even at high doses, are of "point" nature. The results of this experiment are shown in the table, Series I.

As the table shows, the frequency of recessive sex-linked lethals induced in these immature female germ cells by the lower dose, 600r, is even lower than the spontaneous frequency. By reason of the large number of tests carried out at this dose, however, it was possible to attain a relative accuracy almost as great for this induced frequency, after subtraction of the spontaneous frequency, as for that induced by the higher dose, 2400r. The frequency per roentgen, shown in the 9th column, turns out to be substantially the same for both doses, i.e. the relation is linear. Since the two doses differ by a factor of 4 the statistical errors indicated are less important in allowing possible deviations from linearity than they would have been if one dose had been only twice the other. Thus if we calculate, for example, what the expectations could have been if the frequencies of the lethals had actually been proportional to the 1.5 power of the dose, as those of structural changes are, we find that the observed data depart far too much from these expectations to represent a random sample derived from any such material. It is evident, then, that the present results not only give no indication of a threshold but also no indication of a reduction in the mutagenic effectiveness of the radiation as the dose is reduced to levels at which the induced frequency is lower than the spontaneous one.

These data also make it clear that the induced mutations have here been added to the spontaneous ones. That is, the total mutation frequency found at any given dose does not represent some sort of exaggeration, magnification, or multiplication of a "natural" frequency: an exaggeration that becomes proportionately more pronounced as the dose is increased. Instead of this, the total mutation frequency found is made up of two separate components: (1) the spontaneous mutations that would have arisen even without the radiation, plus (2) a contingent of radiation-induced mutations that are of entirely independent origin from the spontaneous ones, even though the mechanisms of origination of the two categories may well have been related in kind. Similarly, the induced mutations themselves are mutually independent.

It was thought desirable to extend our knowledge of the frequency-dose relation not only in the direction of cells having a much lower susceptibility to radiation mutagenesis than spermatozoa but also, contrariwise, to those having an unusually high susceptibility. The work of a number of authors, to be mentioned subsequently, had pointed to the spermatid stage as

the most susceptible. This inference was confirmed by direct comparisons in which Oster[13] found that at least twice as many recessive lethals were induced in spermatids as in the most susceptible spermatozoan stage, that present in inseminated females. Oster has therefore made a special investigation of the frequency-dose relation for recessive sex-linked lethals arising in spermatids. For convenience and repeatability in treating the spermatid stage, Khishin's procedure[14] was followed, of irradiating pupae 48 hours after their pupation and, after emergence of the males, utilizing them as parents for not more than the next three days. The doses used were purposely kept low (250r and 1000r) so that even at the higher dose the great majority of the lethals found would represent point mutations.

The results are shown in Series VIII of the table. The frequencies obtained after subtraction of the control frequency are again seen to conform satisfactorily with a linear relation, and we have calculated that they are not to be reconciled with the postulate of proportionality to the 1.5 power of the dose. On the other hand, a parallel experiment showed that the frequencies of gross structural changes of chromosomes, represented by translocations between chromosomes II and III, did conform clearly with the 1.5 relation expected on the basis of earlier findings[3] on translocations arising from irradiated spermatozoa. The present data on translocations, derived from the same groups of treated spermatids as the lethals, were as follows: 45/3421 for 250r and 148/1533 for 1000r (no controls being necessary since their spontaneous frequency is known to be so low that it need not be taken into account in such work). These results for translocations show that there were no peculiarities of the conditions such as might have tended unduly to reduce the frequencies observed at high doses below those that had actually been produced. Finally, the present results for lethals, similarly to although less strikingly than those for oogonia, show that the induced mutations arise independently of the spontaneous ones and are added to them.

As there are grounds for regarding all the above results for lethals as having reference mainly to point mutations it was thought desirable to get additional evidence concerning the frequency-dose relation for very minute rearrangements. Although earlier work[15] had provided evidence of linearity, for such tiny deficiencies as are usually met with inside of or bordering on heterochromatic regions, doubts and seeming counter-evidence[16] had been raised. Using an X-chromosome of the scute-8 type, that has heterochromatin in the neighborhood of its left end, in which lies the normal allele of yellow-body color, Sara Frye has during the past two years tested the frequency of mutants showing yellow body, among the daughters of untreated males and of those irradiated with 1000r and 4000r, respectively[17,18]. Sperm of the 2nd to 4th day after irradiation were used.

As is to be seen from the table, Series VII, the large-scale results fit well with the expectation for linearity. Analyses of the mutants showed that the great majority of them involve losses of at least three loci (those of a lethal, yellow, and achaete) and constitute minute deficiencies. It is true that some cases of "half-translocation" were also present, and that these must have been derived from two widely separated breaks and have had a frequency dependent on the 1.5 power of the dose at these doses. But since such cases could be reckoned to constitute fewer than 20% of all the mutants

scored their inclusion did not perceptibly affect the linear relation observed for the totals. In contrast to these findings, most deficiencies that are readily visible in euchromatin have their breaks further apart than those here usual, and accordingly follow the 1.5 power rule[19,9].

It should be added that two loci in the main euchromatic region of the tested X-chromosome, those of white and forked, were simultaneously scored for mutants in Frye's experiment. These mutants, which were in large majority point mutations, also showed frequencies closely conforming with a linear relation to dose, the counts being 1/263,694 for the controls, 17/180,942 for 1000r, and 13/41,310 for 4000r. The per-locus induced frequency here, $0.4(\pm 1) \times 10^{-7}/r$, is as expected far lower than that of about $8 \times 10^{-7}/r$ found for the yellow deficiencies involving a break in heterochromatin, but distinctly higher than the frequencies of point mutations induced in female germ cells (Series III).

2. DIFFERENT SUSCEPTIBILITIES OF DIFFERENT CELL TYPES

Space precludes a historical treatment here of the evidence gathered in the last six years, first by Luning and his co-workers[20-29], then by Auerbach[30-32] and her student Khishin[14], by Baker and von Halle[33], and in the last four years by our own group also[34-38], showing that the post-gonial stages of Drosophila germ cells not only are much more susceptible than the gonial stages to mutagenesis by ionizing radiation, as had long been known[39], but differ greatly amongst one another in susceptibility. The identification of which stages in the germ cell development of the male correspond to the differing susceptibilities found has been made by Auerbach and Khishin by a combination of genetic and histological methods. On the basis of their comparative results, checked and added to by our own and those of the other workers mentioned, the sequence of varying susceptibility for the production of recessive lethals (that is, the relative frequencies produced by a given dose) may be roughly indicated as follows for the male germ cells: (A) spermatogonia and early spermatocytes 1, (B) meiotic division stages 8, (C) spermatids 12, (D) spermatozoa more than a day before their ejaculation 3, (E) spermatozoa within a day of their ejaculation 4 to $4\frac{1}{2}$, (F) spermatozoa within inseminated females 5 to 6; and for the female germ cells: (A') oogonia and early oocytes 1, (B') late oocytes (last 3 to 4 days) 2 to 3.

The actual frequencies may be illustrated for A' and C by Series I and VIII in our table, but the values found for a given stage and dose often differ by a factor of nearly 2 for different experiments, so that our unit 1 (applying to A and A') may vary between values of 50 and 100×10^{-7} induced sex-linked lethals per roentgen. As for Series VI of our table, although the induced value here given has often been used for reference it is now evident that it represents a compromise between D and E (somewhat nearer to E), since it is based on work done before the distinction between these stages was known. Undoubtedly differences would be found to exist between still finer subdivisions than those of the present series if there were means of studying them separately. This is illustrated by Oster's finding[40] that a distinctly higher frequency is evinced by oogonia if they are irradiated while halted in mitosis by colchicine or acenaphthene.

A recent example of a comparatively fine distinction in stages is given by the data of Whiting and Murphy[41]. These show that in the wasp Habrobracon recessive lethals are produced at about the same rate per roentgen (if we make the assumption, not contradicted by the data, that the effect is approximately linear at the dose studied) in spermatozoa irradiated in the male and in eggs irradiated at meiotic metaphase I, whereas in eggs irradiated at prophase I a dose nine times as high is required for the same effect. Here, as in the Drosophila results, there is a correlation between the amount of chromosome condensation and the degree of susceptibility. However, the existence of additional factors is shown in Drosophila by the much higher susceptibility of spermatids than of spermatozoa, despite the somewhat lesser condensation of chromosomes in the former than in the latter stages.

Whatever the factors differentiating the susceptibilities of different stages may be, it is unlikely that the pattern found in Drosophila is unique. Thus, in mice, to add to the long-existing evidence that in them as in Drosophila there is a far higher production of translocations and dominant zygotic lethals in spermatozoa than in spermatogonia, we have a recent statement by Russell[42] that the mutations at specific loci studied by him are produced at "approximately two to four times" the frequency in male germ cells irradiated between 19 and 23 days before mating than in spermatogonia. This result agrees quantitatively with the relative frequencies of point mutations and of total recessive lethals induced at the probably corresponding Drosophila stages A and D.

The comparative susceptibilities dealt with above are, as previously mentioned, those for point mutations, in the sense of changes on a scale too small to be definitely detectable by microscopic examination of the extended chromosomes of the salivary glands[9]. The comparative susceptibilities of these different stages to having demonstrable structural changes of a viable kind induced in them differ from one another in the same direction, on the whole, but much more than those above given. Moreover, the frequency-distribution of the different types of structural changes found also varies with the stage irradiated. Not only chromosome breakability differences but also differences in the factors affecting the joining of the broken ends and the recoverability of the products of union play important parts in the determination of the observed frequencies of structural changes, in a manner too complicated to be entered into here. But there are grounds, which will be mentioned subsequently, for inferring that these other factors are not relevant to the production of the point mutations. We may also infer that even at the most susceptible stage, that of spermatids, the recessive lethals remain a valid criterion, at low and moderate doses, of point mutations.

3. THE "DOUBLING DOSE" AND THE SPONTANEOUS FREQUENCY

The doubling dose for point mutations, i.e. the dose inducing as many as arise "spontaneously" in the course of one generation, is subject to enormous variations (see the table). Thus if the spontaneous rate is held constant, the induced rate and with it the doubling dose can vary by a factor of at least 12, depending upon which stage is irradiated. If on the other hand the induced rate is held constant, the spontaneous rate and with it the

doubling dose can vary by a factor of at least 20, depending upon the genetic composition of the stock and the history of the germ cells used. Accordingly, if neither rate is held constant the doubling dose can vary by the product of these factors, i.e. by at least 240. And by alteration of conditions (such as oxygen tension) its variation can be still further increased.

There has long been evidence that differences as great as ten fold in the spontaneous point-mutation rate, caused by differences in genetic factors, since termed mutator genes, are not uncommon in Drosophila[43]. And even with the same stock, the spontaneous rate, typically represented by .2% of recessive sex-linked lethals in the first spermatozoa released by the newly emerged male, is reduced to .06% in the spermatozoa released subsequently to these, but if the spermatozoa are stored in the female their rate is increased by about .06% per week over their original rate; meanwhile, however, the eggs of all periods show an approximately constant rate of about .17%.[44]

The paradoxically higher frequency found among the earlier released spermatozoa is probably to be referred to the longer duration of the spermatid stage which they underwent in the pupa, as compared with that undergone by the later released sperm in the adult, if we grant that the spermatid stage is not only unusually susceptible to ionizing radiation and some chemical mutagens[31,33] but probably susceptible likewise to some of the mutagenic agents naturally present. Whether or not this interpretation is correct, it is evident that different groups of spermatozoa from the very same individual may show spontaneous frequencies differing by a factor of 7: e.g. from .06% in second-week sperm engaging in fertilization within a few days to .44% in first-week sperm stored in the female for a month. The doubling dose therefore has a meaning only in relation to given stocks bred under given conditions and when the radiation has been applied to given stages in a given manner. However, one may speak of average values for given circumstances.

A considerable source of error that sometimes hampers the determination of the spontaneous rate lies in the inordinately high mutability of the stage that includes fertilization and the nuclear divisions of the newly formed zygote. Evidence for this is found in (1) the disproportionate prevalence of large "clusters" of mutants of common origin among the offspring of given individuals[44-46], (2) the high frequency of mutationally mosaic offspring[45,46], and (3) the strong influence of maternal genotype on the frequency, in chromosomes of paternal origin, of mutations common to the entire germ track of an individual and hence scored as having arisen in the father.[47] Estimates based on the first effect indicated a spontaneous mutation rate per unit of time at this early stage of the order of several hundred times that obtaining in the germ cells throughout the major part of the organism's life.[44] Whether this stage is similarly susceptible to the induction of mutations by radiation is not known. But the phenomenon as it pertains to the spontaneous rate leads in several ways to large errors in the determinations of frequency and of doubling dose.

The large-scale experiment by Schalet[45,46] that included the observations on mosaics above referred to was primarily undertaken to determine the frequency of spontaneous mutations involving specific loci that arise in chromosomes derived from the father (Series IV of the table). The results gave

an average for point mutations per locus that was just within statistical range of the higher value previously obtained from chromosomes derived from the mother[48] (Series III of the table), after allowance had been made for the different mutation rates of the stocks used, as ascertained by the lethal tests. It turns out that for a stock with an arbitrarily assumed standard rate of .2% for sex-linked lethals the per-locus point-mutation rate is of the order of $.3 \times 10^{-5}$.

The relation of the point-mutation rate induced at a given stage to the spontaneous rate, when the latter is converted in the manner mentioned into the value it would have for an arbitrary standard of conditions, is (so far as may be judged from present data) about the same for mutations detected by the specific locus method as for recessive lethals, as may be seen by a comparison of Series III and I or by our unpublished comparisons of corresponding data obtained by irradiation of spermatozoa. This result may be expressed by saying that the "standard" doubling dose for either specific-locus point-mutations or recessive lethals induced in oogonia is about 350r while that in late spermatozoa is about 75r. There are indications[49] (Series V) that for back-mutations the doubling dose in spermatozoa may be higher than for the more usual types, but this is uncertain since no standard values were established here by tests of lethal rates.

For small structural changes (mainly deficiencies) the spontaneous rate, like that induced in gonial cells by low doses, is smaller than the corresponding point-mutation rate by one or two orders of magnitude. However, the data obtained in the scoring of specific-locus mutants give the spuriously high ratio of about one such structural change to three point mutations. This is because the likelihood of detection of a structural change by this means is proportional to the average number of loci that are lost or changed by it.

4. MUTATIONAL MECHANISMS AND CONDITIONS

Among studies on the production of chromosome aberrations recently carried out by our group have been a number on oocytes. Despite the rarity of recoverable gross aberrations from oocytes their chromosomes are readily broken. But the broken ends, except when restitutionally united, usually remain free until, presumably after forming dicentric and acentric isochromatids, they occasion the death of the ensuing zygote. Even in those cases where a broken end succeeds in uniting with another one, derived from a different break, union of the reciprocal broken ends seldom occurs. Thereby a half-translocation is formed which except in special instances results in a lethal degree of aneuploidy in a zygote that receives it[50-54].

The types of structural changes induced in oocytes that are oftenest to be detected in viable offspring are (1) those that have involved interhomologue exchanges in the heterochromatic region[55-57] ("pseudocrossovers" that seldom act as recessive lethals), and (2) deficiencies, acting as recessive lethals. We infer the deficiencies to have usually been caused by two breaks in the same homologue. For they do not have a tendency to be associated with crossing over. Moreover, their frequency varies more steeply

than the dose and falls off if the dose has been given in a much protracted or fractionated form, thus following the pattern of multi-break changes[58-59]. This inference fits in with an observation of Lüning's[26] that more Minute-bristle changes (known to be usually deficiencies) arise after irradiation of oocytes than of spermatozoa.

This exception to the rule of a linear relation between dose and recessive lethals raises anew the question of why, after irradiation of spermatozoa, the relation appears linear even at high doses. For lethals thus produced include a sizeable proportion of deficiencies and other multi-break structural changes. Our interpretation is that this linearity at high doses is a synthetic product of two oppositely acting factors: (1) the presence of multi-break deficiencies and positional lethals that cause the total frequency to rise above the straight line, and (2) the operation of selection in eliminating, via dominant-lethal chromosome aberrations, the offspring of more of the germ cells that at irradiation were in a more susceptible stage. The latter factor, causing a depression of the frequency ever further below the line at high doses, could mask the influence of the first factor. When irradiation is applied to groups of germ cells known to be highly heterogeneous, the second factor predominates[60] and there can be a marked drop below linearity when high doses are applied to post-meiotic male germ cells. It is to be expected that the more homogeneous in its susceptibility the material is, the more evidence there will be of a rise above linearity, as appears to have been the case in experiments of Edington's[61] on Drosophila spermatozoa. It is quite possible that oocytes are usually of more homogeneous susceptibility than spermatozoa. Moreover, for doses giving the frequencies of recessive lethals in question, fewer dominant lethals are produced and there is correspondingly less opportunity for selective elimination among the products of irradiated oocytes than spermatozoa[25].

On the above interpretation, lethal and other induced point-mutations do not ordinarily represent restitutions of breaks that occurred at or near the affected locus. Were that the case not nearly so many recessive lethals should be induced in ring than non-ring chromosomes, because of the greater difficulty of exact (non-torsional) restitution of broken rings, yet recent retests of this question by Oster (unpublished) confirm the fact that at low and moderate doses applied to spermatozoa as many lethals are produced in the rings as in the non-rings. At very high doses, to be sure, the frequency in the non-rings does become greater. But this is to be expected in any case because of the inclusion in the non-ring data of many cases of structural change which, had they happened in rings, would have formed aneucentric, inviable combinations.

If we grant the above thesis that point mutations, unlike structural changes, do not involve "effective" chromosome breaks (i.e. breaks, the ends derived from which are capable of union with ends derived from distant breaks), then it is also to be inferred that those accessory conditions, such as oxygen tension, that influence the production of both point mutations and structural changes in the same direction and to the same degree, exert their effect on the primary mutational processes of point mutation and breakage, which are obviously related, rather than on the process, subsequent to breakage, of union between "effectively" broken ends. Whether or not

an influence in question is of the same degree for these two general categories of mutations can be determined by the method used in the following example. In Oster's tests[13] of sex-linked lethals induced in the spermatozoa of late pupae, their frequency after irradiation in oxygen was 2.5 times that after irradiation in nitrogen. For translocations the corresponding figure was 3.8 times. Since the lethals vary with the dose and the translocations with its 1.5 power, an agent that increased lethal production by 2.5 times would, if it affected translocation production to the same degree (i.e. that of a dose 2.5 times as great), increase translocations by $(2.5)^{1.5}$, that is, by 3.9 times. This result is in good statistical agreement with the one obtained. Turning now to the results on the irradiation of spermatids in these two kinds of atmosphere, one finds the effect on lethals much larger than before, namely, about 6 times. This gives an expectation of $(6)^{1.5}$, that is, of 14 times, for the translocation enhancement in spermatids. Again the calculated ratio is in good agreement with the observed one, of 11(±2) times.

Complications can arise in the interpretation of the mode of operation of accessory conditions when, as in the case of oxygen tension, they are able on occasion to affect more than one step of the mutational process. Thus, in Abrahamson's work on the production of two-break structural changes (detachments of attached X-chromosomes) in oocytes, the oxygen tension existing at the time of irradiation had an effect of the same kind as above noted[62,63]. However, a reduction of oxygen tension following irradiation or between two irradiations caused an enhancement of the frequency, presumably by delaying unions between broken ends and thereby making restitutional unions less likely.

The difference in the mechanism of origination of point mutations and structural changes does not justify the conclusion that the former are immediately and irrevocably completed at the moment of irradiation. The conception of a "recovery" process for chromosome breaks in spermatozoa contained in the male, which has been founded on evidence supplied by Baker and von Halle[33] and by Lüning's group[27-29], may well prove applicable even to point mutations, as indicated by work of Nordback and Auerbach[64]. However, a stage of completion and stability of the mutation is finally reached. Except in the special cases where germinal selection operates, these finished mutations must accumulate accurately as a result of repeated or chronic irradiation.[8]

Despite the conditionality of the mutational process and the great influence exerted by genetic, developmental and extrinsic factors in the origination of both "spontaneous" and radiation-induced mutations, there seems in many respects to be a remarkable agreement between these phenomena in Drosophila and mammals, except for the far higher per-locus radiation susceptibility of the mammalian genes tested[65]. Selection has apparently been at work to produce a similar over-all spontaneous mutation rate per generation in the two groups, in the face of their great differences in life span, DNA content, and other respects, and has likewise resulted in similarities in the mode of expression of their genes. Support for these inferences is to be derived from the fact that recent analyses of human mutational load[66] lead to mutation-rate estimates like those earlier based on a quite different type of extrapolation from Drosophila than those here made use of. This agreement attests to the significance of the studies on Drosophila in relation to the problems of the genetic effects of radiation on man.

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