THE GENETIC BASIS OF X-RAY INDUCED RECESSIVE LETHAL MUTATIONS[1]

IRWIN H. HERSKOWITZ

Department of Surgery, Louisiana State University, School of Medicine, New Orleans, Louisiana

Received September 22, 1950

X-RAY induced recessive lethals have been visualized as resulting from point mutation, deficiency, and position effect. The question arose as to the proportion of such lethals associated with chromosomal breakage and the proportion produced independently of breakage. Although it is possible all three types of lethals occur at or near points on chromosomes that have been broken, only point mutation would seem to be capable of arising without chromosomal breakage as a prerequisite. LEA and CATCHESIDE (1945) and HERSKOWITZ (1946) have analyzed the available data and concluded that the great majority of recessive lethals occurred in association with the phenomenon of breakage. At that time it did not seem necessary to postulate any appreciable number of lethals were due to point mutation unassociated with breaks or to position effect. Since then, however, a number of inconsistencies have been noted in this hypothesis, chiefly by FANO (1947) and MULLER (1950), and accordingly, it seemed desirable to reexamine the problem at this time.

Several studies with *Drosophila melanogaster* have shown an intimate relationship between the occurrence of chromosomal rearrangements and recessive lethals. MULLER and ALTENBURG (1930) found that many translocations were accompanied by a recessive lethal effect. OLIVER (1930, 1932) and HERSKOWITZ (1946) irradiated mature sperm of the Canton-S stock, detected lethals on the X chromosome, and subsequently tested them for association with gross chromosomal rearrangements. Using the same criteria, they found the percent of lethal cultures showing rearrangement increased with dosage and that, in almost all cases, it was impossible to separate the lethal from the rearrangement by crossing over. The same type of observation was made by DEMEREC (1937). After examining a number of lethal cultures for their association with chromosomal rearrangements, he found an increase with dosage in the percent of lethals which were associated with rearrangements. DEMEREC also found that in more than 90 percent of the cases the lethal was at or very close to a breakage point of the rearrangement. Other data relative to this are found in DEMEREC and FANO (1941).

There are two ways a lethal associated with a chromosomal rearrangement might arise. Either it is produced directly or indirectly by the ionizing radiation that causes the break or it results when broken chromosome ends unite

[1] This study has been supported by a grant from the NATIONAL CANCER INSTITUTE, U. S. PUBLIC HEALTH SERVICE.

in new arrangements. The former type of lethal is due to an association with the process of breakage, the latter to the position effect following breakage. No attempt is made, in this paper, to determine the proportion of those lethals connected with breakage resulting from point mutation and from deficiency.

Lethals not associated with rearrangements might also have two origins. They may have been produced at or near breakages that returned to their old position, or they may be due to point mutations which arose without connection to radiation that produces breaks.

In order for an hypothesis of lethal origin to be considered adequate, it should conform with the data on the increasing proportion of lethals associated with rearrangements with increased dose and provide a linear increase for lethals in viable sperm with dose (see CATCHESIDE 1948, fig. 19). It should also be consistent with the number of breaks postulated according to the dominant lethal calculations of HALDANE and LEA (1947), which are considered to be essentially correct. It should be noted also at this point, that MULLER has repeatedly emphasized that appreciable numbers of lethal break-free point mutations and lethal break-dependent position effects occur in Drosophila from X radiation (see MULLER 1950).

Nevertheless, LEA and CATCHESIDE and I adopted the hypothesis that all lethals arise in connection with breaks and relegated to position effect and non-breakage mutation a relatively small and undetectable role in recessive lethal production. LEA and CATCHESIDE reasoned that if lethals associated with rearrangements are not fundamentally different from other lethals and are included in rearrangements only because the ionizing particle causing the lethal also caused the break entering the rearrangement, the total observed number of lethals would be proportional to dose, the number of lethals associated with gross structural change would increase as $(\text{dose})^{3/2}$, and the residual number would increase less rapidly than the first power of the dose. This hypothesis seemed to fit the data and was accepted by them. It followed, from this view, that a number of lethals unconnected with rearrangements would really represent lethals located at breaks which had restituted. LEA and CATCHESIDE then proceeded to see how far a consistent picture could be obtained on the basis that all lethals were connected with breakage with position effect playing no role. On the assumptions that lethals associated with minute rearrangements are lethal because one or more loci are deleted and that when a chromosome is broken in two places there is equal chance that the segment between the breaks shall either be deleted or inverted, they were able to calculate from their data on lethals at 3000 r that the number of breaks of all sorts in the euchromatin of X chromosomes of 100 viable sperm should be 22.6. This number of breaks is perhaps subject to considerable error since, as was pointed out by KAUFMANN (1947), the data at 3000 r came from a study of a small number of lethals.

It was possible for LEA and CATCHESIDE (1945) to obtain another estimate of the number of breaks in the X chromosome euchromatin of viable sperm from their studies of the proportion of sperm remaining viable after X ray

treatments. Although possibly an oversimplification, it was assumed that dominant lethals are solely the result of single unjoined breaks and asymmetrical interchanges. A mathematical expression for the yield of dominant lethals with dose was derived after the problem was simplified by neglecting the possibility that more than one break may occur on a single chromosome arm and by supposing that joining between various broken ends is at random. The values they arrived at agreed with the data on the percent of dominant lethals as a function of dose as well as the percent of viable sperm having gross structural change at different doses. Taking .162 as the fraction of all breaks that occur in the euchromatin of the X chromosome, (deduced by FANO 1941, from the observations of BAUER 1939), the number of breaks they postulated at 3000 r was 19.9 per 100 viable sperm. HALDANE and LEA (1947) developed less simplified equations, involving five chromosome arms and without limiting the number of breaks thereon, that fit the same data. They postulated about 19.5 breaks under the same experimental conditions.

HERSKOWITZ (1946) has also assumed that all recessive lethals are produced at points of breakage, with position effect and point mutation unassociated with breaks contributing a negligible number of lethals. Arguing that if the probabilities of a break being a recessive lethal or of entering a viable gross rearrangement are independent, the product of these frequencies would be the frequency with which a break produced a lethal and also entered into an observed gross rearrangement. It may be calculated from this, using the available data, that 23.2 breaks should occur per 100 viable X chromosomes at 3000 r (see CATCHESIDE 1948).

A number of objections can be raised to this hypothesis. If the number of rearrangements that are inviable increases in an exponential manner with dose, the number of breaks lost in inviable cells becomes more important as the dosage mounts and should cause the frequency of breakage in viable sperm to increase less than linearly with dose. This tendency is manifested, in the dominant lethal theory calculations of LEA and CATCHESIDE as well as of HALDANE and LEA, by the number of breaks postulated in viable sperm increasing slower than (dose) [1]. If all lethals occur at breaks, with position effect playing no part, LEA and CATCHESIDE pointed out that the number of recessive lethals in viable sperm should increase with dose in the same manner as the mean number of surviving breaks. According to their dominant lethal theory calculations this would mean lethals should increase as the 0.84 power of the dose, and according to HALDANE and LEA as the 0.92 power of the dose in the interval 1500–6000 r. Since the observed recessive lethal mutation rate shows a rather exact proportionality to dose there would still seem to be a real discrepancy between the postulated and the observed mutation rates. FANO (1947) and MULLER (1950) also recognized, according to this hypothesis, that the frequency of recessive lethals should increase less than linearly with dose because of the inroad that inviable rearrangements make into the original number of lethals. Using general arguments, FANO estimated that at 3000 r the observed mutation rate is at least 20–25 percent

higher than the theoretical rate. In my 1946 paper it was implied incorrectly that the number of breaks postulated was the number originally produced in sperm. These breaks really represent the number in viable sperm, interpreted correctly by CATCHESIDE (1948), and, in view of the discussion above, should increase slower than the first power of the dose. MULLER (1940) cites work done in collaboration with OFFERMANN in which the lethal mutation rate was as high in irradiated ring chromosomes as in non-rings. If all recessive lethals occurred at places of breakage, one would expect a decrease in lethal rate for rings as compared with non-rings due to the factor of torsional restitution which reduces the frequency with which restituted ring chromosomes are recovered. It is evident that the hypothesis that all lethals result from breakage alone is inadequate.

A primary difficulty to overcome in devising an acceptable hypothesis is that whereas the number of breaks in viable sperm is not expected to increase linearly with dose such a relationship does hold for the number of recessive lethals. Discussion of several modifications of the LEA and CATCHESIDE and the HERSKOWITZ hypothesis for lethal origin now follows.

It may be supposed that while all lethals occur only at or near breaks lethals associated with rearrangements are entirely the result of position effect. Certain arguments may be presented against this interpretation.

First, the frequency of observed lethals unassociated with gross rearrangements should be almost linear with dose, whereas, in practice, it increases less rapidly than the first power of the dose (see CATCHESIDE 1948). Second, LEA and CATCHESIDE (1945) have determined at 3000 r, if the mean number of lethals per chromosome is the sum of a (dose) ^{1} and a (dose) $^{3/2}$ term, the maximum proportion of (dose) $^{3/2}$ lethals which can be admitted without disagreement with experiment is only about 17.5 percent. Moreover, this value must also include some lethals associated with minute rearrangements resulting from two ionizing particles. They calculated that 35 ± 4 percent of the lethals experimentally produced at 3000 r are associated with gross structural change. HERSKOWITZ (1946), using a different combination of data, obtained a value of about 23 percent. Thus, there seem to be more lethals associated with rearrangements than can be due to position effect alone. Third, and finally, FANO (1947) has calculated, using general arguments, that the observed mutation rate at 3000 r is about 33 percent lower than it should be on this hypothesis. In view of these arguments one may reject the hypothesis lethals associated with rearrangements are due entirely to position effect.

Another hypothesis to be considered is that some lethals associated with rearrangements are due to the breakage and others due to position effect (see VALENCIA and MULLER 1949, and MULLER 1950). Assuming that lethals can arise only in chromosomes that have been broken, there may be a certain chance that a break not, or not yet, included in a rearrangement shall bear a lethal, and an additional chance for lethality, due to position effect, for breaks that enter into gross rearrangements. According to LEA and CATCHESIDE's analysis up to about 17 percent of the lethals at 3000 r might be due to

a mechanism such as this. How well does this interpretation fit the data and the HALDANE and LEA calculations? The frequency that a breakage point of a gross rearrangement shall bear a recessive lethal condition is 30/80, or about 38 percent, at 3000 r according to LEA and CATCHESIDE (1945). This value must be, then, the combined chance a break free from rearrangement shall bear a lethal plus the chance a break which has entered a rearrangement shall bear a lethal due to position effect. Thus, the chance that a break unassociated with rearrangement has of being a lethal is somewhat less than 38 percent. When LEA and CATCHESIDE estimated the number of breaks per 100 viable sperm from their study of lethals at 3000 r, on the basis that all breaks due to single ionizing particles had a 38 percent chance for lethality, they obtained a value of 22.6. According to the dominant lethal theory less than 20.0 breaks were postulated (LEA and CATCHESIDE 1945, HALDANE and LEA 1947). In the hypothesis under discussion many breaks have less than a 38 percent chance of being lethal so that the number of breaks postulated would have to be still larger and this would increase the discrepancy. Looking at this hypothesis another way, if the chance any break shall bear a lethal is 38 percent, and 19.5–19.9 breaks postulated to occur in 100 viable sperm at 3000 r according to dominant lethal theory, the number of lethals expected is 7.4–7.6 percent as compared with 8.7 percent obtained experimentally. Since the chance some breaks bear a lethal is less than 38 percent, as it would be if the hypothesis under discussion obtains, the same number of breaks would furnish still fewer lethals and fall short by a greater percent. It is possible to select certain values for the chance breaks in and not in rearrangements are lethal that will furnish the correct number of observed lethals and of breaks at one X-ray dose. However, when such values were applied at other doses, the number of breaks required to obtain a linear lethal mutation rate was always greatly at variance with the number expected by HALDANE and LEA. Therefore, on this hypothesis, position effect lethals cannot increase rapidly enough with dose, to produce a total mutation rate that is linear, without overestimating the number of breaks required.

Still another hypothesis may be suggested. There may be two independent origins for recessive lethals, one group resulting solely from breakage and another independent of breakage, both increasing linearly with dose according to the simplest expectation. This is possible according to LEA and CATCHESIDE's analysis for there is no reason to suppose that all lethals unconnected with rearrangement must result from breakage. However, this idea is subject to the same criticisms as the original one. The number of breaks postulated would be reduced proportionately at all doses but the mutation rate observed would not become linear.

Qualitatively, the problem can be solved by adopting a triple origin hypothesis for recessive lethals. The majority of lethals would result solely from breakage with position effect contributing enough lethals to cause a linear increase in viable sperm with dose despite the less than linear increase in the number of breakages that survive. In addition, lethals would be pro-

duced independently of breaks, thereby reducing the number of breakages required. Given these three origins for recessive lethals, it also becomes possible to formulate a mathematical system which will provide values at different X-ray doses that are consistent with the observed lethal mutation rate and the number of surviving breaks. Because of the considerable error to which certain data to be employed are subject, the calculations that follow are meant to serve more as a model for the hypothesis proposed rather than as an attempt to determine actual values.

Recapitulating, some lethals would be produced independently of breakage, due to point mutations, and would increase linearly with dose. Others would result from breakage alone, and still others from position effects following rearrangement of the breaks. Were there no position effect the number of lethals produced at breakages would increase linearly with dose. Although it is possible that a break might in some cases be lethal if it restituted and not be lethal if transposed to a new location, it is postulated that the net chance for lethality is increased when a break enters a rearrangement, so that the total frequency of lethals resulting from breakage would increase in an exponential manner with dose. Only gross rearrangements will be considered as contributing to the number of position effect lethals. Although it is probable that a number of minute rearrangements also add to this class of lethal (MULLER 1950), this is neglected since it is not feasible to determine what proportion do so.

The data and calculations to follow are summarized in table 1. According to HALDANE and LEA the mean number of primary breaks in 100 sperm per 1000 r is 78.0. Taking their value of aq (0.57), the mean number of primary breaks per sperm per 1000 r which do not undergo sister-union, that best fits the data for dominant lethals and viable rearrangements at doses up to 4000 r, the number of breaks in 100 viable sperm can be calculated for various doses using the 5 arm formulae and table 2 from their paper.

TABLE 1

Analysis of data on recessive lethals in viable sperm according to the triple origin hypothesis for lethals (see text for discussion).

On the X chromosome of 100 viable sperm								
Dose r × 1000	Observed number lethals[1]	Number point mutation lethals	Number lethals associated with gross rearrangements[1] (a)	Number breakage lethals not in gross rearrangements (b)	Number breaks postulated a/.316 + b/.23 = Total			Number breaks[2]
1	2.9	0.6	0.3	2.0	1.0	8.7	9.7	10.1
2	5.8	1.2	0.9	3.7	2.8	16.1	18.9	18.6
3	8.7	1.8	1.8	5.1	5.7	22.2	27.9	26.7
4	11.6	2.4	2.7	6.5	8.5	28.2	36.7	34.9
5	14.5	3.0	3.9	7.6	12.3	33.0	45.3	43.3

[1]CATCHESIDE (1948), fig. 19.
[2]HALDANE and LEA (1947).

Since 20.4 percent (325/1596) of all breaks occur on the X chromosome (BAUER 1939), this percent of the total number of breaks in viable sperm furnished the values entered in table 1. The number of lethals observed in 100 viable sperm is about 2.9 per 1000 r, including a correction for the number of X chromosomes bearing several lethals (see CATCHESIDE 1948, fig. 19). The number of lethals associated with gross structural changes at different doses is also summarized in figure 19 of CATCHESIDE (1948). Experimentally, LEA and CATCHESIDE (1945) found at 3000 r that there were 3.0 lethals associated with gross rearrangements per 100 viable X chromosomes. The number of breaks in the euchromatin of the X chromosome under these conditions which take part in recoverable gross structural change is 9.5 according to BAUER (1939). Therefore, 3.0/9.5, or about 31.6 percent, of all breaks in gross rearrangements bear lethals. The values calculated by HERSKOWITZ (1946) ranged from 30–33 percent for doses covering 1000–6000 r. Accordingly, 31.6 percent was taken to represent the chance a break entered into a gross rearrangement has of resulting in a lethal. To get as good an apparent fit as possible, it is assumed that 0.6 percent lethals per 1000 r occur due to break-free point mutation, and that a break not, or not yet, rearranged has a 23 percent chance of being lethal.

The number of breaks postulated according to the assumptions just made may now be calculated. For the safe of clarity, the calculations made for a dose of 1000 r are presented in detail. Of the 2.9 lethals produced in 100 viable sperm, 0.3 are lethals associated with viable gross rearrangements. These must have arisen from 0.3/.316 or 1.0 breaks. Since 0.6 lethals are assumed to result from point mutation there would remain 2.0 lethals due to breaks not involved in gross rearrangements. These should have come from 2.0/.23 or 8.7 breaks. Thus, the total number of breaks postulated is 9.7 which compares favorably with the 10.1 expected by HALDANE and LEA. The numbers of breaks postulated for other doses are also in satisfactory agreement. At 3000 r, 20.7 percent of the total observed recessive lethal mutation rate would be due to point mutation, 73.7 percent to breakage alone, and 5.6 percent to position effect.

SUMMARY

Several hypotheses concerning the origin of X-ray induced recessive lethals are discussed. It is found that those postulating a single, or even a double, origin for recessive lethals are not consistent with the available data and theory.

An hypothesis is suggested that recognizes lethals arising from three origins: point mutation, independent of breakage; breakage alone; and position effect following rearrangement of breaks. When the number of X chromosome break-free point mutation lethals in 100 viable sperm per 1000 r is assumed to be 0.6, the chance for a break not involved in gross rearrangement to bear a lethal 23 percent, and the chance for lethality for a break included in a viable gross rearrangement 31.6 percent, the number of breaks

involved corresponds to the number postulated by HALDANE and LEA (1947) for different doses and a linear increase in recessive lethals with dose is obtained for viable sperm.

LITERATURE CITED

BAUER, H., 1939 Röntgenauslösung von Chromosomenmutationen bei *Drosophila melanogaster*. I. Bruchhäufigkeit, -verteilung und -rekombination nach Speicheldrüsenuntersuchung. Chromosoma **1**: 343–390.

CATCHESIDE, D. G., 1948 Genetic Effects of Radiations. Advances in Genetics **2**: 271–358.

DEMEREC, M., 1937 The relationship between various chromosomal changes in *Drosophila melanogaster*. Cytologia, Fujii Jubilee Volume: 1125–1132.

DEMEREC, M., and U. FANO, 1941 Mechanism of the origin of X-ray induced Notch deficiencies in *Drosophila melanogaster*. Proc. nat. Acad. Sci. **27**: 24–31.

FANO, U., 1941 On the analysis and interpretation of chromosomal changes in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **9**: 113–120.

1947 Note on the theory of radiation-induced lethals in Drosophila. Science **106**: 87–88.

HALDANE, J. B. S., and D. E. LEA, 1947 A mathematical theory of chromosomal rearrangements. J. Genet. **48**: 1–10.

HERSKOWITZ, I. H., 1946 The relationship of X-ray induced recessive lethals to chromosomal breakage. Amer. Nat. **80**: 588–592.

KAUFMANN, B. P., 1947 Review of "Actions of radiations on living cells" by D. E. Lea. Quart. Rev. Biol. **22**: 330–331.

LEA, D. E., and D. G. CATCHESIDE, 1945 The relation between recessive lethals, dominant lethals and chromosome aberrations in Drosophila. J. Genet. **47**: 10–24.

MULLER, H. J., 1940 An analysis of the process of structural change in chromosomes of Drosophila. J. Genet. **40**: 1–66.

1950 Some present problems in the genetic effects of radiation. J. Cell. and Comp. Physiol., **35**, Suppl. **1**: 9–70.

MULLER, H. J., and E. ALTENBURG, 1930 The frequency of translocations produced by X-rays in Drosophila. Genetics **15**: 283–311.

OLIVER, C. P., 1930 The effect of varying the duration of X-ray treatment upon the frequency of mutations. Science **71**: 44–46.

1932 An analysis of the effect of varying the duration of X-ray treatment upon the frequency of mutations. Z. I. A. V. **61**: 447–488.

VALENCIA, J. I. and H. J. MULLER, 1949 The mutational potentialities of some individual loci in Drosophila. Proc. 8th Internat. Cong. Genet., Hereditas Suppl. Vol.: 681–683.