THE PROBLEM OF DOMINANT LETHALS[1]

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IRRADIATION of spermatozoa with X- or gamma-rays results in the death of a proportion of the embryos arising from the irradiated spermatozoa (Hertwig, 1911; Muller & Settles, 1927), and the question arises how much of this effect may be accounted for by structural changes induced in the paternal chromosomes. The evidence, derived mainly from work on *Drosophila*, allows a discussion of some aspects of the problem.

On the one hand we are faced by an experimental curve showing the correlation between the dosage of irradiation, in Roentgen units, and the percentage of eggs hatching from those laid. Recent measurements by Sonnenblick (1940) and by Fano & Demerec (1941) suggest an approximately 'one-hit' survival curve for low dosages. With high dosages the curve is definitely bent downwards as if some effects due to two, or more, hits played an increasing part.

On the other hand, there is the bulk of evidence collected, from work on both *Drosophila* and plants, on induced chromosome changes and the schemes put forward for the interpretation of their mechanism (see mainly: Bauer, 1939; Muller, 1940; Delbrück, 1940; Sax, 1940; Darlington & Upcott, 1941).

The situation may be summarized as follows:

Irradiation produces breaks in the sperm chromosomes more or less at random. Each broken end acquires a non-polarized capacity for fusion with any other broken end, including its previous partner. But movement is necessary to allow broken ends to come into contact and unite. Hence breaks are 'stored up' in the sperm head until fertilization, when chromosomes become capable of movement; in addition they then undergo reduplication.

The manner in which a broken end will unite is determined by 'competition' between all broken ends according to their proximity, and by the intervening of chromosome splitting. The enormous amount of 'restitution' (Fabergé, 1940) and the disproportionate frequency of changes with two or more breaks in the same chromosome are effects

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of proximity. 'Fractional' changes are effects of the overlapping, in time, of reunion and chromosome reduplication.

The origin of unions between sister chromatids broken at identical loci is still not clear. Sister union may be merely one of the alternatives open to broken ends after splitting of the chromosome; or, as suggested by Darlington & Upcott (1941), it may be caused by failure of the broken chromonema to divide at the end.

Another obscure point is whether, by a process of 'healing', broken ends may lose their capacity for sister or non-sister reunion. In the maize plant, this process is usual for some, but not all, tissues (McClintock, 1941). In *Drosophila* all attempts by Muller and co-workers (Muller, 1940) failed to detect such an occurrence; hence the conclusion that 'healing' rarely, if ever, occurs in *Drosophila*. In his review on chromosomal changes at the last Cold Spring Harbour Symposium (July 1941), Fano put forward the working hypothesis that failure to detect terminally deleted chromosomes, by a special genetic arrangement, may be due to their having some particular lethal action beside that of being deficient.

To summarize, a broken end may unite in several alternative ways: in the original or in a new way; before or after splitting; between sister or non-sister chromatids; and in the same, or in different ways, for the two sister ends. Of these ways only one, restitution, leads to no permanent change, whilst some lead to viable and others to lethal changes.

When one tries to determine what part of 'dominant lethality' can be accounted for by structural changes a main difficulty arises: lethal changes cannot be detected directly by genetic means because of their very lethality. Their frequency must be inferred from that of viable changes by making questionable assumptions. Nor can they be detected by direct observation of early cleavage divisions, because *Drosophila* eggs are not suitable for anything more than a roughly qualitative analysis (see Sonnenblick, 1940). We are exploring the possibility of using the eggs of more suitable species, but even if successful this will only help to a limited extent, because in no other species is so much information of other kinds available as in the case of *Drosophila*.

Some attempts have been made to overcome these difficulties and measure lethal changes. One made recently by Bishop has been referred to by Fano & Demerec (1941). Hyperploids are obtained by the usual system of crossing irradiated males to multiple recessive attached-*X*'s females. Viability is not affected when the deleted *X* 'covers' only a small distal and proximal region. Hence the frequency with which such large deletions are produced can be safely compared with that of inver-

sions having breaks in the same regions. Preliminary results, it is reported, suggest equal frequencies for the two types of change. This would mean that the chance is equal for four broken ends *A*, *B*, *C*, *D* of the same chromosome to unite as *A*, *C*, *B*, *D* (inversion) or *A*, *D* and *C*, *B* (deletion).

Should these results be confirmed, it will be possible to estimate the frequency of *all* deletions from that of inversions. The difference between all deletions (estimated in such a way) and those acting as recessive lethals (to be determined by direct observation) would represent dominant lethal deletions.

However, a clear cut limit between the two types would hardly be expected on theoretical grounds. In other words, it is probable that some deletions reduce the viability of the embryo but not to such an extent as *always* to cause its death. Evidence in support of this is given by the observation (Pontecorvo & Muller, unpubl.) that deletions of the autosomes, lethal in diploids, are often viable in triploids. External conditions would be expected to shift the effect of these 'borderline' deletions one way or the other; in fact, there is some indication that of eggs fertilized by irradiated sperm, fewer hatch at high than at low temperatures.

Another attempt, by Muller and the writer, aims at a *direct* detection of changes that are usually inviable (Muller, 1940; Pontecorvo, 1941; Pontecorvo & Muller, 1941). It concerns the other two types of lethal changes: aneucentric translocations and sister chromatid reunions.

Both these owe their lethality to the deficiencies, and in lesser degree duplications, produced when the affected chromosomes go through mitosis. It is known, in fact, that generally acentric fragments are not included into the telophase nuclei. The dicentric bodies also may not be included into the daughter nuclei of the first cleavage division, or they may undergo repeated breakage-fusion and eventual loss as described by McClintock (1938).

If the loss of paternal irradiated chromosomes could be compensated by extra chromosomes from the mother, an otherwise inviable embryo might survive and, by suitable markers, show its matroclinous origin. With this purpose, aneuploid eggs from triploids were used to compensate for the loss of autosomes, and eggs from attached-*X*'s females for the loss of *X*-chromosomes. Indeed, with the latter arrangement losses of the *Y*-chromosome, for which of course no 'compensation' is needed, are also detected and cannot be discriminated from losses of the *X*-chromosome.

With this technique, flies have been obtained in which a particular

paternal chromosome has been eliminated after simple breakage followed by sister chromatid reunion. Their frequency depends linearly on the dosage of irradiation and with 4000 r.u. is of the order of 1 % for each major chromosome. On the other hand, using the same technique we failed to produce appreciable numbers of imagines in which *two* paternal chromosomes had been eliminated, as would be expected when a dicentric chromosome is formed in the male pronucleus as a consequence of two or more breaks followed by aneucentric translocation. The four cases of this type obtained, among several thousand F_1 flies from triploids, are hardly more than could be expected for coincidence of two losses independently originated. If dicentric chromosomes were usually eliminated, several hundreds of these matroclinous flies should have been obtained.

The survival of embryos in which a dicentric *chromatid* is produced by sister union, when compared with the non-survival of those in which a dicentric *chromosome* is produced by translocation, raises two questions. First, the cause of this different behaviour. Second, the possibility that sister chromatid dicentrics also are not *always* successfully eliminated. In other words that only a part of sister chromatid reunions result in a viable fly detectable with the technique adopted.

Assuming, as a working hypothesis, the latter to be true, then the first question would become: why are sister chromatid dicentrics so much more readily eliminated than translocated dicentric chromosomes? What is known of the behaviour of dicentrics, especially from plant material, offers a basis for explanation.

One must keep in mind that, with the particular genetic arrangement adopted, the fewer the number of cleavage divisions for the total elimination of the dicentrics, the greater the chance of survival of the embryo. This total elimination is achieved by eventual non-inclusion in either or both of the telophase nuclei. The latter case—in other words, *lagging* of the dicentric—is favoured by the small distance apart of the two centromeres (McClintock, 1938). This small distance may be an original feature of any given dicentric, or may be the consequence of a series of successive breakages and fusions. A sister chromatid dicentric, having sister centromeres, will always orientate in such a way as to give an anaphase bridge. Its chance of lagging, with or without passing through the breakage fusion process, depends only on its size. On the other hand translocation dicentrics, which, it must be noted, are always in pairs at anaphase, may become orientated with both centromeres of each dicentric towards either the same pole or opposite poles. The only appreciable chance of at least one daughter nucleus getting rid of the dicentric is when orientation

is of the first type, and the two chromatids make a twist around each other. A small distance between the centromeres favours both this orientation and lagging, but hampers the necessary twisting of the chromatids (Husted, 1936). The required coincidence of too many conditions, some of them contrasting, may well be the cause of the rare elimination of translocation dicentrics.

Some indirect evidence of the mechanism proposed above is at hand. In the first place, the frequency of non-lethal loss was found to be the same for two *X*-chromosomes, one truncated left of *Bar* (a translocation to the tip of IV), and the other of normal composition, both with identical proximal regions. This indicates that only sister union dicentrics originating from breaks near the centromere offer a chance for the embryo to survive.

A second type of evidence comes from a fourfold comparison between non-lethal losses and the amount of change in sex ratio when a rod- and a ring-shaped *X*-chromosome, respectively, are irradiated. In brief, the double size ring dicentrics, or the interlocked rings which are produced by particular types of reunion of broken ends in the case of a ring-chromosome, appear to be mostly lethal. The centromeres of these structures are always at the maximum distance apart, wherever the break occurs, unless a coincident large deletion takes place in the same chromosome. We should expect them to lag only rarely, hence, on our assumption, to be generally lethal to the embryo.

A third type of evidence, only preliminary, is provided by the fact that low temperature during cleavage seems to increase very considerably the frequency of non-lethal losses. It is probable that low temperature increases the chance of lagging, by its known effects on the formation of the spindle.

The three types of independent evidence reported above are far from final. However, on the whole, they support the idea that, with the technique adopted, imagines showing the loss of a chromosome are only the surviving fraction, we do not know how small, of the embryos in which by simple breakage in the sperm a sister chromatid dicentric has been formed on the first cleavage spindle.

It is, therefore, an open question whether sister unions are so frequent as to cause a considerable portion of dominant lethality. Should this be the case, the trend of the curve of dominant lethality could be explained. Most dominant lethality would be determined by single-break sister unions at low dosages, and as the dosage increased lethal changes of the other two types (translocations and deletions) would come to

play an increasing part. Hence the approximately 'one hit' type of the curve for low dosages and the 'two- and more-hit type' for higher dosages.

In conclusion, a clear knowledge of the mechanism of dominant lethality seems necessary as a means for the analysis of the more general process of radiation necrosis. When somatic tissues are irradiated, an extremely complex series of effects is produced. Some have their primary origin in the cytoplasm, others in the nucleus, perhaps others in the achromatic constituents of the mitotic apparatus. Irradiation of the sperm and detection of what follows in the embryos offer a means of discriminating between some of these effects.

The comparative use of X-rays and ultraviolet radiation should enable the analysis to go still further.

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