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The production of chromosome structural changes by radiation

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Summary. This paper attempts an update and comment upon some of the topics of chromosome aberration formation which Lea raised in Chapter VI of his classic work 'Actions of Radiations on Living Cells'[24]. Only the first nine sections of this chapter are covered, which deal primarily with the qualitative aspects of aberrations, their formation, classification and interrelationships. In commenting upon these topics, pertinent references are made to work with mammalian and human cells.

Increased knowledge of the importance of DNA as a fundamental target and the integral part it plays in the complex structure of the chromosome, coupled with cellular techniques not available to these earlier workers necessitate some revision and modification of early ideas. However, inspite of the enormous accumulation of data and ideas since the original work was published in 1946, the foundation that these early workers laid is still very solid. Surprisingly, we are still puzzled by many of the problems that perplexed them.

Key words. Chromosomes; chromosome aberrations, ionizing radiation; radiation effects; cells.

One of my valued possessions is a 1946 first edition of Lea's book, 'Actions of Radiations on Living Cells'[24]. No one who has read this book and the papers of those early radiation biologists which underly it, can fail to be impressed, almost awed, by the exhaustive and penetrating analysis to which they subjected available data. Every possible avenue was explored and they saw and discussed topics which are still in vogue today, though with a somewhat changed terminology.

The title of this paper is of that of Chapter VI of Lea's book. Limitations of space preclude a full-scale review of this enormous field, so I propose instead an update/annotation on some of the facets raised in the early parts of that Chapter. I shall use the section headings he used (with page reference numbers), but select items for comment which are pertinent to present day interests.

Experimental materials (p. 189)

"For the detailed study of structural changes it is necessary to use nuclei in which chromosomes are large and few in number." There is no doubt that aberration structure can be visualized better in such cells, and plant materials offer a much wider range of cytological fixatives to produce crisper chromosomes. Equivalent mammalian cell cytogenetics was nonexistent in Lea's day but the plethora of work since has produced no surprise aberra-

tion types that were not well documented then, except perhaps the human fragile X[46].

There are, however, two disadvantages with small numbers of large chromosomes. One is the enormous amount of DNA involved (diploid *Tradescantia* with 12 chromosomes, has ~60 pg compared to ~6 pg in humans with 46) and the consequent packed chromosome structure tends to obscure smaller aberrations and precludes recognisable G-banding. The second is the loss of chromosome-type exchange aberrations as the radiation dose increases, because of the high probability of an arm being involved in more than one exchange. This leads to underdispersed aberration distributions and a distorted and saturating dose-response curve[30, 31].

"Chromosomes are, in general, only observable at metaphase" (p. 189) but recently, the technique of precocious chromosome condensation (PCC) has allowed the examination of chromosomes in some parts of interphase, and attempts are being made to quantify very early radiation damage and to investigate its repair[14, 21].

Lea and his colleagues were well aware of the uncertainties introduced by mitotic perturbation and delay, when trying to relate the damage seen to its time of induction in the cell cycle. This point has been largely disregarded by subsequent workers. If there is *any* element of inhomogeneity of radiation sensitivity amongst cells of the population, then delay and perturbation play havock with the observed aberration frequencies and yield-time profiles[36, 37]. This is why no reliable dose-response curve for chromatid-type aberrations has ever been produced.

Another problem Lea touches on in this introductory section is that of cell selection. Referring to *Drosophila* salivary gland chromosomes he recognised that "since many divisions occur between irradiation ... and fixation ... it is evident that inviable chromosome changes will not be observable by this method" (p. 191). This is another problem often overlooked. *A truly random, unbiassed, sample of the original target cells, observed at first post-irradiation division,* is an essential for critical work with aberrations. Only at first division can one see primary structural changes with all parts present. This ideal, the elimination of all selective bias, is seldom achieved in practice, but it should always be our aim. The recently introduced 'harlequin' staining method is one valuable step in this direction[26, 43].

Structural changes and physiological changes in chromosomes (p. 192)

The early radiation cytologists made a clear distinction between structural rearrangements – 'the joining in various fusions of the several breakage ends present in a nucleus' and the 'alteration of the surface properties of the chromosomes so that they tend to stick together' leading to clumping and a curious form of anaphase bridge ('side-arm' bridge).

The former were regarded as the 'direct' (i.e. localized to the vicinity of the ionizing track) effect of radiation, the latter to a generalized effect on the whole surface of the chromosome and interpreted as a depolymerization of the nucleic acid matrix. The integral relationship of DNA to chromosome structure was not recognised at this period. This stickiness was termed the 'physiological effect' of radiation and it was known to be confined to irradiation of cells in mitosis, mid to late prophase to be exact (N. B. Lea's use of the term 'prophase' is not that of current literature, see Savage[31] for discussion of this point). Individual cells are unscorable and anaphases, if completed, show a high frequency of sticky bridges. In contrast, true structural changes arise only in cells irradiated before division, in what we would term G_2 phase and before. In favourable materials, the two effects are clearly separable. It was soon realised that some forms of stickiness could be related to exchanges between subunits of a chromatid (half- or sub-chromatid exchanges, and a considerable spate of interest ensued (see Evans[17] for historical review).

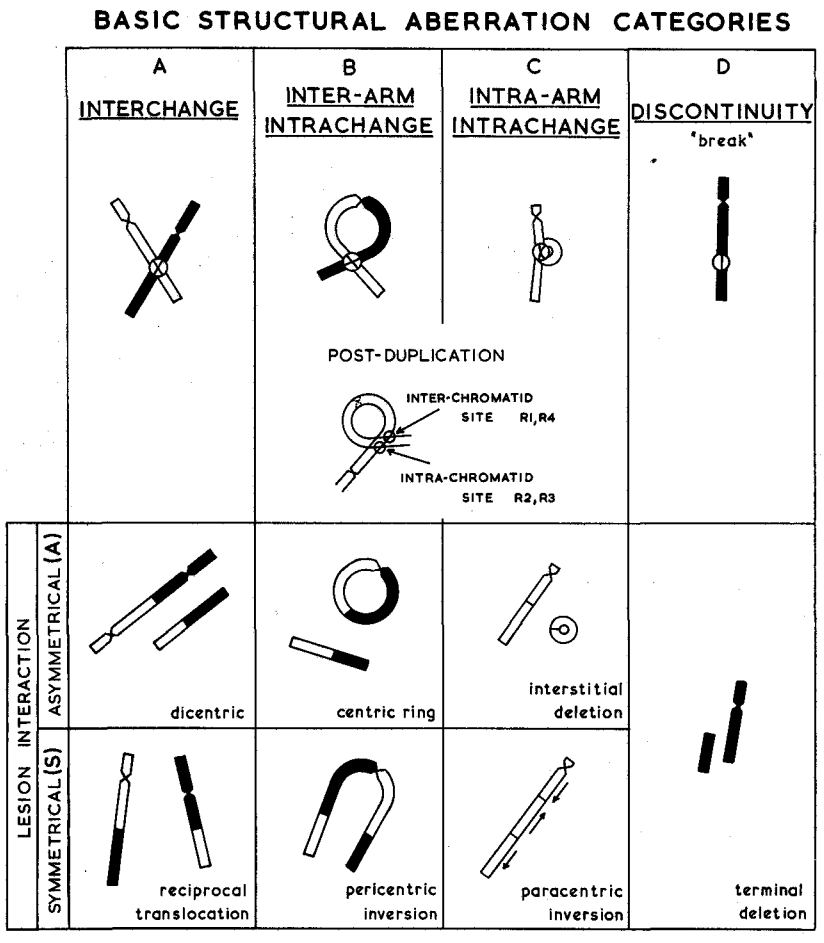
The existence of such sub-units is still a debatable point. Recently, there has been renewed interest in the effects of radiation given close to division, and new ideas are emerging with the recognition that there are a variety of important and sensitive non-histone proteins associated with the condensed chromosome structure[18].

Types of structural change (p. 197)

The various kinds found have been described and documented many times in numerous publications[17, 28, 31–33, 41]. Leaving aside achromatic lesions, all primary types can be reduced to four categories, depending upon the disposition of the lesions or breaks in relation to the chromosome arms (fig.). In the case of 'exchanges' (categories A–C – please note that these do not correspond exactly with Newcombe's categories, discussed by Lea on p. 211) rejoining may be asymmetrical (*A*), which always gives rise to an acentric fragment, and which entails genetic loss and sometimes mechanical problems at anaphase or, rejoining may be symmetrical (*S*) which never, unless incomplete, gives rise to an acentric fragment. The rejoining may be incomplete in one or other direction, but very rarely in both.

The four categories are seen in their simplest form when irradiation is given to unreplicated ('unsplit') chromosomes. Replication then duplicates the exchange so that *both* sister-chromatids are affected at the same locus. These are *chromosome-type* aberrations.

Irradiation of replicated ('split') chromosomes or chromosome regions, leads to aberrations which affect *single* chromatids at any one position, *chromatid-type* aberrations. The presence of sister-chromatids provides for additional kinds of rejoining not possible in unreplicated regions, so there are many more kinds of chromatid aber-



The four basic aberration categories referred to in the text. (From Savage[34], by permission of Elsevier Science Publishers).

rations. However, all these are still reducible to one of the four basic categories.

Given twice the number of arms, plus extra interactions, plus also the fact that *A* and *S* chromatid-types can be scored with equal facility, it is not surprising that, for a given radiation exposure, the frequency of chromatid aberrations exceeds considerably that of chromosome aberrations. Whether the factors mentioned above totally account for the difference, or whether duplicated chromosomes are intrinsically more sensitive to radiation, is a question that has never been answered satisfactorily, mainly because there is no cell system available which displays a constant sensitivity with time for chromatid-type changes.

Lea discusses yield-time relationships, in particular the change from chromatid to chromosome aberrations, as a means of determining when chromosomes 'split', concluding that in *Tradescantia* microspores, it is "between 26 and 32 h prior to metaphase" (p. 199). This was one of the earliest demonstrations of the interphase location of replication. Since then, autoradiographic methods with ^{3}H-thymidine[27] have become available, allowing us to pin-point 'S-phase' with a high degree of accuracy and providing the now conventional division of the cell cycle. More recently, bromodeoxyuridine (BrdU) replication banding methods have provided an even finer division of the cell cycle and the facility to investigate actual programmes of replication at the chromosome band level[9,16,39]. Contrary to the ideas of the early workers, the chromosome does not split along its length like a zipper, but replicates at many positions along its length according to a very precise, repeatable programme, unique to each chromosome. As a general rule, pale G-bands replicate early in S, dark G-bands later and heterochromatin and pericentric chromatin last of all. These BrdU methods can be combined with radiation to investigate aberrations during transition in great detail[35].

Chromosome breaks (p. 199)

These correspond to category D of the figure. The break was considered as the primary lesion from which all other aberrations derive. It had three possible fates. (Note the terminology used; misuse has caused much confusion in cytological literature).

The break can remain open or *unjoined*, to be seen as a terminal deletion. Very few do. Lea estimated ~1/20 in *Tradescantia*. The break can *restitute* to reform the original configuration, and so is not visible at metaphase. The vast majority follow this pathway, >90%. If there happens to be another break close in space *and* time, the two may *rejoin* (in an *A* or *S* manner) to produce a structural *rearrangement* which may or may not be visible at metaphase. Most of Lea's theoretical calculations are based upon the assumption of competition amongst these three fates.

Judging from the literature, chromosome terminal deletions are more common in mammalian cells than in *Tradescantia*. The difficulty, however, of distinguishing them from interstitial deletions (which appear to be *rarer* in mammals than in *Tradescantia*) in small chromosomes may lead to confusion. There are enormous discrepancies for frequencies of acentric fragments in human radiation cytogenetic literature.

Chromatid breaks (p. 201)

"The chromatid breaks vary in distinctness, sometimes appearing only as achromatic lesions in the chromatid, and are not easy to score with accuracy." Here lies the hint of a problem and a seed for a stormy controversy that is still unresolved today. The problem is reducible to three questions: a) Is there a true difference between 'breaks' and 'achromatic lesions' or are they just different manifestations of the same phenomenon as Lea's statement implies? b) Given a difference, what is the nature of each? and c) what are the distinguishing criteria for scoring purposes?

With regard to a) it was soon realised that for similar doses of radiation, chromatid breaks were far more common than chromosome breaks relative to other aberration types. Moreover, they increased dramatically in cells irradiated close to division. Controversy came to a head when Revell[28, 29] introduced his 'exchange hypothesis' which presented a direct challenge to the 'classic' or 'breakage-first' hypothesis, exemplified by Lea and universally held. Briefly, he argued that the primary event was not a 'break' in the chromosome thread, but an unstable lesion. Left to itself, this would decay (today we would say 'repair') but some would enter into an unstable association with another lesion close in space and time. This association (which is termed a 'state of exchange initiation') was committed to exchange but was not the exchange itself – this latter could occur at some later stage in the cell cycle. Divorcing 'initiation' from the 'exchange process' meant that exchange was not irrevocable within a short time of irradiation, and thus post-irradiation modification of aberration yields (a known phenomenon) could be accommodated (It was precluded by the classic hypothesis). At the time of exchange, a proportion would fail and be incomplete (i.e. some parts fail to join up). Following chromosome condensation, these failures would *appear* as breaks ('discontinuities') but these breaks were *secondary*, not primary as required by the classic hypothesis.

Certain implications about numerical relationships between chromatid intrachanges and breaks followed from the hypothesis, and these were immediately challenged, because chromatid breaks were more common than any stretching of the hypothesis would allow. Arguing that *real* breaks would lead to anaphase fragments Revell showed that such fragments were relatively rare, so that most achromatic lesions ('gaps') were not true breaks. Once this distinction was made, the numerical predictions of his hypothesis were in good agreement with observation. Conger[12] made a test in the *Tradescantia* pollen grain system and found real breaks very common. Tests in *Tradescantia* pollen tubes were, however, in very good agreement with Revell's predictions[40].

There have been many tests of the hypothesis since, and to date, the predictions appear to hold only with irradiation given to post-S cells, and not at all for chemically induced aberrations.

The relative frequency of true chromatid discontinuities varies from species to species, but is never very high, and this is borne out by the relatively low frequencies of micronuclei found when actively dividing cell populations (where chromatid-type aberrations will predominate) are irradiated.

Our knowledge of DNA and radiation effects upon it, the real existence of single- and double-strand breaks and the fact that there appears to be a well-established (but controversial) relationship between breaks and aberrations[3, 10] (and see also recent work on restricition enzyme double-strand breaks and aberrations [7]) means that the idea of an initial unstable radiation lesion which is not a break is difficult to sustain. But, side by side with this, we have to put the weighty evidence that repair mechanisms abound in the cell and the operation of these on lesions that are *not* breaks, can certainly (e.g. in the case of ultraviolet (UV) light and chemicals) lead to aberrations[1, 2].

In all favourable materials, it can readily be seen that some of the breaks are indeed incomplete intra-arm intra-changes (category C) but for mammalian cells, the current concensus must be that not all are.

Turning to the nature of the chromatid achromatic lesion, let us summarise the facts.

1) Overall frequency varies with stage of cell cycle irradiated. They are maximal in cells very close to division, very infrequent in early S and pre-S cells. In contrast, true structural changes appear only when achromatic lesions are declining.
2) They show great variety in expression. Some are very long, others failing to traverse the width of a chromatid. The majority are about a chromatid-diameter in length.

3) They are visible with phase-contrast and are Feulgen negative, indicating very reduced or missing DNA content in the region.
4) They are often found associated with structural aberrations, e.g. at the presumptive break-point site in an exchange (like a scar).
5) They are capable of being stretched at anaphase and some will obviously contribute to acentric fragments by *secondary* breakage.
6) They do not appear to give rise to structural aberrations in the subsequent cell generation (though some really critical experimentation is needed to establish this point unequivocally).
7) When cells with plentiful achromatic lesions are G-banded, the frequency drops, suggesting that many occupy pale G-band sites.

Many attempts have been made to solve the gap/break problem. Higher resolution methods using UV have given clear evidence of chromatin continuity through the region[42].

Transmission and scanning electron microscope studies (particularly those where light and EM methods are compared[4]) show both breaks and gaps with and without continuity of the basic 30 nm chromosome fibre! Clearly, at this point in time, an absolute distinction cannot be made. The current criterion for distinction with the light microscope, i.e. that breaks show a clear dislocation of the fragment whilst gaps retain alignment, is useful, but likely to underestimate true breaks. The fact that gaps are maximal at the time of maximum chromosome condensation, suggests that, in part, they represent errors in packing. What the lesion is that gives rise to them is still a mystery. Those who see all aberrations in terms of damage to DNA, have suggested that they could be manifestations of unrepaired single-strand breaks[3].

Purists try to make a distinction between discontinuities that are clearly the result of failed intrachange and those that are not – "any complete severance of the chromatid thread showing clear dislocation and not obviously derived from an incomplete exchange process"[32]. However, from what has been said above, the element of subjectivity cannot be eliminated and each worker has to decide and then rigidly adhere to, his own criteria.

Isochromatid breaks (p. 202)

"... instances where both chromatids are broken at the same level". This could also be taken as a definition of a chromosome-type terminal deletion and Lea raises the question "whether we are justified in regarding isochromatid breaks as twin breaks produced at the stage when the chromosome is already split or whether they are ... produced by breakage and *subsequent* splitting of a chromosome which is unsplit at the time of irradiation" as had been suggested by Darlington[15].

He urges two arguments as evidence for their post-split origin. First that they are found at the same time, and often in the same cells as chromatid interchanges (which must be post-split aberrations). This argument is considerably weakened when we remember that all S-phase cells contain a mixture of split and unsplit chromosome regions. We can now eliminate S cells from a sample by using ^{3}H-thymidine or BrdU and score only post-S cells, which have virtually no unreplicated regions. In these we find high frequencies of isochromatid deletions.

Isochromatid aberrations almost always show sister chromatid fusion, 'sister union', at both ends (SU) or in the centromeric or acentric fragment (NUd, NUp). Incompleteness at both ends (NUpd) appears to be very rare. In contrast, chromosome terminal deletions *never* show sister union. Taking advantage of this fact, Thoday[49] was able to demonstrate an exact parallelism with other, unambiguous, chromatid aberrations.

Careful examination in good materials often shows the presence of 'gap' scars at the presumptive site of sister union joining. Almost invariably, the scars are offset and not terminal. Moreover, in the NUp and NUd types, the unjoined ends are not always of equal length. These facts indicate that the breaks are not strictly 'isolocus' and that we are dealing with a chromatid intra-arm intrachange[29,32]. This would be further evidence that the lesions are introduced into already duplicated chromosome regions.

Lea's second argument, based upon his belief that the majority of isochromatid breaks were produced by the passage of a single ionizing particle through both threads, came from the work of Swanson[47,48] who had demonstrated that UV light could induce chromatid, but not isochromatid breaks in *Tradescantia* pollen tube chromosomes. Ionizing radiation produced both kinds plentifully. The UV quantum absorptions, being random, would only produce breaks at the same locus by chance, so isochromatid aberrations would be very rare in split chromosomes. Unfortunately, subsequent work in this and other cell systems indicates that *all* forms of chromatid type aberrations are produced with UV, including isochromatid breaks complete with sister unions[1,23].

The principle lesion produced by UV is well characterised, a cyclobutane dimer formed between adjacent pyrimidine bases in DNA. This can be repaired by several known enzymatic pathways, and in subsequent S-phase, chromatid-type aberrations are produced. Thus, like many other known DNA damaging agents, the conversion of UV lesions to aberrations is S-dependent, and therefore, strictly speaking, *chromatid* aberrations are arising from lesions in pre-duplicated ('unsplit') regions. In the light of this, it is rather remarkable that the early, successful, work with UV was done in *Tradescantia* pollen tube generative cells, since this is a *pure* G_2 system; generative cell DNA synthesis is completed some 48 h before the pollen becomes available for germination[31].

In mammalian cells aberration frequencies are very low in post-S cells [1].

The relative frequency of radiation-induced isochromatid: chromatid breaks varies as the cell transits the cycle. In general the ratio is higher closer to division, mid to late G_2 and falls as we move back into S. This is most probably a reflection of changes in aberration completeness, perhaps linked with changes in time for, or capacity of, repair (together with proximity/condensation factors) rather than a simple change in the duplication status, as Lea suggests.

The various forms of isochromatid incompleteness also vary with cycle transit. The frequency of doubly incomplete (NUpd) forms is a real problem area, and quoted values a widely even within the same cell type. The fact that complete, multilesion, intrachanges occur, which can simulate simple intrachanges [32] serves to cloud the issue and add uncertainties to data.

The effect that radiations of different linear energy transfer have on incompleteness, is also a problem area. It is not simply a case of 'unrejoinability' of broken ends; there may be differential effects on different kinds of aberrations [40].

Completeness is difficult to assess accurately in mammalian chromosomes. The recent introduction of silver staining for the chromosome 'core' [22], looks as if it will help here.

Chromosome intrachanges (p. 205)

Both breaks within the same chromosome: in opposite arms, *inter-arm* intrachanges (category B, fig.) or in the same arm, *intra-arm* intrachange (category C, fig.). The rejoining may be *A* or *S*. The *A* forms (centric ring, interstitial deletion) are readily visible, the inter-arm *S* forms only if they produce some obvious change in centromere position. Intra-arm *S* forms cannot normally be observed in conventionally stained chromosomes.

The introduction of chromosome banding in the 1970s [44, 45] has greatly facilitated the detection of *S* types. Paracentric inversions, however, are missed, since most are very small and, moreover, their detection will vary throughout the karyotype depending upon the longitudinal distinctiveness of the local band pattern [33]. In passing, it is worth noting that although these are *S* types, without chromatin loss or mechanical problems at mitosis (and therefore readily transmitted) they could have important genetic consequences. On the assumption of a unineme model for the chromosome (i.e. effectively one molecule of DNA running from end to end), and strand polarity preservation in exchange, this aberration involves not only segmental inversion, but also strand interchange, such that an inverted, non-reading (nonsense) segment will be encountered by the reading apparatus. Segregation of strands will produce two different kinds of altered proteins in progeny.

The anaphase segregation patterns of centric rings have been the subject of several studies. All the familiar duplication patterns of the Moebius strip with different numbers of half twists up to 3 ('trefoil') have been reported. Chromosome duplication was envisaged by Lea and these early workers as splitting along a fixed cleavage plane and twists were included in, or introduced into, this plane at the time of ring formation. We would anticipate, therefore, a relationship between ring-size and anaphase configuration; only large rings being interlocked and small ones always 'falling free'. This appears to be the case.

An alternative to cleavage plane twist is the occurrence of sister-chromatid exchanges (SCE) within the ring at the time of replication [6] which would also produce ring-size related frequencies. In theory, it is possible to distinguish between the two hypotheses, by studying the interlock patterns of dicentric-rings in a subsequent division. However, the proven existence of SCE and the presence of topoisomerases which can decatanate interlocked DNA [18], will confound any experimental test.

Lea says (p. 206) "It is of some interest to know whether the alternatives (*A* and *S* rejoining) – are equally probable, but it is not very easy to obtain information on this point". However, the advent of chromosome banding, which allows efficient detection of *S* events when the breakpoints are well separated (as in the case of chromosome-type translocations and pericentric inversions, fig.) can provide the data, and tests in human cells have shown that *A* and *S* are true alternatives [38]. This does not, of course, imply that at a particular locus and at a particular time, there is not a predisposition to one or other mode.

The modal size of 'about 1 μm in diameter' quoted for interstitial deletions in *Tradescantia* is probably an overestimate because of the limits of microscopical resolution [31], but it is clear, in all organisms investigated, that the break points are usually very close together.

Chromatid intrachanges (p. 207)

Lea devotes two short paragraphs to this category and whilst recognising the existence of intra-arm forms, he neither illustrates nor discusses them. Interestingly, it was this dismissed category which was to form the spearhead for the attack on the most fundamental of Lea's tenets – 'breakage and reunion'. The alternative 'exchange hypothesis' [28, 29] has been briefly described above. All the chromatid intrachange forms are figured and discussed by Savage [32, 33]. In scoring these forms, it is necessary to recognise that chromosome condensation and concomitant torsional changes can modify and disguise these rather small changes and readily lead to misclassification. The isochromatid deletion really belongs in this category.

The genetic consequences of the various forms are very varied and can readily explain some of the more bizarre changes found in clinical situations[32].
A and *S* interarm forms which Lea acknowledges are difficult to distinguish can only be resolved by early anaphase analysis[32].

Interchanges (p. 207)

Interactions between breaks in different chromosomes (category A, fig.). Here again *A* and *S* forms are possible, the former leading always to an acentric fragment which is compounded from *two* chromosomes and leading to genetic loss and mechanical difficulties at division. The latter are transmissible, and form a large part of surviving radiation damage.
In the case of chromosome types *A* or *S* are equally likely[38] though the latter are only detectable with any efficiency using chromosome banding.
Both forms (*A* and *S*) are equally detectable for chromatid interchanges without banding. Since Lea's original work, there has been uncertainty over equal likelihood. If we separate pollen grain and pollen tube data in *Tradescantia*, the former can be shown to have equal likelihood of *A* and *S*[31]. In other materials, results are variable, and there is an indication that the relative frequency may vary with sampling time after irradiation[5]. There are also additional problems raised by configurational complexities and intrinsic differences between animals and plants[50].
Lea argues that the equal frequency of *A* and *S* is evidence for a lack of polarity in the chromosome and 'makes it improbable that breakage and union occur in linkages in a polypeptide chain, since such linkages are polarized' (p. 210). Using methods that demonstrate the semi-conservative segregation of DNA at the chromosome level, there is now abundant evidence that rejoining is restricted by molecular polarity considerations[3,25] and that the prime target is the DNA[1,2].
Like centric-rings, the anaphase separation properties of chromosome dicentrics have been much studied. The relative frequencies of the three modes ('fall free', 'criss-cross bridge', 'interlock') differ between species, so generalizations are not possible. The rates of elimination have been worked out[8], since these are important in fields such as biological radiation dosimetry.
The bridges and fragments that arise from dicentrics (and rings) are sometimes used as a substitute for detailed aberration scoring, when assessing radiation damage. However, whilst the aberrations which produce them show a curvilinear relationship to dose, anaphase bridges and fragments are almost linear, or saturating, even when scored from the same slides. Lea noted this (p. 237 and fig. 36) but it was a long time before the reason was investigated[11,13,20,51]. There appears to be a dose-dependent loss of bridges (and probably fragments) which results in a flattening of the dose-response curve. There is, as yet, not satisfactory explanation for this loss.
A proportion of all aberrations are 'incomplete', at least as judged from LM studies. EM studies raise considerable difficulties for the meaning of this term[4]. In general, incompleteness is rare (1–2%) for chromosome-type aberrations; higher but very variable (10–40%) for chromatid-type. In chromatid interchanges, incompleteness is also a function of the configuration of the exchange[17,50]. The quality of the radiation can also affect the degree of incompleteness; it is always greater for very high LET radiations, as Lea originally noted.

Concluding sections

In the remainder of chapter VI, Lea moves on to discuss the quantitative aspects of aberrations, first the relationship between the various types, then the distribution of breaks within chromosomes and of aberrations between cells. All of these have been very fruitful areas in subsequent research.
He then considers external factors which can modify observed yields and we can now add many more of which he was unaware.
Radiation intensity, fractionation and dose-response relations form several large sections, for it was upon these results that so much of his later theory rested.
The chapter concludes with a consideration of radiation quality and relative biological effectiveness (including UV light). It is worth mentioning that the ultra-soft X-rays, which proved so inefficient for aberration induction in his hands, are now known to be very efficient for aberrations, mutation and cell killing[19] and this finding has led to considerable revision of ideas about aberration production.
Space limitations preclude any detailed discussion of these concluding sections, but sufficient has been touched on from the early sections to show how solid was the foundation these early workers built. One wonders if the same will be found for contemporary radiobiology in 40+ years time.

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