Classification and relationships of induced chromosomal structural changes

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Summary. A detailed survey is given of the types and classification of primary structural changes that can be induced in chromosomes and observed at the first metaphase after the initial damage. Comments upon identification and scoring are given for the benefit of new workers. The annotation concludes with a brief discussion of the potential relationships between the primary types, and the secondary or derived types encountered in clinical studies.

1. Introduction

Structural changes are being increasingly used in the screening of drug and environmental hazards, and this requires an agreed classification of the aberrations which may be observed. The tendency seen among some workers coming new to these fields to invent their own classifications and criteria, or to lump together different kinds of aberrations under some blanket name like 'breaks' or 'fragments' should be avoided.

The underlying construction of the chromosome makes it unlikely that any structural alteration will be found that has not already been described and documented in the vast literature on radiation-induced chromosomal aberrations. Since this literature (much of which involves plant chromosomes) is not readily accessible to new workers, the purpose of this annotation is to provide a comprehensive survey of the kinds of aberrations observable with visible light microscopy *at the first post-treatment metaphase*, the principles of their classification, and some characteristics which may be of help in identification and scoring. Also included are some brief comments on current controversies with regard to the formation of aberrations so that those wishing to consult original papers may have a working vocabulary with which to start.

In all critical qualitative and quantitative work with aberrations, it is necessary to confine attention to the first metaphase following aberration induction,

as this is the only time when the structural changes can be observed in their entirety (*primary* aberrations). Many kinds lead to mechanical separation problems and genetic loss when the cell attempts division and result in the death of one or both daughter cells. Such aberrations are not seen again. Some kinds of structural change, however, may not cause problems at division, and may be transmitted to further cell generations. These persistent (*secondary*) aberrations are frequently modified in various ways, and it is seldom possible to infer with any certainty their primary form (see *Section 10*). It is the secondary types which are encountered in clinical work.

There have been numerous descriptions of primary *chromosome-type* aberrations induced in human cells as these are the kind most frequently studied (UNSCEAR, 1969; Buckton and Evans, 1973). *Chromatid-type* aberrations in human cells have hitherto received only scant attention, but the increasing use of chemical mutagens and chemotherapeutic agents in experimental work is bound to bring this kind into prominence in the future.

Wherever possible, the descriptions and discussion in the following sections refer to human cells, but it is clear from the large body of data available that, although the relative frequencies of specific aberrations may differ between organisms, the kinds observed are the same whatever cells are used.

2. General principles of classification

The *chromosome* as seen at metaphase of cell division consists of two parallel threads or *chromatids*, those composing one chromosome being termed *sister chromatids*. In each chromosome, the sister chromatids are held together at a definite point, the primary constriction or *centromere* (synonyms: kinetochore, spindle attachment region), which may be situated anywhere along the thread. The centromere partitions the thread into two *arms* (often referred to as *opposite arms* with respect to the centromere). The ratio of the lengths of these two arms (*arm ratio*), expressed as long arm (*q*)/short arm (*p*) or as short arm $\times 100$ /total chromosome length (the *centromeric index*) forms an important diagnostic character in identifying individual chromosomes.

At anaphase of cell division, the sister chromatids of each chromosome are freed by longitudinal division of the centromere, and they migrate to opposite poles of the cell, where they regroup to form daughter nuclei. The key factors in this migration are (1) the presence in the cell of an organized spindle, and (2) the possession by the chromosome of a functional centromere which is attached to certain fibres of the spindle which determine its movement to the poles. Any chromosomal piece without a centromere fails to move, and is usually left out in the cytoplasm where it may form (alone, or with other fragments) a *micronucleus* in one or other of the daughter cells.

In the interval between successive divisions (*interphase*, or resting phase), the daughter cells grow, and each chromatid duplicates (or 'splits'), so that it is once again a double structure when the cell re-enters division.

From early work with ionizing radiations in plants (Mather, 1934; Riley, 1936; Sax, 1938, 1940), it was established that, when cells are examined at the first metaphase after exposure, two broad categories of permanent structural change were distinguishable in chromosomes, *chromosome-type* and *chromatid-type*.

In the former, the changes induced always appear to affect both sister chromatids of a chromosome at the same location, whereas in the latter—with one notable exception (the *isochromatid deletion*)—only one of the sister chromatids is affected at a given location.

Chromosome-types were thought to arise from damage of the chromatid thread in its unduplicated or 'unsplit' stage (corresponding to the bulk of G_1 , the pre-DNA synthesis stage of the cell cycle), and this damage was duplicated along with the chromosome. Damage which affected only one of the pair

of sister chromatids at a particular location, as seen in chromatid-type aberrations, was taken as an indication that the chromosome region involved had already split or duplicated at the time of exposure. Such types arise from irradiation of cells in very late G_1 and throughout the DNA synthesis phase, S and the post-synthesis phase, G_2 .

Classification into these two major categories is still valid, though the passage of time has raised some questions as to their precise relationship to the state of chromosome duplication.

Aberrations may be classified according to their appearance or according to their presumed method of formation. The former 'descriptive' kind of terminology is more usual, and the names and symbols used are intended to be without implications as to the precise mode of origin (for example, Sax, 1941; Catcheside *et al*, 1946; Lea, 1946). The latter kind is exemplified by the scheme of Darlington and LaCour (1945) in which emphasis is placed upon the numbers of breakage and rejoining events required for the aberrations within a cell, rather than individual aberration kinds. The full form is seldom used these days, though a number of the symbols have been carried over into descriptive systems.

The recognition of the highly complex nature of chromosomal organization (particularly at the molecular level); of the complex biochemical repair mechanisms present in the cells; of the great variety of agents which can produce qualitatively identical types of structural change; and, not least, the fact that the limits of optical resolution are such that only a small proportion of structural changes are actually detected has led to the almost universal adoption of a descriptive terminology.

2.1 Definitions of terms

Since structural chromosomal changes can be produced by a very wide variety of agents, the non-committal term *lesion* will be used for the damage or event within the chromosomes which is a necessary prerequisite for aberration formation.

The following basic terms apply to all conventional structural chromosome-type or chromatid-type aberrations.

2.1.1 Exchanges When there is an exchange of chromosome parts after the occurrence and interaction of two lesions:

Interchange: when the interacting lesions occur in the arms of different (homologous or non-homologous) chromosomes,

Intrachange: when the lesions are within one

chromosome. Intrachanges may be further subdivided into:

Inter-arm intrachanges where the lesions are in opposite arms with respect to the centromere.

Intra-arm intrachanges where the lesions are within one arm. In duplicated chromosomes there is the possibility of interaction between lesions induced in sister chromatids. Chromatid-type intrachanges can therefore be further subdivided into:

Inter-chromatid intrachanges where the exchange involves both sister chromatids, and

Intra-chromatid intrachanges where the exchange occurs within one chromatid while its sister remains unaffected in the exchange region.

Interaction of lesions in chromatids or unduplicated chromosomes may lead to an *asymmetrical* exchange, which always gives rise to one or more *acentric fragments* (symbol *ace* Chicago Conference, 1966, Paris Conference, 1971)—that is, pieces of chromosome without a centromere and which therefore tend to be left behind and excluded from daughter nuclei (with consequent loss of genetic information) when the chromosomes move to the spindle poles at anaphase. Asymmetrical exchanges are invariably scorable. Interaction which produces a symmetrical exchange does not lead to an acentric fragment (except when the exchange process is incomplete, see below) and, unless there is some very obvious disparity produced in the lengths or arm-ratios of the participating chromosomes, such exchanges pass undetected in the case of chromosome-type aberrations with the usual staining techniques. In marked contrast, some forms of symmetrical exchange can be scored with an efficiency approaching 100% in the case of chromatid-type aberrations.

More than two lesions may be involved in exchange and so give rise to some very complex configurations.

In all kinds of exchange aberrations, the actual exchange process may be *complete* (*C*)—that is, all the chromosome parts have joined up in their new configuration leaving no free or 'broken' ends, or it may be *incomplete* (*I*), in which case the exchange of parts may be potential rather than actual. In the case of asymmetrical forms, it is possible to designate the incompleteness as *proximal* (*p*) or *distal* (*d*) with respect to the centromere (see Figs. 3 and 5). In all organisms so far investigated, chromatid-type exchanges always show a much higher frequency of incompleteness than chromosome-type exchanges.

2.1.2 Breaks Any descriptive classification must include a category for the *break* or *discontinuity*, a

simple severance of the chromosome or chromatid to give an acentric fragment, and which is not clearly associated with any exchange process.

This category will always comprise a heterogeneous mixture, for whatever hypothesis is adopted for the origin of aberrations (see Section 4) this class will always be augmented by certain incomplete interchange and intrachange events which simulate (and in many cases are indistinguishable from) simple breaks.

2.2 Aberration names and symbols

The terms defined in Section 2.1 form a convenient basis for a systematic descriptive classification of structural changes, and will be used as such in this annotation.

However, over the course of the 50 or so years of research into radiation-induced chromosomal aberrations, certain names or symbols have become assigned to particular aberration kinds and are frequently used in the literature. The more important of these (and their synonyms) are given in the following sections, but it must be remembered that the same name may well be used differently by different authors. Similarly, several different kinds of aberration may be included under one term, such as 'break' (see above).

3. Achromatic lesions ('gaps')

Before embarking on detailed descriptions it will be as well to consider an area of controversy which can be confusing to a new worker.

When chromosomes are examined at the first metaphase after a dose of radiation, or of chemical (and even in the absence of any aberration inducing agent), some of the chromatids may have, in addition to obvious structural changes, small non-staining regions at various positions in their arms. These regions show considerable variation in size and clarity, ranging from a small 'nick' only partially traversing the chromatid to quite long sharply bordered regions. There may also be indications of *slight* dislocation, bending or stretching at the same location.

Most of the gaps are confined to one of the sister chromatids that make up the chromosome arm (*chromatid gap* or *achromatic lesion*, *g*) but occasionally they are found paired at the same location on the arm (*chromosome gap*, *isolocus gap*, *paired achromatic lesion*, *G*).

They are visible with nearly all staining methods (in particular they are Feulgen negative) under bright-field illumination, and with phase contrast. With ultraviolet illumination and with scanning electron microscopy they can be seen to be traversed

by twin strands in the chromosomes of some organisms (Scheid and Traut, 1970, 1971).

Larger gaps are very easily (and frequently) scored as breaks at metaphase, and were, in fact, included in this category by a number of early workers. This can lead to very big discrepancies in break frequencies between different laboratories (for information see Evans, 1962, 1963; Revell, 1974). Nevertheless, when chromatids carrying them are observed at anaphase, it is seen that acentric fragments are not released, and therefore these achromatic lesions cannot constitute a true discontinuity.

It must be admitted straightaway that there is considerable difficulty in distinguishing achromatic lesions from true breaks *when chromosomes are observed at metaphase*, and this difficulty is aggravated by cytological methods like air drying which exert tension on chromosomes during spreading. The accepted criterion for a real chromatid discontinuity is that *a very distinct dislocation and misalignment must be present* between the parts of the arm on either side of the lesion. Even so, there is still an element of subjectivity involved, and each worker must establish by experience (and then rigidly adhere to) his own criterion. It cannot be emphasized too strongly that, in critical work, scores obtained at metaphase must be checked against scores at anaphase. Such checking is normally precluded by the usual cytological methods which involve pretreatment of cells with a spindle inhibitor like Colcemid to accumulate metaphases before sampling.

3.1 Nature of achromatic lesions

In spite of the fact that achromatic lesions have been recognized for a very long time (Sax, 1938), their precise nature still remains a mystery. Almost certainly they must represent a mixture of different types of induced damage, but they are not infrequent in untreated cells in culture (spontaneous frequency ~ 0.022 per cell in cultured blood lymphocytes (Evans, 1970)).

In addition to the facts already mentioned the following are pertinent in any discussion:

a. The frequency is highest in cells closest to division at the time of irradiation—that is, in cells that were in early prophase or late G_2 —and declines as the frequency of true structural changes increases but not in a simple reciprocal manner. They are rare in cells irradiated while in G_1 .

b. They appear to be completely reparable, since corresponding structural aberrations are not seen when cells which would have carried gaps at first division appear in their second division.

c. The majority are not associated with any obvious structural change, or noticeable elongation of the arm in which they occur. However, they are sometimes found in exchange aberrations located in the presumptive region of the actual exchange, rather like 'scars'.

d. Very occasionally, achromatic lesions on different chromatids are associated in pairs by 'stickiness' and accompanying deformation of chromatids, almost as if the process of interchange had been initiated but was not completed.

e. Radiation-induced chromatid gaps show a reduced frequency if the dose is given in the absence of oxygen—that is, a classical oxygen effect—and their radiation dose response curve appears to be linear, suggesting that they are predominantly 'one-track' events (Neary and Evans, 1958). However, arguments based on linearity are very insecure (Savage and Papworth, 1973).

Several suggestions have been made as to the nature of achromatic lesions. Early workers thought that they might represent partial or sub-chromatid breakage (Section 7.4), or 'scars' marking sites of rejoining or points at which exchange was realized. Alternatively, from the observation that they are Feulgen negative regions, it has been suggested that they may represent a loss or depolymerization of DNA, analogous to the DNA steresis observed after ultraviolet microbeam irradiation of chromosome (Zirkle *et al*, 1956). To be visible, such loss would involve millions of nucleotides, and it is difficult to see how this could be without genetic significance and leave a viable cell. Yet another suggestion is that they represent errors in chromosome coiling and condensation, particularly as their frequency is maximal when this process is most active. This would perhaps account for their reparability and apparent lack of genetic significance.

4. 'Breakage-and-reunion' and 'exchange' theories for origin of chromosomal aberrations

As far as classification for scoring purposes is concerned, the origin or mechanics of aberration formation is of minimal importance. Nevertheless, it is impossible to go far in the literature, or into the discussion of aberration types, without some reference or allusion to the two major opposing theories. For the guidance of new workers I have attempted in this Section a simple summary of the salient points of the controversy which I hope will provide a basic framework. For fuller treatment, the reviews of Evans (1962) and Revell (1974) should be consulted.

The first theory (often referred to as the Classical

Theory) is that of breakage-and-reunion, proposed by Sax (1938, 1940, 1941) and developed in detailed mathematical mode by Lea and his colleagues (see Lea, 1946). It is still the most widely held theory. The radiation energy-loss event is thought to result in a *primary break*—that is, a severance of the unduplicated chromosome or chromatid thread producing a pair of broken ends. These broken ends have one of three fates. They may join up again to reform the original configuration (*restitution*), and therefore no structural aberration is detectable at metaphase. It is estimated that >90% of breaks follow this pathway. Secondly, if two primary breaks happen to be in close spatial and temporal proximity, the four broken ends may *rejoin* in a variety of ways to produce the exchange aberrations, some of which will be seen at metaphase. Thirdly, the residuum of primary breaks which have neither restituted nor rejoined remains to be seen at metaphase as simple *breaks*. Thus, on this theory the break is *primary*; everything else is derived from it.

In 1959 Revell formalized and advanced an alternative hypothesis, known today as the Exchange Theory. Basically, this states that the initial damage is not a break (as defined above) but an unstable lesion—a *primary event of damage*, which tends to decay towards a normal or undetectable state. If, however, such events occur, or are brought together, in pairs, they may take part in an exchange process, and so give rise to the aberrations seen at metaphase. In a proportion of these exchange processes, the actual exchange of parts may fail or be incomplete, and a *discontinuity* in the chromosomal thread will result. As the chromosomes proceed towards mitosis, coiling and contraction tend to disrupt the original configuration of the arms that existed at the time when the exchange was established, so that many of these discontinuities when seen at metaphase will be indistinguishable from simple breaks. All breaks are therefore *secondary*, being the result of failed exchanges.

A primary event which is not involved in an exchange process cannot give rise to a break at metaphase, and it decays to a stable, undetectable state. Thus, strictly speaking there is no such thing as 'restitution' as defined for the Classical Theory, for the 'decay' process may be quite different biochemically from the exchange process.

All workers nowadays are agreed that *some* of the breaks observed at metaphase arise from failed exchanges; the remaining controversy turns on the frequency of those that do so. Protagonists of the Exchange Theory believe *all* do, while those of the Classical Theory believe that only a small proportion do so.

The diagrams in this annotation illustrate most of the ways in which breaks can be derived from exchange failure.

5. Chromosome-type structural aberrations

These arise from the irradiation of unsplit or unduplicated chromatids in the early pre-DNA synthesis period (early G₁) of active cells, or from non-dividing cells with unduplicated chromosomes—for example unstimulated blood lymphocytes, liver cells. They are very rarely found after chemical or drug exposure when cells are examined at the *first* post-exposure metaphase. However some (modified) kinds may be found at the second or subsequent divisions, but these are secondary forms derived from primary chromatid aberrations.

Most of the structural aberrations encountered in clinical work are of the chromosome-type, but again these are secondary forms (Section 10) and may not conform in detail to the primary types encountered at first post-exposure division, and which are described in the following sections.

5.1 Exchanges: interchanges (Fig. 1).

5.1.1 Asymmetrical interchange (Synonyms: dicentric, C/C, Paris Conference (1971): dicentric *dic*) When complete, this interchange results in a chromosome with two centromeres accompanied by a single acentric fragment, which however is actually compound, being made up from the terminal pieces of the chromosomes involved.

The distance between the centromeres can vary from being undetectable up to almost the total length of the two arms involved. Some human Robertsonian translocations may in fact be dicentrics with an extremely small intercentromeric region (Mikkelsen, 1973).

The proximally incomplete form gives rise to two chromosomes with shortened arms and a single acentric fragment (Fig. 1). Unless the arm shortening is noticeable, the aberration will probably be scored as a simple break or *terminal deletion* (Section 5.3). The distally incomplete form is a dicentric with two fragments. The frequency with which this occurs suggests that incompleteness is relatively rare, less than 2% in most organisms examined.

The dicentric is probably the most easily recognized and unambiguous aberration. The commonest scoring category in aberration literature is the combined group of 'dicentrics plus centricrics' (for the latter see Section 5.2.1 below).

CHROMOSOME INTERCHANGES

lesions in different chromosomes

pre-duplication disposition
	ASYMMETRICAL	SYMMETRICAL
COMPLETE	(C/C) <u>dicentric</u>	(-) <u>translocation</u>
INCOMPLETE	("C") incomplete proximal (C/C+C) incomplete distal	(C) <u>terminal deletions</u>
COMPLEX INTERCHANGES more than two chromosomes involved		
pre-duplication disposition
examples:-	asymmetrical exchanges in opposite arms <u>tricentric</u>	asymmetrical exchanges in a common arm <u>dicentric</u> with unrelated acentric fragment + <u>complex translocation</u>

FIG. 1. Chromosome-type interchanges.

At the anaphase immediately following the metaphase at which they are observed, the acentric fragment is usually excluded from the daughter nuclei and the dicentric portion may form a double or interlocked bridge with concomitant mechanical difficulties in cell separation. Both daughter nuclei will be genetically deficient, so that cells containing dicentrics are rapidly lost from a cell population. In cultured human lymphocytes the rate of loss is estimated at 40–50% per division (Sasaki and Norman, 1967; Carrano and Heddle, 1973). However, if the intercentromeric distance is very small, bridges may

not form, and dicentrics may persist and be transmitted. The Robertsonian translocations cited above are examples, and there are several cases of dicentric Y chromosomes known in man (Cohen *et al*, 1973).

Complex exchanges The opposite arms (with respect to the centromere) of a given chromosome may take part in different asymmetrical interchanges leading to a range of *polycentrics* (tricentric, tetra-centric, etc.), each exchange event contributing a single compound fragment.

The same arm can also take part in more than one

asymmetrical interchange, but never more than one of these can be observed at metaphase. In such cases, as is illustrated in Fig. 1, the acentric fragment which accompanies the dicentric is unrelated to it, in the sense that it contains a terminal region from a chromosome not participating in the observed dicentric. There will also be present in the cell a chromosome with a complex, non-reciprocal translocation.

5.1.2 Symmetrical interchange (Synonyms: reciprocal translocation, Paris Conference (1971): reciprocal translocation *t* or *rcp*) The symmetrical counterpart of the dicentric which, when complete, leads to the reciprocal transfer of terminal portions of two separate (homologous or non-homologous) chromosomes (Fig. 1). Any length up to an entire arm may be involved and the lengths are not necessarily the same in both chromosomes.

Unless the exchange results in chromosomes with anomalous arm lengths, it will go undetected with conventional staining techniques. It is estimated that efficiency of detection of this form and the pericentric inversion (see below) is not greater than 20% in irradiated human cells (UNSCEAR, 1969). The advent of banding techniques has greatly increased the efficiency of detection but it is still nowhere near 100% (Seabright, 1973).

Reciprocal translocations can be (and frequently are) transmitted to subsequent cell generations because no genetic loss is involved, and there are no mechanical separation difficulties at anaphase. This has obvious genetic implications.

Incompleteness is infrequent, and the resulting acentric fragment will be indistinguishable from a terminal deletion, although it will be unrelated to the chromosome from which it will be judged to have arisen (see Fig. 1).

Because of the inefficiency of detection, reciprocal translocations are commonly omitted when scoring for aberrations.

As with asymmetrical interchanges, a chromosome or chromosome arm can participate in more than one interchange leading to some very complex rearrangements.

5.2 Exchanges: intrachanges

5.2.1 Inter-arm intrachange (Fig. 2) The complete *asymmetrical inter-arm intrachange* is a centric ring (Synonyms: $\bigcirc C$, Paris Conference (1971), ring chromosome, *r*). It is analogous to an asymmetrical interchange where the two arms involved belong to the same unduplicated chromosome, being separated by the centromere. It is

helpful to think of the two lesions involved as being brought into proximity by a loop (see Fig. 2), and in this case the loop contains the centromere. The consequence of the exchange is a ring-shaped centric chromosome and a compound acentric fragment formed from the two terminal regions of the chromosome beyond the point of exchange.

The ring portion may include almost all the chromosome, when the acentric fragment will be very very small, or it may be little more than the centromeric region. In this latter case, unless centromeres in the cell are counted very carefully, a small centric ring may be scored as an interstitial deletion (see below 5.2.2).

Proximal and distal incompleteness is infrequent, the latter (a ring and two fragments) is usually recognizable with care. If banding techniques are not used, proximal incompleteness is likely to be classified as a terminal deletion.

At anaphase the ring portion may separate freely, or may form a variety of loops or interlocking rings, leading to bridges. This, together with the loss of the acentric fragment, usually leads to the death of the cell which contains it. However, persistent ring chromosomes occur in many organisms including man, where examples are known in each of the seven chromosomal groups. The problems of mechanical separation encountered at each successive anaphase lead to considerable variation in size and morphology of a persistent ring in different cells (Smith-White *et al*, 1963; Kistenmacher and Punnett, 1970; Moore *et al*, 1973).

Just as with interchanges, an arm of the chromosome involved in the ring may participate in a second exchange. Either an *A* or an *S* interchange will convert the centric ring to a dicentric with a very complicated arrangement of its component parts, and once again the observed acentric fragment will not be related to the dicentric.

Symmetrical inter-arm intrachanges (Synonyms: pericentric inversion, Paris Conference (1971), pericentric inversion, *inv*) lead, when complete, to the reversal or inversion of a region of chromosome containing the centromere. Unless this results in a very obvious change in the centromere position (a change in arm ratio, or centromeric index) the aberration will be undetectable with normal staining methods. With chromosome banding techniques the larger inversions can be seen, but in practice the majority will be missed with both methods.

The incomplete forms will be recorded as simple terminal deletions, but, as with incomplete symmetrical interchanges, the acentric fragment is not derived from the observed shortened end of the chromosome which accompanies it (Fig. 2).

CHROMOSOME INTRACHANGES

lesions within one chromosome

INTER-ARM lesions in opposite arms			
	ASYMMETRICAL	SYMMETRICAL	
pre-duplication disposition		centromere in loop
COMPLETE	<u>centric ring</u>	<u>pericentric inversion</u>	
INCOMPLETE	incomplete proximal	incomplete distal	<u>terminal deletions</u>
INTRA-ARM lesions within one arm			
	ASYMMETRICAL	SYMMETRICAL	
pre-duplication disposition		centromere outside loop
COMPLETE	<u>interstitial deletion</u>	<u>paracentric inversion</u>	
INCOMPLETE	(M+C)	(M or C')	<u>terminal deletions</u>

FIG. 2. Chromosome-type intrachanges.

5.2.2 Intra-arm intrachange (Fig. 2) *Asymmetrical intra-change* (Synonyms: interstitial deletion, intercalary deletion, minute, double minutes, double dot deletions, **M**, Paris Conference (1971): interstitial deletion, *del*).

The interaction of two lesions within an unduplicated chromosome arm can result in the removal or deletion of a region between the centromere and the end of the arm. The ends of the deleted portion almost always rejoin to form an acentric ring, while

the terminal and centric portions of the parent chromosome rejoin to give a shortened chromosome arm.

The ring structure can be identified by the presence of a distinct lumen but this is obvious only in the larger deletions. Some workers classify these larger forms in a separate category, *acentric rings*. However, the distinction must be arbitrary, as there is a continuous size distribution down to (and almost certainly below) the limits of optical resolution. The majority of interstitial deletions are very small, and most appear as paired dots. In plants, there is evidence of a modal size of about 1 μm , but this may be an artefact of microscopical resolution. No quantitative information is available for size distribution in human cells, but observation shows that the smaller deletions are much more frequent than the larger.

Since the deleted segments do not usually remain associated with their site of origin, but lie free in the metaphase spread, it is rarely possible to assign them to the parent chromosome. For this reason the proximally incomplete forms will appear as simple terminal deletions. Distal incompleteness—that is, an incomplete ring—will be scored as a terminal deletion when large, but, since most deletions are very small, most will still appear as ‘double dots’ and be recorded accurately. As with other aberrations, incompleteness is infrequent.

In published data from human cells, the relative frequencies of interstitial deletions show considerable variation, much larger than that found for dicentric and centric-rings. In part, this must reflect differences between observers in scoring efficiency for this category.

At anaphase, interstitial deletions, like other acentric fragments, are invariably excluded from the daughter nuclei, and the larger ones may form micronuclei. The genetic loss occasioned by large deletions is probably lethal to the cell, but whether the smaller deletions are also lethal is a matter requiring investigation. There is some evidence from plants which indicates that loss of small interstitial deletions does not impair cell survival.

Larger acentric rings may, on separation, show the complex looping and interlocking seen in centric rings.

Symmetrical intra-arm intrachange (Synonyms: paracentric inversion, interstitial or intercalary inversion, Paris Conference (1971): paracentric inversion, *inv*)

Instead of being deleted, the interstitial segment between the two lesions may be reversed (inverted), so that its proximal end is now distal and *vice versa*. No change in arm length is involved, so that even the

largest are wholly undetectable with normal staining methods. The larger examples are resolvable with chromosome banding techniques.

The two incomplete forms will usually be indistinguishable from simple terminal deletions.

5.3 Breaks

(Synonyms: terminal deletions, chromosome breaks, rod deletions, chromosome discontinuity, fracture, **C**; Paris Conference (1971): terminal deletion, *del*)

The *complete* severance of the terminal region of a chromosome arm which gives rise to a shortened chromosome and an acentric fragment, and which is *not associated* with any obvious exchange process. The fragment lies free among the chromosomes of the metaphase spread.

The size of the fragment may vary from a whole arm down to (and presumably below) the limits of optical resolution. Very small terminal deletions will be indistinguishable from interstitial deletions, so that in practice it is impossible to obtain a complete separation of these two aberration forms. Because of this, many workers lump terminal and interstitial deletions together in a single category of ‘fragments’ or ‘deletions’.

The diagrams show that many incomplete aberrations cannot easily be distinguished from a simple terminal deletion, and in Section 4 we have considered whether there is a *true* terminal deletion apart from failed exchanges. However, for scoring purposes, a category for breaks is necessary, but it must always be remembered that it constitutes a heterogeneous mixture of aberration types.

It needs to be emphasized that, when scoring, the acentric fragments derived from asymmetrical exchanges of the dicentric and centric-ring classes are *always excluded* from the category of breaks, for they are not simple terminal deletions, but compound fragments made from two terminal deletions. Moreover, they are an integral part of an aberration, and to include them with the breaks would be to count the aberration twice. Since incompleteness is infrequent in chromosome-type aberrations, most workers adopt the principle of associating *one* fragment with every ‘major exchange’—that is, a dicentric or a centric-ring (UNSCEAR, 1969).

6. Chromatid-type structural aberrations

These arise from irradiation given at the time of, or subsequent to chromosome duplication in interphase.

All the kinds of chromosome changes discussed in Section 5 occur also at the chromatid level, but the

dual nature of the duplicated chromosome opens up the possibility for many new kinds of exchange. Moreover, in marked contrast with chromosome-types, the majority of symmetrical forms can be observed and recorded, and this increases the number of categories available for scoring purposes.

For a given radiation exposure, about two to three times as many chromatid aberrations are found as chromosome-types. Not all of this difference can be accounted for in terms of greater detectability of symmetrical forms and the new interactions possible in duplicated chromosomes. Exact numerical com-

CHROMATID INTERCHANGES				
lesions in different chromosomes				
(c/c)	CENTROMERES POLARISED (P)	ASYMMETRICAL		SYMMETRICAL
		U-TYPE		X-TYPE
	COMPLETE (C)	PUC		PXC
	INCOMPLETE (I)	PUIp	PUId	PXI
	CENTROMERES NON-POLARISED (N)	X-TYPE		U-TYPE
	COMPLETE (C)	NXC		NUC
	INCOMPLETE (I)	NXIp	NXId	NUI
COMPLEX INTERCHANGES				
more than two chromosomes involved.				
examples:-	ASYMMETRICAL	SYMMETRICAL	MIXED	
OBLIGATE (c/c/c)
NON-OBLIGATE (2 c/c)

FIG. 3. Chromatid-type interchanges.

parisons are difficult, however, since the radio-sensitivity of the cell varies in different stages of the cell cycle.

Incompleteness is higher than in chromosome-types, amounting to about 10–25% depending on organism and also with the cell stage exposed and the aberration inducing agent used.

Nearly all chemicals which result in aberrations produce chromatid-types (Kihlman, 1966) and induction appears to be *via* metabolic pathways at specific periods of the cell cycle—in particular, the DNA synthesis phase. The majority of spontaneous aberrations are also chromatid-type.

6.1 Exchanges: interchanges (c/c, Fig. 3)

Exchanges involving two or more lesions situated in the chromatids of different (homologous or non-homologous) chromosomes. *Asymmetrical* (A) and *Symmetrical* (S) forms can be scored with equal facility because the pairing affinity between sister chromatids is retained until late metaphase.

When viewed at metaphase, the centromeres of the exchanged chromatids may both lie on the same side of the exchange (whence they are said to be *polarized*, P) or be opposed to one another (*non-polarized*, N). Occasionally exchanges may have the arms arranged in a cross-like configuration and are sometimes referred to as *quadriradials*.

Polarized exchanges are thought to be a reflection of the polarized condition of the centromeres. This is initiated at anaphase when the centromeres form the leading points of the chromosomes as they move to the spindle poles, and the resulting arrangement of arms appears to be retained within the nucleus during interphase (Evans, 1962). This *centromeric polarization* should be carefully distinguished from *intra-arm polarization* which restricts the re-joining and exchange of chromosome parts (Olivieri *et al*, 1973).

When the disposition of the chromatids at the presumptive point of exchange is examined, it can usually be assigned to one of two forms, U-shaped or X-shaped, the latter being similar to the form of a chiasma at early prophase of meiosis.

Utilizing these factors of centromeric polarity (P/N), exchange form (U/X), and completeness (C/I), all chromatid interchanges can be fairly easily assigned to one of 10 categories. Twelve are shown in Fig. 3, but the symmetrical incomplete types of both polarized and non-polarized interchanges cannot be distinguished. It is the interplay of these three factors which really determines exchange symmetry.

The 10 categories do not occur with equal frequency, though the reasons why this is so are not at

all clear. The predominant type varies with the organism (Savage *et al*, 1973), and may also vary with the stage of the cell cycle from which a metaphase sample may be drawn.

In general, U-type exchanges are favoured over X-types, and there is a tendency for polarized exchanges to be more frequent than non-polar ones, though this does not appear to be the case in human lymphocytes (Savage *et al*, 1973).

Some forms ($PU \rightleftharpoons NX$, $NU \rightleftharpoons PX$) could be interconvertible during the cytological process of metaphase preparation, and this may confuse the interpretation of numerical results. Such interconvertibility will not, however, account for the overall differences between frequencies of the 10 sample kinds.

6.1.1 Complex interchanges (Fig. 3) A chromosome may be involved in several interchanges of the same or different kinds. Unlike *chromosome-type* interchanges, multiple participation can be observed and recorded with considerable accuracy. The number of possible combinations is very large and detailed classification would be tedious. For scoring purposes, we can group complexes into two broad categories: *obligate complexes* (c/c/c) where *the same* lesion is participating in two different interchanges, and *non-obligate complexes* where each lesion is participating in only one interchange. In the latter case, each exchange counts as a separate entity for numerical purposes (2 c/c, 3 c/c etc.).

In general, non-obligate complexes are the more frequent type.

6.2 Exchanges: inter-arm intrachanges ($\widehat{C}_{\text{Cter}}$, Fig. 4)

Since lesions may occur on the same or different chromatids and a lesion pair may interact in U or X exchange mode, four basic forms are possible. However, for practical scoring purposes, these may be reduced to two because it is impossible to trace a particular chromatid through the centromeric region at metaphase. The two asymmetrical forms can be distinguished at anaphase, and all four forms will give quite different products if they survive to subsequent cell generations.

6.2.1 Inter-chromatid inter-arm intrachanges

The asymmetrical form *appears* at metaphase as a centric ring, and is so classified for scoring purposes. In reality, however, it is a *chromatid dicentric*, and this is clearly seen at anaphase, since the two centromeres always go opposite poles of the spindle and form an anaphase bridge. Both daughter cells therefore will be affected by the damage. The

CHROMATID INTER-ARM INTRACHANGES

FIG. 4. Chromatid-type inter-arm intrachanges.

dicentric is derived from a *duplication-deletion*—that is, a centromere containing length has been deleted from one chromatid and inserted in tandem fashion (without inversion) into one of the arms of the other sister-chromatid. The terminal regions left after deletion have rejoined to produce a compound acentric fragment.

The proximal and distal incomplete forms (not illustrated in Fig. 4) are distinguishable if sister-chromatid pairing affinity is retained until metaphase, otherwise the proximal form is likely to be scored as a terminal deletion.

The symmetrical form is indistinguishable from the symmetrical intra-chromatid counterpart, but is quite different from it in resultant structure. It is generally referred to as a *double duplication-deletion*, and is best understood by unravelling the loop diagram shown in Fig. 4.

The complete intrachange results in two chromatids whose terminal regions beyond the point of exchange are mirror-images. Thus, if the original constitution of chromosome were $a\ b\ c\ d-e\ f\ g\ h\ i$ with exchange points between $b\ c$ and $f\ g$, the two derived chromatids would be $a\ b\ c\ d-e\ f::b\ a$ and $i\ h\ g::c\ d-e\ f\ g\ h\ i$.

It is easy to see that, if the loop containing the centromere were very small, an *isochromosome*—that is, a chromosome whose opposite arms are genetically identical mirror images (Paris Con-

ference (1971): Isochromosome, *i*)—would be generated as one of the products. Isochromosomes can also arise from other kinds of aberrations (Bate-man, 1968).

As with other types, the incomplete forms can simulate chromatid terminal deletions, but, again, the fragment and apparent broken end are unrelated.

6.2.2 Intra-chromatid inter-arm intrachanges

The asymmetrical form analogous to an *X*-type exchange produces a chromatid *centric-ring* and a compound acentric fragment derived from the opposite ends of the same chromatid. The sister chromatid is unaffected.

Ring size may vary, sometimes including almost the entire chromosome length.

The proximal and distal incomplete forms are usually distinguishable, although incompleteness may lead to a degree of unravelling by the time the aberration reaches metaphase. In this case, the former may be scored as one, the latter as two chromatid terminal deletions.

The symmetrical counterpart, answering to a *U*-type exchange is a *pericentric inversion* in one chromatid, while the sister chromatid remains unaffected. Here again a retention of sister chromatid pairing to metaphase will prevent the incomplete forms from being scored as simple terminal dele-

FIG. 5. Chromatid-type intra-arm intrachanges.

tions. At anaphase, the acentric fragments from both incomplete types will appear as terminal deletions, but it must be remembered that the fragment is not directly related to the observed shortened chromosome arm.

6.3 Exchanges: intra-arm intrachanges ($c\overline{c}$, Fig. 5)

Just as with inter-arm forms, there are four basic types of simple—that is, single—intra-arm intrachanges, based on lesions in the same or different chromatids, and lesion interaction in U or X mode.

Unlike the inter-arm (centric) forms, the majority of intra-arm types are small, and seldom retain much of their original interphase conformation through to metaphase, for this is lost as the chromosomes coil and contract in preparation for mitosis. Consequently, the scoring of the four types is not equally efficient.

In each type, failure of the exchange process can lead to two incomplete types, making eight in all. Rarely, doubly incomplete types are found, suggesting that the whole exchange process has failed. This point is in dispute, however, and there are valid alternative mechanisms to account for these doubly incomplete forms.

Revell (1959), who has studied intra-arm intrachanges in considerable detail, has numbered the four basic types (R_1 – R_4 in the diagrams, Fig. 5) and his notation is now usually employed in critical work. Convention has assigned names to the more obvious forms, and these are given below.

6.3.1 Inter-chromatid intra-arm intrachanges

Isochromatid deletions (synonyms: isochromatid breaks, isolocus breaks, Revell type 4, i: distinguish carefully from the symbol i used by Paris Conference (1971) for isochromosomes) are the asym-

metrical form resulting from a *U*-type exchange between the sister chromatids. Visually it appears as if both sister chromatids have been severed at the same point, and that the broken ends of the sisters have rejoined (*Sister Union, SU*). This aberration constitutes the most obvious and least ambiguous form of intrachange.

The two single incomplete types are readily distinguishable: non-union of the centromeric portion (non-union proximal, *NUp* Revell type 4a) or non-union of the acentric portion (*NUd* Revell type 4b).

Doubly incomplete types (*NUpd*) are indistinguishable from *chromosome-type* terminal deletions, unless there is marked inequality in the lengths of the sister chromatids of the acentric and/or the centric portion. *Chromosome-type* aberrations never show either sister union or inequalities in sister-chromatid lengths. The frequency of true *NUpd* is a matter in dispute. They appear to be rare, but there may well be differences between organisms, and between cells from different regions of interphase. There are certainly differences when radiations of different Linear Energy Transfer (LET) are employed to induce chromatid aberrations in plant cells (Lea, 1946; Savage *et al*, 1968).

The loop origin shown in the diagrams can often be inferred from eccentrically placed achromatic lesions which presumably mark the regions of exchange, and also that the two acentric fragments of *NUd* types are often unequal. However, the existence of true isolocus lesions giving rise to *SU* without loop origin cannot be ruled out, especially for irradiation during S phase, and for chemical aberration induction.

The symmetrical form is a *duplication-deletion* (Synonym: Revell type 1) derived from an *X*-type exchange between sister chromatids. It results in the removal of an intercalary segment from one sister chromatid arm, and its insertion in tandem fashion (without inversion) in the other. The consequent inequality in lengths of the two chromatids leads, in the complete type, to buckling or a 'dog-leg' bend at the point of the exchange.

With care, both singly incomplete forms are recognizable, each contributing a terminal deletion. In type 1a, the deleted arm is incomplete, so that there is an obvious separation between fragment and centric portion where the insertion containing arm has straightened out (Fig. 5). In the other type (1b, Fig. 5) the insertion arm is incomplete, and there is an obvious overlap and dislocation in the region of the breaks. In some cases, the fragment will contain the duplication.

Of course, ability to detect type 1 intrachanges presupposes that a reasonable length of chromatid

is involved. The smaller complete ones will tend to be overlooked.

6.3.2 Intra-chromatid intra-arm intrachanges

The asymmetrical form is a chromatid *minute* (Synonyms: interstitial deletion, dot deletion, *m*, Revell type 2). It arises from an *X*-type exchange and results in the deletion of a small (frequently very small) intercalary segment of one chromatid. The ends of the deleted portion are nearly always united to form an acentric ring-fragment, but this is obvious only in the larger deletions where a distinct lumen can be observed. The ends of the parent chromatid rejoin to give a shortened chromatid. The other sister chromatid is structurally unaffected, except that, when a large piece has been deleted, sister chromatid pairing leads to buckling or bending at the site of deletion.

The two singly incomplete types differ, proximal incompleteness (type 2a) leads to a terminal deletion plus a minute. In the case of small deletions, distal incompleteness (type 2b) will not be distinguishable from the complete type 2 because the limits of resolution preclude the direct observation of a ring structure. Larger deletions will appear as a terminal deletion without a very obvious source. In this case, one looks for a bent or buckled chromosome.

Minutes are very often dislodged from the site of the intrachange during cytological metaphase preparation, and come to lie loose among the other chromosomes. Thus they are frequently overlooked or obscured, and scoring efficiency can be considerably reduced. One other effect of the displacement of the minute is that complete types with buckling, and proximally incomplete types (2a) can be mistaken for types 1 and 1a respectively (see Fig. 5).

The corresponding symmetrical form arising from a *U*-type exchange produces a *paracentric inversion* (Revell type 3) of an intercalary segment. No differences in relative lengths between the sister chromatids result and, unless the inverted segment is long enough to cause breakdown in the pairing affinity of sister chromatids (shown by a bowing-out of the affected region), the change will not be detected. Since most inversions are small, scoring efficiency for complete type 3 intra-changes is very low.

Both singly incomplete types (3a, 3b) are indistinguishable from one another and will be scored as simple terminal deletions.

6.4 Breaks

Chromatid *terminal deletions* (Synonyms: chromatid break, chromatid discontinuity, c).

For scoring purposes, any complete severance of the chromatid thread showing clear dislocation, and not obviously derived from an incomplete exchange process, constitutes a simple terminal deletion. Since incompleteness is much higher for chromatid than for chromosome-type aberrations the class of observed terminal deletions will always be inflated by the inclusion of failed exchanges, particularly from the intra-arm intrachange class.

Protagonists of the exchange theory (Section 4) argue that this inflation is sufficient to account for all observed breaks.

The situation is, however, much more complex than was at first thought. The contribution of the various incomplete intrachange types appears to vary throughout the cell cycle, and also with the agent used to induce aberrations. There may also be real differences between organisms. Add to this the existence of more complicated types of multi-lesion intrachanges (some of which are discussed below, Section 7) many of which can simulate the simple types, and it can be seen that tests of aberration theories based on the numerical relationships of the various simple types, as most tests are, are likely to be subject to considerable error.

7. Additional chromatid aberration types

So far we have dealt with the types most frequently encountered in aberration experiments where radiation is used as the inducing agent. There are, however, other types which occur relatively infrequently after radiation, but which are likely to become important as experiments with chemical mutagens increase. Many of these mutagens act at the time of, or require the processes of, DNA synthesis to form structural aberrations, and the possibility of multi-lesion aberrations is greatly increased. There is plenty of evidence in the literature that the forms discussed below are much more common among chemically induced aberrations.

7.1 Triradials

(Synonyms: isochromatid—chromatid interchanges, i/c) (Fig. 6)

As the name suggests, these appear as a three-armed configuration involving two chromosomes, and are normally accompanied by an isochromatid deletion fragment which shows sister union.

The simplest explanation on classical theory is that they arise from the interaction of an isochromatid break and a chromatid break—that is, they are three-break aberrations. For exchange theory derivation, see Revell (1966).

Since the isochromatid portion involved in exchange may be centric or acentric, there are two

categories, the *dicentric triradial* and the *monocentric triradial*, and these are shown in Fig. 6.

Metacentric chromosomes can form a triradial between opposite arms—that is, an *isochromatid-chromatid inter-arm intrachange* (i/c , Fig. 6).

Triradials can occur together with other interchanges in the formation of Complexes (Section 6.1.6).

7.2 Isochromatid-isochromatid exchanges (i/i , Fig. 6)

The joining of the centric portions of two isochromatid deletions to give what appears to be a chromosome-type dicentric. It is only distinguishable from a true dicentric by the fact that there are always two fragments both of which (very rarely only one) show sister union. Inter-arm isochromatid-isochromatid intrachanges (i/i) also occur, and these will simulate chromosome-type centric rings.

7.3 Insertion intrachanges

These comprise certain members of a whole class of intrachanges, some kinds of which occasionally figured in early literature, but which were first described in detail by Fox (1967) for G_2 induced aberrations in the locust *Schistocerca*.

They involve the interaction of three or four lesions variously situated in the sister chromatids of a single chromosome arm.

For the three lesion forms, Fox's derivation makes them equivalent to isochromatid/chromatid intra-arm intrachanges—that is, the interaction of a chromatid and isochromatid deletion within an arm (cf. 7.2 above).

The whole family can also be derived from two intrachanges of the same or different types occurring very close together in the neck of a Revell loop of the kind shown in Fig. 5 (Revell, 1974).

When viewed at metaphase, the majority are indistinguishable from simple intrachanges. There are, however, some very characteristic types, a few of which are drawn in Fig. 6, and careful scoring of these in good preparations of plant chromosomes suggests that the frequency of insertion intrachanges and related types is much greater than has hitherto been realized. This is particularly true when radiations of high LET are used.

If this finding be generally true, then, as already mentioned, numerical scores of 'simple' intrachanges are going to be very difficult to obtain. Moreover, since there is an increased chance of incompleteness when two intrachanges are involved, the 'contamination' of the terminal deletion category will be augmented. In several cases,

TRIRADIALS isochromatid/chromatid interchange (i/c)		
	DICENTRIC FORM	MONOCENTRIC FORM
fragment with sister-chromatid union (SU)		
fragment without sister-chromatid union (NU)		
isochromatid/chromatid inter-arm intrachange (i/c̄)		
examples:-		
INSERTION INTRACHANGES multiple intra-arm intrachange		
examples of some more obvious forms:-		
most simulate simple intrachanges		
isochromatid/isochromatid exchanges		
	INTERCHANGE (i/i)	INTER-ARM INTRACHANGE (ī/ī)
examples:-	 "dicentric"	 "centric ring"

FIG. 6. Additional chromatid-type structural changes.

incompleteness in both intrachanges leads to a *NUpd* configuration (Section 6.3, and Fig. 6).

7.4 Sub-chromatid aberrations

Irradiation of cells very close to mitosis (very late G_2 , early prophase) results in a class of aberrations

which appears to involve only part of the thickness of a chromatid at any one point. Since there is always some degree of stickiness and chromosome clumping produced by radiation at this time (Section 8.3), usually only the exchange forms are seen when cells enter anaphase. The most characteristic

type is a single bridge, with fragments attached to it ('side-arm' bridges).

The structure of those bridges can be seen with particular clarity in some plant materials (Wilson *et al*, 1959; Wilson and Sparrow, 1960) and from such studies it has been inferred that the exchange involves a longitudinal sub-unit of the chromatid.

Kihlman and Hartley (1967) have challenged this concept of the sub-unit and come to the conclusion that these aberrations are in reality true chromatid aberrations disguised by the processes of chromosome contraction which precedes mitosis.

The recent demonstration (Wolff, 1970) that G_1 (unduplicated) chromosomes in human lymphocytes treated with such compounds as urea before irradiation can yield unambiguous *chromatid* aberrations at first post-treatment mitosis indicates, however, that the question of chromosome sub-units is by no means settled.

8. Unconventional aberrations

From time to time, various chromosomal changes are reported which cannot be classified within the framework outlined above. Some of these have been given descriptive names, but the exact nature of the changes is unknown and their occurrence serves to show how much more remains to be learnt about chromosome structure and the mechanisms of aberration formation.

8.1 Shattering

This was first described in detail for G_2 pollen cells irradiated with particular wavelengths (~ 265 nm) of ultraviolet light (Lovelace, 1954). However, the phenomenon occurs in the same material exposed to ionizing radiations of high LET, and is occasionally encountered in mammalian cells in culture even with low LET radiations.

The affected chromosomes, as seen at metaphase, appear to have broken up into many small pieces of varying length. Some of them can be seen to be minute acentric rings, and other pieces may show partial exchange and isochromatid-type changes.

Sometimes all the chromosomes in a cell are affected, but frequently only one or two are shattered, the rest appearing normal, or showing conventional chromatid-type structural changes.

In the case of high LET radiations, the phenomenon may reflect the highly localized deposition of energy along the particle tracks, but this suggestion will not suffice for UV.

8.2 Pulverization

The original descriptions for plant cells suggest that the phenomenon was very similar to shattering,

but the term has since been redefined and applied to mammalian cells for an effect often noted after certain virus infections—for example, Nichols *et al* (1965); Henry *et al* (1971).

The chromosomes are reduced to masses of very small, *thin* fragments, and individual chromosomes are seldom recognized. Occasionally longer fragments of uncoiled chromosome lie among the debris.

The phenomenon appears to be confined to cells that have fused in culture or formed syncytial masses. In this respect, one cannot help but be struck by the similarity of the published photographs of pulverization, and the phenomenon of precocious chromosome condensation (PCC) seen when interphase cells, particularly those in S phase, are fused with cells in division under the action of virus fusing factors (Johnson and Rao, 1970; Sperling and Rao, 1974). Such 'pulverization' does not constitute a degradative effect of virus infection, but corresponds with the visualization of chromosomes in the process of duplication.

8.3 Physiological effects (stickiness)

This is a transient phenomenon found for a short time immediately after the exposure of cells to radiation, and affecting primarily those cells actually in stages of mitosis, especially late prophase. The chromosomes appear to be sticky, often joined to one another by fine threads, and tending to clump into a mass at division.

In some materials, the effect may be present for a number of hours and makes it almost impossible to obtain accurate aberration scores at this time. As noted above, it is concomitant with the period when sub-chromatid aberrations occur.

Early workers suggested that the effect might be due to degradation or depolymerization of DNA. However, the radiation doses which produce stickiness are very low for extensive damage of this sort.

Electron micrographs of condensed chromosomes coupled with the limits of resolution of the optical microscope make it unwise at present to press simple solutions to this problem.

8.4 Agglomerated mitotic divisions

It is now generally believed that ultrasound of the frequencies employed for diagnostic purposes does not induce structural aberrations of the conventional types described in earlier sections. In plant cells, however, it does appear to have an effect very similar to the physiological effects (8.3) acting on cells in or very near division at the time of sonication. Pro-phases are 'bridged' by strands between chromosomes and collapse into a sticky mass. Affected cells fail to complete division. Such cells have been

termed *agglomerated mitotic divisions* (Gregory *et al*, 1974).

As with physiological effects, the phenomenon is transitory, and cells appearing in division at later times are normal.

9. Scoring and scoring categories

The method used for scoring aberrations will depend, of course, upon the purpose for which the data are required. However, 'there can be no question that the maximum information can only be obtained when aberrations are categorized on the basis of their detailed structure. The Committee, therefore, strongly recommends that the detailed system of scoring be employed, particularly in those cases where attempts are made to obtain information on dose-response relationships' (UNSCEAR, 1969, p. 103, para. 50).

Suggested procedures for scoring and recording aberrations have been discussed in several publications—for example, Buckton and Evans (1973). The aberration symbols given in the text and figures of this annotation (derived primarily from the work of Catcheside *et al* (1946) have proved to be a valuable shorthand for scoring purposes, and are widely used in radiation cytogenetics. Set out as column headings (Table I), they enable accurate recording of all types of structural change on a cell by cell basis. Chromatid intrachanges can be assigned Revell numbers and chromatid interchanges designated by the factors outlined in Section 6.1. More complicated or unusual aberrations should be drawn and co-ordinate slide references given to allow rechecking.

TABLE I
COLUMN HEADINGS FOR SCORE SHEETS

Chromosome-type	g, G	C	M	$\widehat{C/C}$	C/C	Complexes, etc.
Chromatid-type	g, G	c	c/c	$\widehat{c/cter}$	c/c	Complexes, i/c, etc.

Before leaving this topic, a few cautionary comments may not be out of place.

a. All slides should be scored under a code, so that the scorer is unaware of the dose or treatment which the cells have received. The bias that knowledge of such facts can introduce can altogether spoil deductions from observation. The subjective element is very real.

b. Beware of cell selection. It is right to select complete (unbroken) cells having a full complement of centromeres, but it is dangerous to select only

cells that are well spread, or with minimal chromosome overlapping. Cells carrying aberrations of the more complicated types seldom have their chromosomes laid out neatly. As far as possible, an attempt should be made to score every complete cell in a sample, and only to reject when it really proves impossible to untangle a configuration.

c. It is usually assumed for statistical purposes that the distribution of aberrations between nuclei conforms to the terms of a Poisson distribution. If this is valid in a particular case (and it is wise to test the assumption, Savage, 1970), then the width of the standard error will depend simply upon the number of aberrations scored. At low aberration yields, therefore, it is better (though more laborious) to score a fixed number of aberrations than a fixed number of cells.

d. In any experiment, tests should always be made for variation in aberration yield with sampling time after treatment. If this be present, as is usually the case with chromatid aberrations, the interpretation of quantitative data becomes extremely complicated, and its usefulness for such purposes as dose-response curves rather limited (Savage and Papworth, 1973).

10. Secondary or derived aberrations

All the structural changes discussed so far are those seen at the *first* metaphase after exposure to the aberration inducing agent.

The majority of these are lethal to the cell that carries them, for they occasion mechanical difficulties and chromosome loss at anaphase with consequent genetic imbalance in the daughter cells. In the case of chromosome-type aberrations, since both chromatids are affected at any one locus, *both* daughter cells will be subject to equal genetic loss, and to death. In contrast, some forms of chromatid-type aberrations, since they affect only one sister chromatid, may lead to genetic loss and death of only *one* of the daughter cells.

Some aberrations of both chromosome and chromatid-types, however (mostly symmetrical forms), can survive division and may be transmitted to future cell generations, either intact or in some modified form. Acentric portions tend to be lost during transmission.

Chromatid-type aberrations which survive the first division are converted by duplication into secondary chromosome types, so that all stable and semi-stable forms observed in later cell generations appear as chromosome-type changes, whatever their primary origin. Moreover, the same secondary change can arise from a variety of different

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