

Chromosomal Aberrations and Bone Marrow Toxicity

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The importance of chromosomal aberrations as a proximate cause of bone marrow toxicity is discussed. Since **chemicals** that can cause nondisjunction are rare, numerical aberrations (aneuploidy, polyploidy) are not ordinarily important. **Many** structural aberrations, however, can lead directly to cell death and **so** are proximate causes of toxicity when **they** occur. The micronucleus test which utilizes the polychromatic erythrocyte is **capable** of detecting agents (**clastogens**) that can cause such structural aberrations. **Many** carcinogens can be detected by this test, and recent changes in the protocol **may** increase the **success rate**. Nevertheless only a small proportion **of** chemicals are clastogens. The importance of cell division in the expression of chromosomal damage and the stage of the cell cycle at the time of exposure on the amount of damage **is** emphasized.

A speculative **mechanism** for the relationship between chromosomal aberrations and carcinogenicity is proposed.

There are two broad classes of chromosomal aberrations: numerical aberrations in which whole chromosomes are either lost or added, (e.g. trisomy-21 or Down's syndrome) and structural aberrations in which pieces of chromosome are lost, added, or translocated, for example the Philadelphia chromosome which leads to chronic myeloid leukemia. Obviously either class of aberration, if inherited, can have a significant influence on human health. These aberrations arise in fundamentally different ways. Aneuploidy, i.e., numerical aberrations involving other than a full complement of chromosomes, arise as a result of nondisjunction which is a failure of the proper distribution of chromosomes to daughter cells. Agents that interact directly with the cell's spindle mechanism are able to cause nondisjunction; there are also scattered reports which suggest that other agents may also be able to cause nondisjunction, perhaps by some indirect mechanism. Similarly, inhibition of the cell's spindle formation can lead to polyploidy, i.e., the gain or loss of whole haploid sets of chromosomes. In most tissues polyploid and aneuploid cells are rare

after treatment by toxic agents, even those agents capable of causing structural chromosomal aberrations. Thus such aberrations are not usually important contributors to the toxic response. Vincristine and vinblastine are probably exceptions to this, but their irreversible binding to microtubules may cause toxicity by other means as well.

Structural aberrations, in contrast, can lead directly to cell death and are often major contributors to toxicity. With respect to toxicity, the kinds of chromosomal aberrations (which from this point on in this paper means structural abnormalities except where otherwise indicated) may be classified in two ways: those that lead to the loss of genetic information at cell division and those that do not. This is shown diagrammatically in Figure 1. Those aberrations (of which only one of many possible types is shown) that involve a rearrangement of gene order rather than a direct loss of a gene are not cell lethal events and, hence, are not contributors to cellular toxicity. In contrast, those aberrations that lead directly to the loss of a section of genetic information are usually cell lethal events and do contribute directly to cellular toxicity. These aberrations may involve an acentric chromosomal fragment whose movement at anaphase is dependent upon cytoplasmic currents so that the

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fragment may or may not be swept into a position from which it can be incorporated into a daughter nucleus. If the fragment is not incorporated into one of the daughter nuclei it will become a micronucleus, a small body that resembles a normal nucleus except for its size and possibly the presence of nucleoli (see Fig. 2). If the fragment is incorporated into a daughter nucleus it will be replicated so that a double fragment is present at the next cell division. If the fragment was already double there will be two fragments present, either or both of which may be lost to become micronuclei. Thus the longer a fragment escapes anaphase loss and micronucleus formation, the more potential micronuclei exist.

The actual mechanism by which the loss of a chromosomal fragment causes death in a diploid cell is not known. It may be that recessive lethal genes existing on the homolog are made hemizygous by

the loss of the fragment. Alternatively, it may be that the genetic imbalance between those loci represented twice and those loci represented only once (as a result of the fragment loss) is lethal. Both mechanisms are undoubtedly involved to some extent, with their relative importance being influenced by the actual chromosomal region involved and the demands placed upon the genome by the stage of cell differentiation involved.

Regardless of the mechanism by which the loss of a chromosomal region causes cell death, the loss itself is dependent upon cell division. According to this idea the induction of chromosomal aberrations should not affect cells that do not divide or have not yet divided. Traditional techniques do not permit a direct test of this idea because the aberrations cannot be detected unless the cells divide. Indirect lines of evidence rather strongly favor this viewpoint, however. For example, numerous tests of

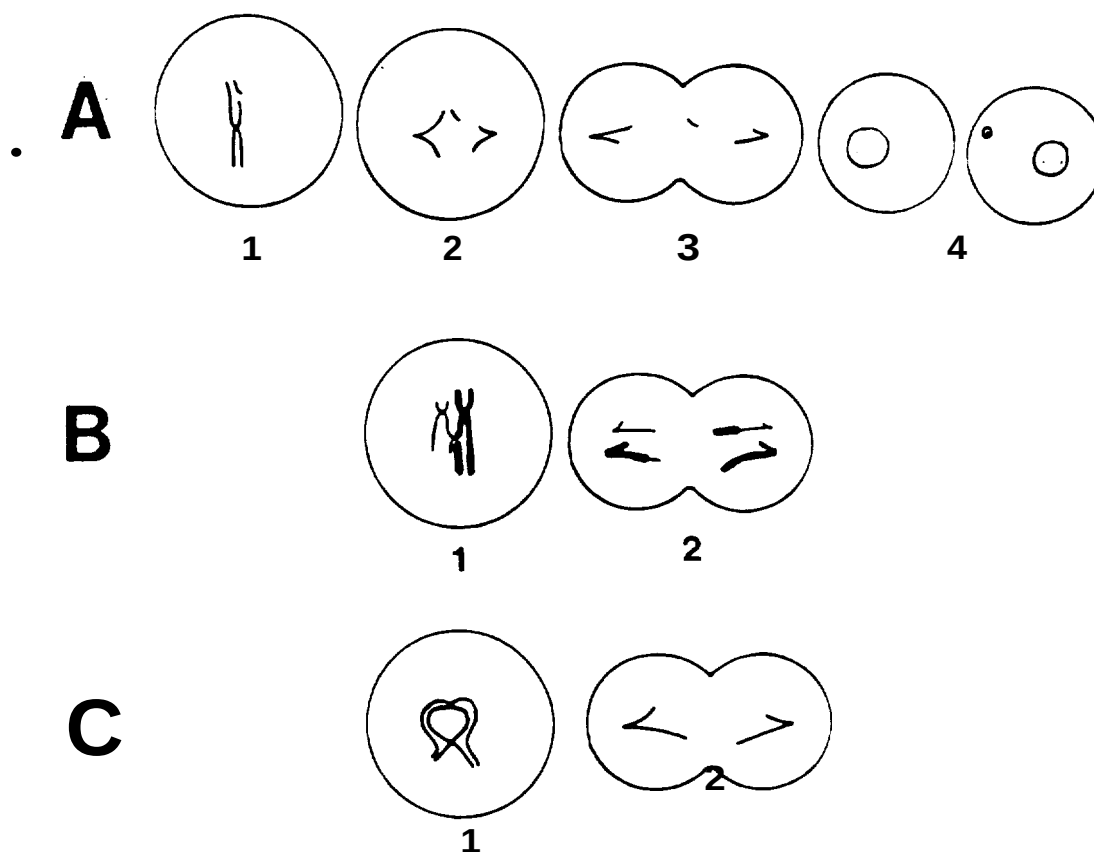


FIGURE 1. (A) Schematic diagram of a chromatid deletion and its fate (1) at metaphase, (2) at anaphase, (3) at telophase, and (4) the resultant micronucleus at interphase. The genetic information contained in the micronucleus is ordinarily unavailable to the cell which may be either the daughter cell with the deletion or the daughter cell with the normal chromosomal complement. (B) A chromatid translocation (asymmetrical chromatid interchange) at metaphase which may segregate either (1) in a balanced form, or (2) in an unbalanced form giving a duplication for one region and a deficiency for the other in each daughter cell. (C) A chromatid inversion (asymmetrical chromatid intrachange) at metaphase and (2) at anaphase. This aberration will not ordinarily be lethal or have any other genetic consequence in a somatic cell.

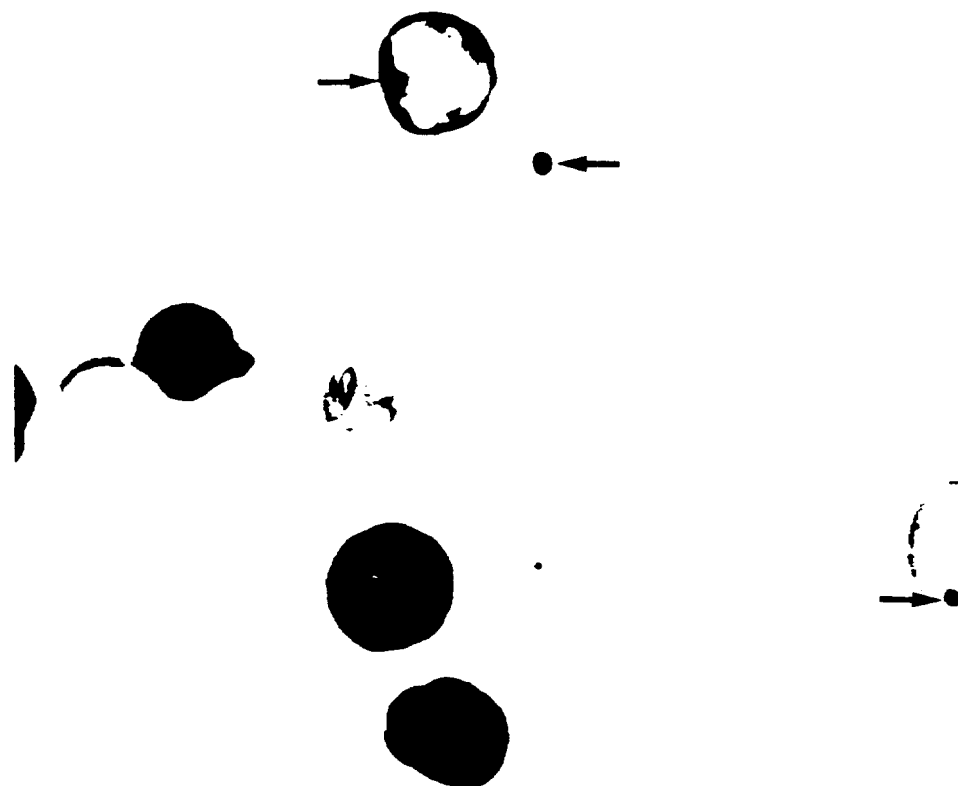


FIGURE 2. Bone marrow preparation showing micronuclei (Howell-Jolly bodies) in two polychromatic erythrocytes and a mature erythrocyte (central cell of the three cells).

the importance of aberrations show that, at doses of ionizing radiation which 10% or more of the cells *can* survive, the majority of cell death in dividing **cellular** populations is attributable to chromosomal aberrations. At these doses virtually no cell death is observed in nondividing cell populations. **How-**ever, if such populations, for example liver, *are* stimulated to divide, then a wave of cell death and necrosis occurs. Furthermore, aberrations that involve rearrangement without loss of genomic material are **often** not cell lethal but are maintained through many cell divisions. Although this view is probably broadly correct, new techniques of cell fusion enable one to examine chromosomes in nondividing cells for aberrations and have thereby revealed at least some cells more heavily damaged than those seen at division (1). It may be, therefore, that there is a reduced division potential in heavily damaged cells even before the first division. It remains true, however, that the tissues sensitive to ionizing radiation and to chemicals that can cause chromosomal damage are normally those undergoing rapid proliferation.

In the case of ionizing radiation, the cells are sensitive to chromosomal damage at all stages of

the cell cycle (2). With chemicals the situation is more complex. Although **DSA** damage may be induced at **any** stage of the cell cycle, the aberrations are often produced only during **S**. **Thus** cells treated with nitrogen mustard in G_2 appear normal at the first division after treatment but may be heavily laden with chromosomal aberrations at the second division (3). Cells of nondividing tissues are normally found in the G_1 phase. Provided that such cells are not stimulated to enter **S** and divide for some **time after** treatment, much of the DNA damage may be repaired prior to **S** and thus much chromosomal damage may be avoided. Possibly this is true for stem **cells** in the bone marrow while the proliferating compartment suffers extensive damage.

Because it is a ready source of dividing cells, the bone marrow has been a favorite tissue for studies of chromosomal aberrations *in vivo*. Traditional chromosomal analysis has suffered, however, from being a time-consuming process and from being a rather specialized field in which many of the basic concepts arose in nonmammalian systems. As a consequence, many of the *in vivo* experiments have been inadequate in the number of cells sampled. the

time of sampling, or both. In addition, inadequate dose levels have often been used. There is a natural reluctance to work at level; too close to lethal levels for fear of nonspecific effects. Unfortunately although aberrations probably occur even at very low doses, too much scoring effort is required at such low doses, especially as a noticeable spontaneous rate does exist. Once aberrations are common enough to detect easily, cell death is the consequence and thus the animal's life is endangered. Therefore it is inevitable that aberrations will be easily detected only at doses close to the LD₅₀. This does not mean that aberrations are a nonspecific consequence of toxicity nor that they do not occur at lower doses. Rather the association between significant frequencies of aberrations and toxicity is a consequence of the statistical problems of detecting low levels together with the importance of high aberration frequencies causing toxicity and death. Nonspecific induction of chromosomal aberrations has never been established as a real mechanism.

Cytogeneticists interested in the mechanisms by which chromosomal aberrations arise place great store on details of the types and kinds of aberrations produced. The classifications are relatively complicated and are not of concern here except to say that they depend upon analysis at metaphase. The question of whether or not an agent can cause chromosomal breakage can be answered much more simply. As outlined earlier, acentric chromosomal fragments become micronuclei when lost during cell division. While these micronuclei do not provide the cytogeneticist with important details of the aberration types from which they arose, they are much more easily and rapidly quantified and so provide a simple means of detecting the occurrence of chromosomal damage. No agents have yet been found that can cause aberrations of any kind without causing those kinds that have associated acentric fragments. Micronuclei should thus be a reliable index of chromosome damage. Micronuclei were used once as an experimental tool in plants and noted occasionally in other circumstances but were not used as an assay until independently proposed by Schmid and co-workers (4) and Heddle (5). The cell type proposed by Schmid was the polychromatic erythrocyte (see Fig. 2). This cell, although not itself a dividing cell is the immediate product of a series of cell divisions. The success of this assay can be judged by the fact that it has largely replaced traditional metaphase analysis in the screening of chemicals for their ability to produce chromosomal damage *in vivo*. More than 200 chemicals have now been tested, about a third of which were found positive. Of course the selection of agents was highly non-random and included a high propor-

tion of known mutagens and carcinogens. Recent advances suggest that a success rate greater than 50% in detecting carcinogens can be expected with few if any false positives (6).

Since micronuclei arise as a consequence of chromosomal loss during cell division, there is inevitably some lag period before they begin to appear. In human lymphocytes *in vitro* this lag is approximately 40 hr because few divisions occur before this (7). The maximum frequency occurs after about three cell divisions in the lymphocyte cultures, but this will depend upon the frequency of fragment loss per cell division. In lymphocyte cultures this has been estimated to be 20% per cell division (8). In systems in which fragment loss is more frequent the maximum should occur earlier. In the polychromatic erythrocyte (PCE) cell assay there is an additional factor: the time between the last division and the expulsion of the main nucleus (9). As a consequence of this, no micronuclei are detectable in PCE for at least 8-10 hr after treatment. Treatments given within this interval are wasted and may even be harmful by depressing PCE production or causing artefacts. Until recently this has not been taken into account so that the majority of testing has occurred under less than optimal conditions.

A second factor of importance in this assay, but not recognized until recently, has been the time course of micronucleus production. Although some mutagens such as x-rays or mitomycin C result in a maximum production soon after treatment, i.e., at about 36 hr, others show maximum frequencies at later times. Dimethylbenzanthracene, for example, gives a maximum at about 72 hr that is more than five times the frequency at 36 hr (Fig. 3). The reason for this variation in the time of maximum response is not clear; we have speculated that it is related to the uptake and metabolism of the agent rather than any fundamental difference in the interaction with the bone marrow (6). The problem raised by this finding is that some agents may not produce detectable increases at 36 hr but are detectable at 72 hr. Indeed a new protocol devised to take account of this possibility led to three carcinogens that were not detectable at early times being detected at later times (6). Because the results even at late times were marginal, this result needs to be confirmed. At the moment, however, it seems likely that the addition of later sampling times to the standard protocol will increase the proportion of carcinogens detected by this assay.

The micronucleus assay seems at the moment to be a robust assay with a moderate success rate. It is robust in that few laboratory to laboratory discrepancies have emerged. The success rate has

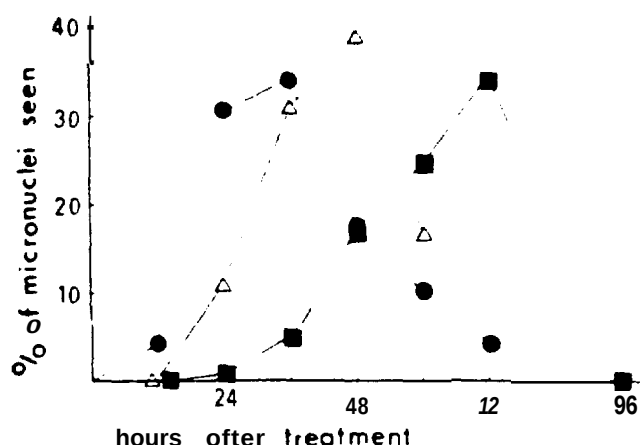


FIGURE 3. Time course of micronucleus production for three agents: (●) mitomycin C; (Δ) cyclophosphamide; (■) 9,10-dimethylbenzanthracene. In each case the proportion of the total number of micronuclei observed is shown as a function of time.

usually been in the 40% to 60% range. Since the protocols have almost always been less than optimal, it is likely that the time success rate will be higher. Nevertheless, it is unlikely that any tissue-specific assay will be 100% successful, and this assay measures only one class of genetic damage. In our opinion, and we must stress that it is only an opinion, the assay detects quite a high fraction of the most potent human carcinogens. We believe that this is true not because the aberrations themselves are the cause of cancer (and certainly not those that lead to micronuclei) but because the cell death that these aberrations cause leads to increased cellular proliferation and hence to an increased likelihood that a quiescent but transformed cell will be called upon to divide. Once called upon to divide it is able to express its cancerous phenotype, i.e., it does not respond adequately to the normal feedback mechanisms that keep cell production in balance with cell loss.

To return from the issue of genetic toxicology to bone marrow toxicity, it should be evident from this discussion that genotoxic agents can contribute greatly to toxicity in any dividing cell population. In the bone marrow in which there are so many different proliferating cell populations, large doses of such chemicals can be expected to produce a pancytopenia; at lower doses some populations may be more or less severely affected than others. Certainly the time course of toxicity arising in subpopulations will differ according to their rate of proliferation and to the fraction of cells in the sensitive phases of the cell cycle, as well as to the dose they receive. Genetic toxicity is to be expected

from a relatively small minority of chemicals, a few per cent of chemicals at most. The rapid advances in genetic testing are making the detection of such chemicals a much more reliable process. It will be interesting to see if diseases other than cancer, birth defects, and genetic abnormalities will be found to be associated with genotoxicity and whether specific genetic events can be shown to be the cause of somatic diseases such as leukemia.

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