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MEDICAL PROGRESS

Induced chromosomal aberrations: Biological and clinical significance

A multiplicity of chemicals, viruses, and ionizing radiations are now known to damage the genetic material of human cells. Chromosomal breaks and rearrangements are relatively gross measures of such damage. However, there is evidence that increases in chromosomal aberrations may be seen in human populations which are at high risk in terms of neoplastic disease. Further, studies of chromosomally abnormal cell populations in vitro have revealed an increased tendency to malignant transformation after exposure to oncogenic DNA viruses. The clinical and biological significance of induced aberrations in man is reviewed in the light of these findings.

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IT HAS BECOME increasingly clear in recent years that we are exposed to a wide range of chemical and physical agents which may damage the genetic material of our cells. Thus future generations of our species may be affected adversely by our exposures to such agents, and the present generation may, in addition, be putting itself at risk in terms of neoplastic disease.

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The description by Moorhead and associates[1] in 1960 of a method for cultivating peripheral blood leukocytes was a critical step in the development of human cytogenetics. This method enabled us to associate specific abnormalities of the karyotype with specific malformation syndromes. It also enabled us to begin to gather data on the frequency and types of chromosomal breaks and rearrangements which appear in cultured white cells. The studies of Buckton and associates[2] and Court Brown and colleagues,[3] in the early

1960's, on the therapeutically X-irradiated patients with ankylosing spondylitis first demonstrated that chromosomal morphology could be used in man, as it had been in plants and animals, as a reasonably sensitive indicator of exposure to a mutagen. It was subsequently learned that cells which respond to the stimulation of phytohemagglutinin in leukocyte cultures are immunologically committed, long-lived lymphocytes, thus providing evidence not only of recent, but also of distant, exposure to chromosomally mutagenic agents.

Our concerns in terms of breakage need, then, to be with both the germ cell chromosomes and the somatic cell chromosomes. To date, no evidence of vertical transmission of chromosomal aberrations, induced by a specific agent, has been unequivocally documented in man. I have, therefore, elected to concentrate here mainly on somatic cell aberrations. We will discuss how such aberrations arise and speculate on what they may mean. (For a more detailed review of this subject, the reader is referred to a recent article by the author which was written primarily for geneticists.[4]) The evidence is now convincing that populations with increased levels of induced aberrations, from whatever source, are at increased risk in terms of the development of malignant disease.[5] Studies of patients with Fanconi's anemia and those with Bloom's syndrome support this idea, as do studies of heavily irradiated populations, such as the Hiroshima and Nagasaki survivors. Further, evidence from congenital syndromes in which chromosomal damage is present, as well as from irradiated cell populations, indicates that somatic cells with structurally altered chromosomes or with an increased number of chromosomes (as in trisomy 21) may be peculiarly susceptible to oncogenic virus infection.

TRANSMISSION TO OFFSPRING

In experimental animals, a variety of chromosomal rearrangements have been induced in meiotic chromosomes and transmitted to the next, or so-called F_1, generation. Most transmissible abnormalities involve either the translocation of chromosomal material from one chromosome to another or an inversion of the gene sequence which results in a repositioning of the normal constriction, or centromere. Those germ cells which are most likely to survive the effects of such breakage and rearrangement are those in which no major loss of chromosomal material has resulted and in which the abnormalities are relatively stable. The ability or inability of a damaged chromosome to survive meiosis (or mitosis) will, in large measure, determine the fate of the daughter cell.

In man, we know of many instances in which an abnormality of chromosomal structure is transmitted through several generations. For example, the presence of a long short arm of a G-group chromosome in three generations of a family would be one such instance. The abnormality has no apparent deleterious effect, and so there is no apparent selection against these germ cells during fertilization. Nor is there a problem in reproduction for the carrier of such a variant chromosome. The result, then, may be the production of a new individual who has one abnormal member of a pair of chromosomes. This sort of structural chromosomal "heterozygosity" is relatively common, with 2 to 4 per cent of the population being heterozygous in this way for one or another pair of chromosomes.[6]

Theoretically, it should be possible for a chromosomal mutagen to damage a germ cell chromosome of a normal individual, with the resultant production of gametes that carry a translocation, or an inversion, or an abnormality of chromosome number. Such aberrations *must* be produced in man, as in other species. The problem is one of our inability to detect such changes. The damaged gamete may be killed outright, or it may be selected against during gametogenesis, or, last, the zygote may be nonviable and may spontaneously abort.

The complexities of this problem of detection of transmissible meiotic chromosomal effects in man are illustrated by the experience in Hiroshima and Nagasaki, where the populations were exposed to high doses, often

in excess of 100 rad, of known physical mutagen (the gamma and neutron radiations). However, the early studies of the offspring of the A-bomb survivors demonstrated no increase in congenital malformations, no increase in abnormalities of early development, and no increase in spontaneous abortion.[7] While these are not *purely* genetic phenomena, genetic factors play a significant role in each of them. The fact that increases in these parameters were not seen in the offspring of Hiroshima and Nagasaki survivors suggests not that genetic effects did not take place, but rather that these parameters are relatively insensitive measures, even in a fairly large population, of genic or chromosomal damage.

For the past four years, we have been studying the cytogenetics of the children of heavily A-bomb–exposed parents, recognizing, for the reasons given above, that a high measure of selection may have been operating against chromosomally abnormal gametes or zygotes. Nonetheless, the detection of an increased incidence of structural heterozygotes or of abnormalities of chromosome number among the children who had at least one parent exposed to >100 rad might certainly be a measure of a radiation effect on the parental germ cell chromosomes. To date, several hundreds of children have been karyotyped, with no evidence of such effects.[8] Of the first 228 children studied, two were chromosomally abnormal, one being a carrier of a familial short arm deletion of a G, the other being XO/XXX mosaic, the latter presumably resulting from a post-, not a pre-, fertilization error. This study is a continuing one[9] and may well require several thousands of individuals to demonstrate conclusively the presence or absence of a radiation effect.

The new techniques of identifying and distinguishing each homologous pair of chromosomes, by use of fluorescent[10] and heterochromatin[11] stains to produce specific banding patterns, provide a considerable increase in the resolution power of the microscope for examination of human chromosomes. With these methods, previously undetectable translocations, in particular, may be readily found. Thus we have reason to be optimistic that screening of an F_1 generation for the effects of chromosomal mutagens on parental meiotic chromosomes may soon be done with far more sensitivity than has been possible until now.

With 0.5 to 1.0 per cent of individuals in "normal" human populations having a major abnormality of the karyotype[12] and 2.0 to 4.0 per cent having a minor abnormality,[6] as determined by conventional cytologic techniques, it seems unlikely that most laboratories will ever be equipped to study routinely the effects of suspected chromosomal mutagens on human populations. The magnitude of the effect is likely to be small, and many persons would have to be studied. Fortunately, a number of groups have systems of automated or semiautomated chromosomal analysis that appear promising, with the computers generating either printouts of chromosome measurements or graphic representations of the karyotype. The latter is clearly the more desirable of the two alternatives, and should aid immensely in our ability to monitor human populations for transmitted chromosomal abnormalities.

SOMATIC CELL ABERRATIONS

It is often difficult for the clinician, as well as for the geneticist, to evaluate studies which purport to demonstrate chromosomal damage after exposure of an individual to one or another virus or drug. What is still lacking, despite the many studies of the effects of physical or chemical agents on human somatic cell chromosomes, is a uniform set of criteria by which an agent shall be judged chromosomolytic. The controversy over the effects of lysergic acid diethylamide (LSD) serves to illustrate several important points. (The excellent review of Dishotsky and colleagues[13] on the subject of the biological effects of LSD in man is recommended for those interested in more detail than appears below.)

Cohen and associates[14] initially studied the chromosomal effects of LSD-25 at concentrations which ranged from 0.001 μg per

milliliter to 10.0 μg per milliliter. Human lymphocytes were exposed in vitro for up to 48 hours to these levels of LSD and the "break rate" was reported to be twice the "spontaneous" level at all but the lowest concentration. The in vitro studies of others which followed these early reports have been far less consistent, with no dose-response relationship being invariably demonstrable in vitro when purified LSD was used.

The confusion over interpretation of the in vitro findings was intensified by the publication of a number of in vivo studies with contradictory results. Among users of "illicit" or "street" LSD, approximately 50 per cent have an increase in chromosomal aberrations. However, among 126 persons treated with pure LSD in hospital or clinic settings, 14 per cent had a higher than control frequency of aberration-bearing cells. These data suggest either that illicit drugs contain one or more contaminants that break chromosomes or that other factors which may be common to heavy drug users—such as inapparent virus infection—are involved.[15, 16]

The best designed studies of the in vivo chromosomal effects of drugs are those in which pre-exposure studies of chromosomes are done on involved subjects, so that each person may act as his own control. For LSD such studies have demonstrated that few persons have an increase in chromosomal damage. Further, five of seven studies involving only post-therapy blood samples have been negative. Pure LSD, at moderate dose levels, does not seem to induce chromosomal breakage.

One of the difficulties in the interpretation of the LSD data occurred because of the use of "break rates" in the presentation of the data. Fig. 1 presents "stick" diagrams of one nonmolecular way of viewing the mechanism of formation of some of the more common chromosomal breaks and rearrangements. One of the simpler types of breaks is termed isochromatid, in that both chromatids of the chromosome are broken at the same level. This type of abnormality may arise as a result of either one or two "hits," rendering a break rate per cell meaningless when this aberration is frequently seen, as it was in the LSD studies.

TYPES OF INDUCED ABERRATIONS AND DOSE RESPONSE

The type of aberrations which are formed on exposure of a cell to a chromosomolytic agent depends on the single or double strandedness of the chromosomal DNA at the time of exposure. Normally, in the G_1 stage of interphase, the chromosome is single stranded; beginning late in G_1 and during the S phase, DNA synthesis takes place, ceasing in G_2 after duplication of the chromosome. Thus exposure of a G_1 cell to ionizing radiation, for instance, produces damage from a single break that affects both chromatids, whereas exposure of a G_2 cell would require two "hits" to produce the same aberration.[17]

The more commonly seen simple and complex chromosomal breaks and rearrangements, and the mechanisms of their formation, are diagrammed in Fig. 1. (A detailed description of these mechanisms is given in references 4 and 17.)

One major criterion of whether a suspect agent is or is not a breaker of human chromosomes must be, in the opinion of this author, the demonstration of a dose-cytogenetic response relationship over some reasonable dose range. Bender and Gooch[18] originally suggested that the frequency of chromosomal aberrations might be used as a kind of biological dosimeter after acute radiation exposure, and this has been substantiated for in vivo and in vitro irradiations of human cells down to doses less than 50r. For example, Evans[19] showed that the frequency of dicentric chromosomes after in vitro exposure of human cells increased as the square of the dose. In our early studies of somatic cell aberrations in residents of Hiroshima and Nagasaki years after their atomic bomb exposure,[20, 21] we demonstrated that the frequency of complex, exchange-type aberrations seemed related to both the age of the survivor and to his estimated exposure dose. Awa and associates[22] have now definitely shown in the survivors that, even this long

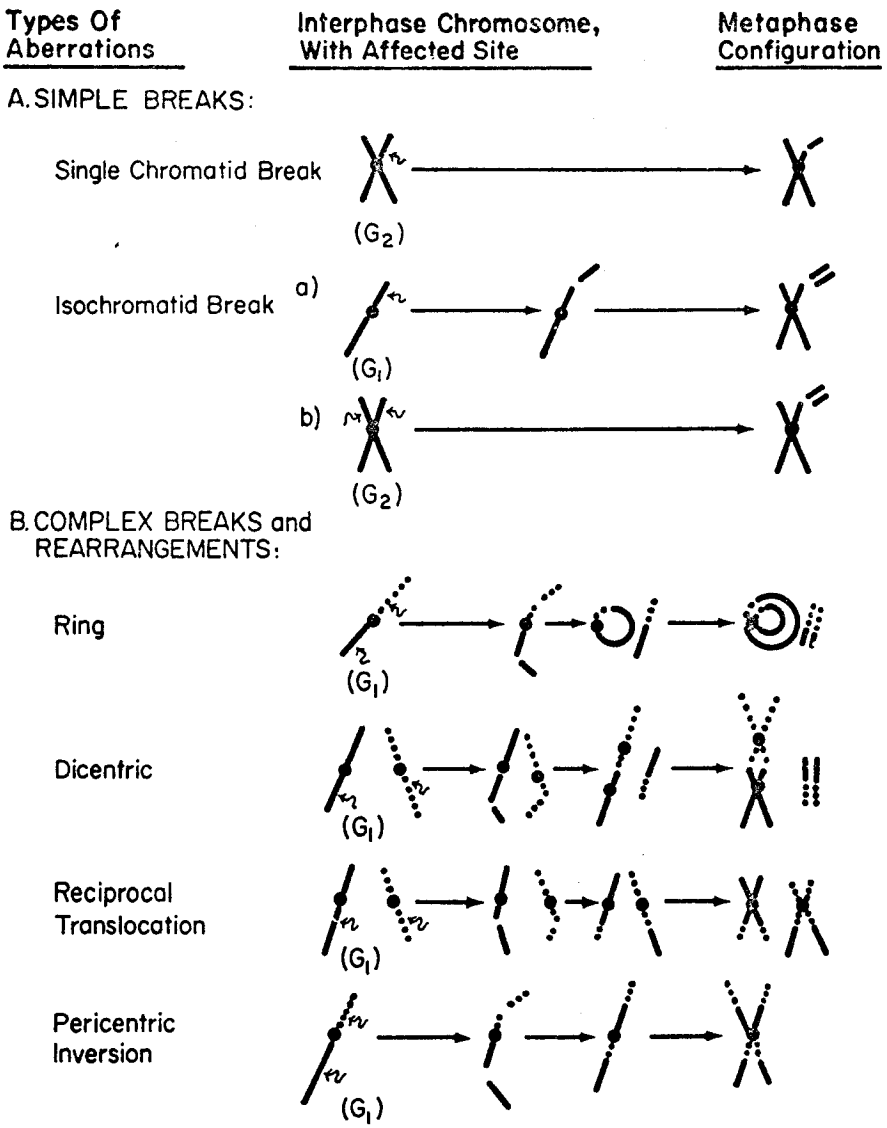

Fig. 1. The mechanism of formation of some of the more common types of induced chromosomal abnormalities is illustrated here in "stick" diagram fashion. The basic distinctions to be made are (a) between single stranded (e.g., G_1) and double stranded (i.e., G_2) chromosome effects, and (b) between simple breaks and more complex rearrangements.

after exposure, a direct radiation dose-cytogenetic response relationship does exist for several classes of complex aberrations, including rings and dicentrics, as well as translocations and inversions.

POPULATION STUDIES

An increased risk of neoplastic disease appears to exist for patients with clinical syndromes which are known to be associated with chromosomal breakage on a congenital basis, such as Fanconi's anemia, Bloom's syndrome, and the Louis-Barr syndrome.[23] Further, considerable epidemiologic evidence is now available which suggests that there is an association between exposure to chromosomolytic agents in a human population and an increase in cancer risk to that population.[23, 24] The data from populations exposed to ionizing radiations are the most substantial. Acute external exposure to high-energy X, gamma, or neutron radiations clearly pro-

duces chromosomal aberrations, even at relatively low total exposure doses.[25] Leukemia and tumors of solid tissues (as thyroid) have been well described in a number of populations so exposed, including the survivors of the Hiroshima and Nagasaki A bombs.[26] Internal emitters with long biological half-lives, such as ^{226}Ra, are similarly associated with chromosomal damage and tumor formation, such as the osteogenic sarcoma seen in radium dial painters.[27]

The association in man between chromosome-breaking viruses, or drugs, and neoplasia is far more tenuous. Viruses, such as rubeola, may damage somatic cell chromosomes, but we do not know of any long-term carcinogenic effects of this damage. Similarly, drugs, such as the antibiotic mitomycin, have been shown to damage chromosomes with no known sequelae. The mechanisms by which such agents act are obviously diverse, ranging from interference with DNA replication to possible digestion of chromosomal nucleoproteins by lysosomal DNAse.[28] But the ultimate effects, if any, of these virus- or drug-induced aberrations in terms of somatic cell disease are unclear.

With respect to LSD, again there is no strong evidence of an oncogenic effect. Among persons taking LSD, three cases of leukemia have been reported, two in therapeutically exposed persons and one in a user of illicit LSD.[13] However, no systematic study has been made and there is presently no evidence to support the idea that LSD is a carcinogen. Even if LSD were to be classified as a chromosome-breaking agent, which it may not now be, this would still not mean that it is by definition a chemical carcinogen. Proof of each of these potential effects of any drug must be independently derived.

CELLULAR TRANSFORMATION

We may tentatively conclude from the experience in man to date that a cell with a chromosomal rearrangement is not by definition a malignant cell and that chromosomolytic agents are not, per se, carcinogenic. Nonetheless, the epidemiologic associations (from studies of irradiated populations) of neoplasia and chromosomal aberrations are beginning to find experimental support, and the evidence merits review here. The growth of human fibroblasts in tissue culture is associated with the rare "transformation" of an occasional cell. This transformation involves: (a) loss of contact inhibition, with resultant stacking up of transformed cells in several layers, unlike normal fibroblasts which grow in monolayer, (b) change in morphology of the cells within transformed colonies, as from fibroblastic to epithelial in appearance, (c) shift in metabolism from the aerobiasis of fibroblastic cultures to anaerobic glycolysis, and (d) the ability of some transformed animal cells to produce tumors in vivo.[29]

The very rare spontaneous transformation of normal human cells may be increased somewhat by exposure of the cells to simian virus 40 (SV40). While the transformability of normal cells by the polyoma-like SV40 is small, Todaro and Martin[30] have demonstrated that cells from patients with karyotypic abnormalities (such as trisomy 21) or from patients with "spontaneous" chromosome breakage (such as Fanconi's anemia) have a 20- to 50-fold increase in susceptibility to transformation in vitro. Further, the cells of heterozygous carriers of genetically determined syndromes such as those of Fanconi and of Bloom have an approximately 10-fold increase in susceptibility to SV40 transformation, with transformation rates which are midway between those of normal and affected persons. The increased susceptibility of irradiated 3T3 mouse cells and of irradiated human cells to SV40 transformation[31] is further evidence that cells which are likely to have considerable chromosomal damage are also more sensitive than normal cells to the effects of oncogenic virus.

The biological basis of in vitro transformation by this DNA virus is currently being actively studies in a number of laboratories. At the present time, it appears that virus is incorporated into the host cell genome and that the presence of the virus in some way derepresses the internal control of cell di-

vision. However, it is unclear why virus infection of chromosomally abnormal cells should be so significantly enhanced.

It should be stressed that these findings on normal and abnormal rates of transformation suffer from a lack of sufficient control data. More information is needed on the rates of SV40-induced transformation of cells from normal males and females of different ages, and living in different environments, before unqualified acceptance of the phenomenon of increased transformability of chromosomally altered cells is possible. Furthermore, induction of transformation by chemical carcinogens has not been successful, despite the fact that several of the chemicals used are clearly carcinogenic in mammals.

CHROMOSOMAL BASIS OF ONCOGENESIS: A HYPOTHESIS

How, then, are we to synthesize our current information on the relationship between chromosomal aberrations and oncogenesis. We may visualize, first, the exposure of a cell, or cell population, to a mutagenic agent, with the resultant induction of either a point mutation, a chromosomal mutation, or both. The mechanism of induction of chromosomal mutations is not well understood at present. If the mutation is sufficiently deleterious, the cell may die; or more likely, some cells of the exposed population will survive, proliferate, and repopulate the tissue involved. The surviving cell lines may have mutations in individual cells, and mutant clones may well be produced. Thus a major result of exposure of a normal cell population to a mutagen, assuming cell survival, may be the establishment of a genically or chromosomally mutant cell population.[32]

It remains to be determined whether or not such cells are as sensitive to oncogenic virus in vivo as they may be in vitro. We clearly need evidence on this point. The epidemiologic evidence cited above does at least raise the possibility that transformation may not be uniquely an in vitro phenomenon. If it is not, then we have the beginnings of an explanation of the relationship between chromosomal abnormality and neoplastic disease, a phenomenon astutely recognized as long ago as 1914 by Boveri.[33]

The suggestion that I write this paper originated with the late Dr. Stanley Wright, and I wish here to acknowledge my gratitude to him.

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