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Persistent Chromosome Aberrations in Irradiated Human Subjects

M. A BENDER AND P. C. GOOCH

Biology Division, Oak Ridge National Laboratory,[1] *Oak Ridge, Tennessee*

INTRODUCTION

A number of investigations have been made of radiation-induced chromosome aberrations in human cells (*1–3*). However, these have been limited to cells grown and irradiated *in vitro*. Although some attempts have been made to correlate the results of these experiments with those of *in vitro* and *in vivo* studies with experimental animals (*2, 4, 5*), there is an obvious need for investigation of the *in vivo* chromosome damage resulting when normal human subjects are irradiated.

The recent development of techniques permitting analysis of chromosomes from short-term cultures of peripheral blood (*6*) has made practical the investigation of damage to the chromosomes of irradiated human subjects. Suitable subjects for a quantitative study are, however, very rare. It would be best if observations were made on otherwise normal human subjects who had received single doses of total-body irradiation. Further, the cells should be examined shortly after the irradiation, during the first postirradiation division before any of the aberrations are lost through bridge formation, etc. Only then can such a study provide the necessary background for the use of chromosome analysis as a biological dosimeter. However, even when these criteria are not met, aberration analyses can yield useful information. Tough *et al.* (*7*) have presented a preliminary study of chromosome aberrations induced in two patients given X-ray treatments for ankylosis spondylitis. In one case just one X-ray dose was given. The irradiation was limited, however, to the region of the spine, and aberration analysis cannot give quantitative dose-effect information. We have made a similar study of the chromosomes of eight men who received total-body doses during a nuclear excursion. Although these cases were not examined until several years after irradiation, and thus the quantitative dose-effect relationships cannot be measured, chromosome aberrations were still present in most of the cases, and with a high frequency in some of them.

[1] Operated by Union Carbide Corporation for U. S. Atomic Energy Commission.

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MATERIAL

Peripheral blood samples were secured from eight otherwise normal, healthy men irradiated in June, 1958, by a nuclear excursion. They received doses estimated to range from 22.8 to 365 rads of mixed γ-rays and fission neutrons. Neutrons were estimated to account for about 26% of the total first-collision dose. The dose calculations and the physical circumstances surrounding the irradiation are described elsewhere (*8*). The patients' ages varied from 24 to 56 years at the time of irradiation. Blood samples were drawn between November, 1960, and January, 1961 (about 2½ years after irradiation) through the cooperation of Dr. B. W. Sitterson of the Oak Ridge Institute of Nuclear Studies Medical Division. Blood samples were also drawn from five normal, unirradiated control individuals during the same period.

METHOD

The culture method was modified from that described by Moorhead *et al.* (*6*) and others. Serum and leukocytes were separated from 5-ml samples of heparinized blood by treatment with 0.1 ml of Phytohaemagglutinin (Difco). The serum containing the leukocytes was then added to a culture medium consisting of 10 ml of Mixture 199, 4 ml of inactivated human AB-positive serum, and 5 mg each of penicillin G and streptomycin. The cultures were incubated for 3 hours at 37°C in a 5% CO_2-95% air atmosphere. Six hours before fixation colchicine was added to the cultures to a level of 10^{-7} *M*. Hypotonic treatment, fixation, mounting, and staining were done as described by Moorhead *et al.* Since few mitoses were seen in material fixed before the third day of culture, most of the cells scored were in their first *in vitro* mitosis.

Two scoring procedures were used. For a survey of gross abnormalities, cells were selected at low magnification and examined at 900 diameters. The number of chromosomes, and the presence of chromatid aberrations, ring and dicentric chromosomes, minute fragments, and chromosomes of otherwise abnormal form were recorded. The easily identifiable pairs of chromosomes (numbers 1, 2, 3, 13, 14, 15, 21, 22, and the Y, by the Denver system of nomenclature) were also checked wherever possible.

The second procedure has been used to determine the presence of less-obvious abnormalities, such as pericentric inversions and small deletions. Suitable cells were selected at random and photographed. Enlargements of the photographs were then used to construct "paste-up" karyotypes for detailed analysis and measurement.

RESULTS

Survey analyses have been made on five control individuals. The results are given in Table I. The frequency of counts other than $2n = 46$, 1.7%, is lower than that found by Tough *et al.* (*7*). The only aberrations seen in the controls were

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TABLE I

CHROMOSOME ANALYSIS OF NORMAL CONTROL LEUKOCYTE CULTURES

Case	*Age (years)*	*Cells scored*	*Chromatid aberrations (%)*	*Cells with $2n \neq 46$ (%)*	*Chromosome aberrations (%)*
1. P.C.G.(♀)	25	100	1	0	0
2. E.H.Y.C.	33	100	5	1	0
3. M.A.B.	31	50	0	0	0
4. A.H.	62	108	2.8	4.6	0
5. A.H.H.	30	100	2	2	0
Total	—	458	2.4	1.7	0

of the chromatid type and must have arisen in culture, since chromatid-type aberrations are either lost or become chromosome types in anaphase of the division in whch they occur. No polyploid cells were seen in the control individuals.

The results for the irradiated individuals are presented in Table II. The identification letters are those used previously (*8*). The frequencies of chromatid aberrations are about the same as in the controls. Cells with counts other than $2n = 46$ are frequent in most of the irradiated persons. Aberrant chromosomes occur in the cell samples for all the irradiated cases except individual "H," who received the lowest (estimated) dose. In one case ("C") the frequency of aberrant chromosomes, particularly dicentrics, was very high. No polyploid cells were seen in any

TABLE II

CHROMOSOME ANALYSIS OF LEUKOCYTE CULTURES FROM IRRADIATED INDIVIDUALS

Case	*Estimated dose (rads)*	*Cells scored*	*Chromatid aberrations (%)*	*Chromosome aberrations*					*Total abnormal cells (%)*
				Cells with $2n \neq 46$ (%)	*Minutes (%)*	*Rings (%)*	*Dicentrics (%)*	*Other*[a] *(%)*	
A	365	100	2	14	5	0	1	1	18
B	270	100	1	20	0	0	1	1	20
C	339	142	1	23.2	2.4	3.2	13.4	1.6	28.2
D	327	100	0	14	5	1	3	1	19
E	236	75	1.3	6.7	0	0	1.3	0	6.7
F	68.5	109	0	14	0	0	0	0	12.9
G	68.5	107	0	8.4	1	0	0	1	8.4
H	22.8	98	2	4	0	0	0	0	4

[a] Chromosomes of abnormal length or centromere position.

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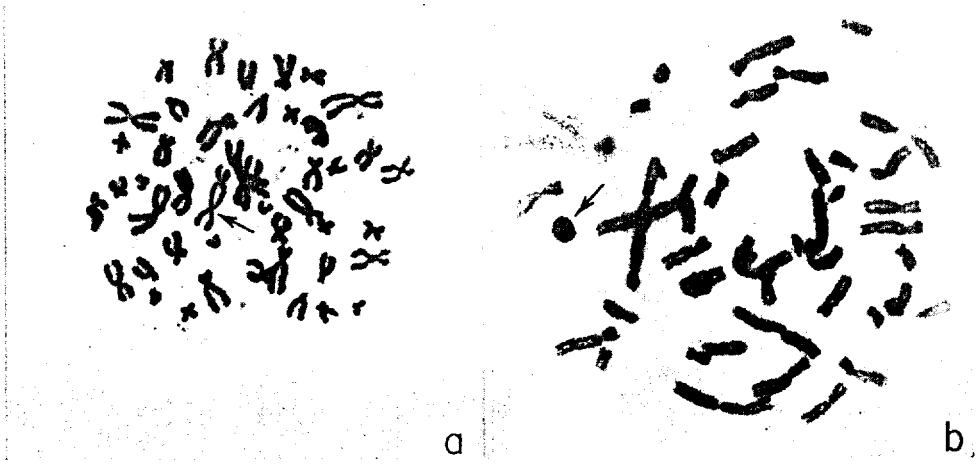

FIG. 1. Aberrant chromosomes of cells from irradiated men. *a*, Cell with dicentric chromosome; *b*, cell with ring chromosome (and also a dicentric).

of the irradiated individuals. Figure 1 shows representative cells with ring and dicentric chromosomes.

Analyses were made of the karyotypes of cells from two of the "high-dose" cases ("A" and "C"), and from one of the "low-dose" cases ("H"). Results are shown in Table III. It is clear that many of the superficially normal cells from the "high-dose" cases are not, in fact, normal. Thus, the total frequency of cells with abnormal chromosomes is actually at least twice that listed in Table II. Figure 2 shows several karyotypes with examples of inversions and deletions. No extensive analyses of karyotypes were attempted on control material, since no examples of variation have been found in the many karyotypes of normal human subjects that have been constructed in the past.

TABLE III

"MINOR" ABERRATIONS IN GROSSLY NORMAL CELLS FROM THREE IRRADIATED MEN

Case	*Number of cells*	*Deletions*	*Inversions*[a]	*Translocations*	*Total abnormal cells (%)*
A	3	4	0	0	67
C	15	11	3	2	53
H	6	5	0	2	83

[a] Chromosomes with an abnormal centromere position, indicating pericentric inversion with the break points unequally spaced from the centromere.

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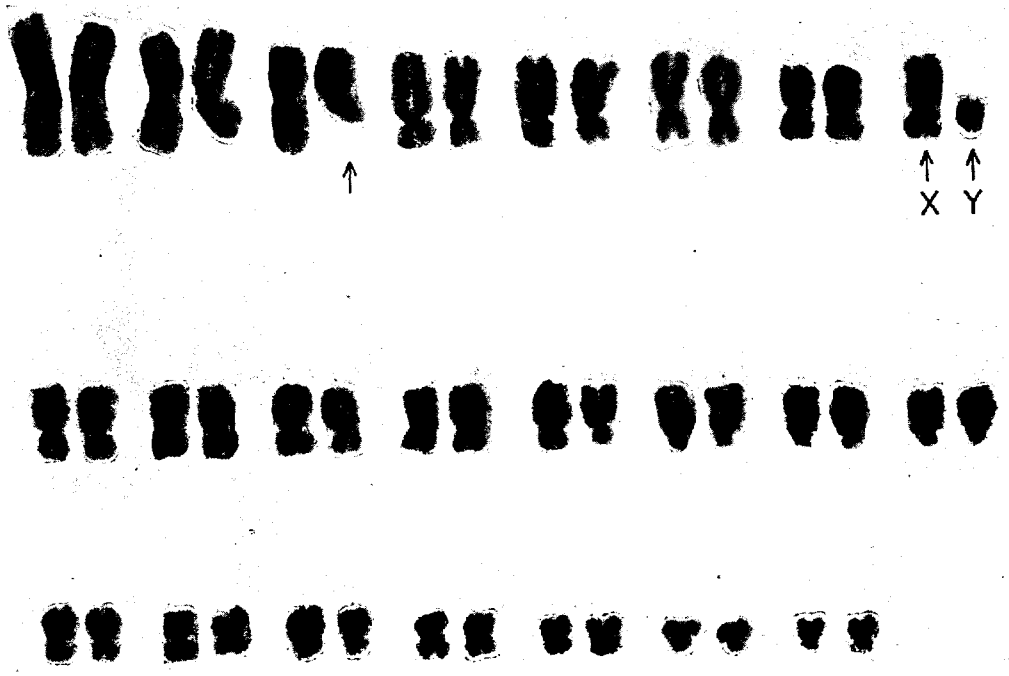

FIG. 2*a*

FIG. 2. Aberrant karyotypes of cells from irradiated men. *a*, Grossly normal cell showing deletion; *b*, cell showing possible pericentric inversion.

DISCUSSION

Irradiation can induce a number of different types of chromosome abnormality. If the chromosome is broken before effective duplication, chromosomal aberrations appear in the next division. In this case each abnormality is present in each of the two daughter chromatids. If the chromosome is broken after duplication, then chromatid-type aberrations are seen, in which only one of the two chromatids need be involved.

Several factors can modify the numbers and kinds of aberrations seen when cells from irradiated persons are examined some time after irradiation. For example, some kinds of aberration are completely lost after the first division; others tend to be lost progressively in subsequent divisions. Chromatid aberrations are either lost completely or become types involving whole chromosomes after the first division. Chromosome-type aberrations, and many of the post-first-division chromatid aberration derivatives, are lost some time after the first division. Many of the deletions are probably cell-lethal and are thus expected to be lost quite rapidly from

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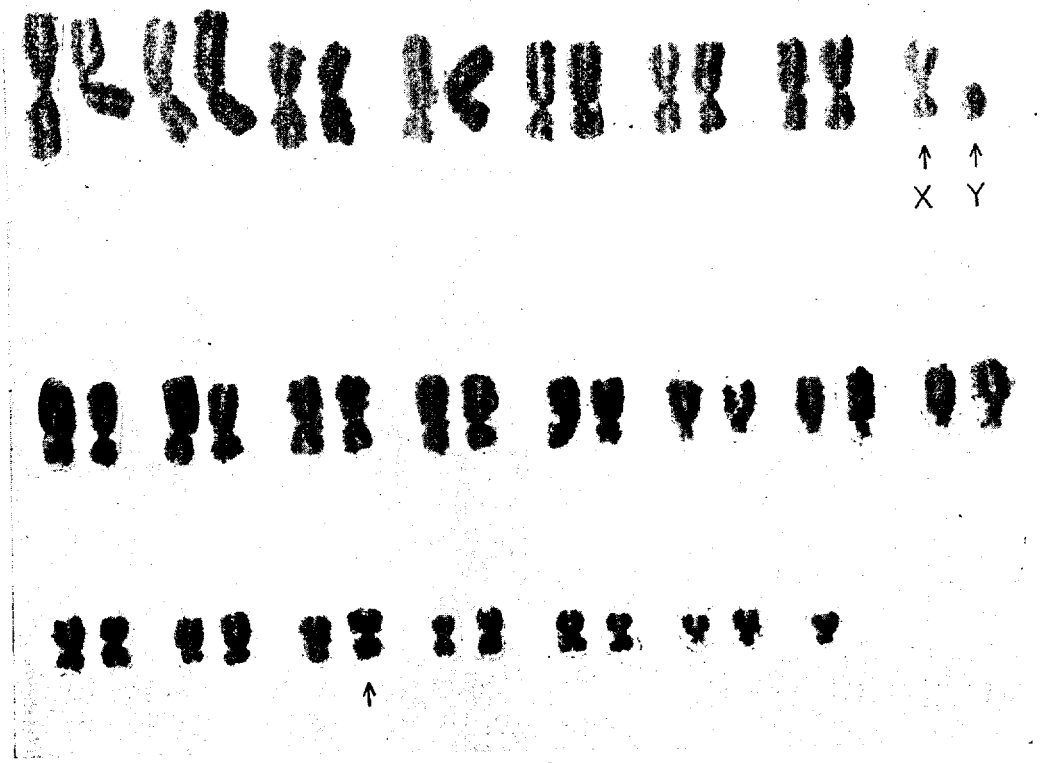

FIG. 2b

the cell population. In addition, cell selection may well operate to increase or decrease the frequencies of various aberration-bearing clones of cells. It is thus impossible to establish any accurate relation between dose and aberration yield except when cells are examined in their first postirradiation division.

The figures for total abnormal cells and total abnormal chromosomes in Table II show a rough correlation with dose. Case "C" shows the largest departure from strict dose dependence. The discrepancies may be explained in a number of ways: by the uncertainty of dose estimation, individual neutron-to-γ-dose ratio, cell selection, on nonuniform dose distributions to various patients, which would leave reservoirs of relatively undamaged hematopoietic cells that might well repopulate the damaged tissue and lower the numbers of aberrant cells seen in the peripheral blood. If selection were increasing the yield of certain types of aberrations in some of the present cases, we might expect that their frequencies would be greater than predicted by previous work on aberrations induced in human cells.

Our earlier experiments with both human and other mammalian cells (*1, 2, 4, 5*)

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suggest that the hematopoietic cells which gave rise to the circulating leukocytes examined in the present study probably had a coefficient of breakage of about 0.005 breaks per cell per roentgen for low doses of hard X-rays. Since total detectable breakage rises as somewhat more than the first power of dose, this value yields an estimate of breakage that is too low at higher doses. There is no information on aberrations in human chromosomes irradiated with neutrons. If, however, we accept the same relative biological effectiveness (RBE) for fission neutrons that was used in estimating the doses received by the irradiated cases (2.0), we should expect the estimated doses to have produced *at least* the breakage frequencies listed in Table IV, and substantially more breakage at the higher doses. We may assume that the breaks are distributed according to the Poisson formula $[e^{-m} \cdot (m^r/r!)]$ (*4*). From the coefficient of break production, then, we may calculate the maximum percentage of cells *without* breaks. These values, calculated for the exposed men, appear in Table IV, column 4. Thus, at higher doses, most of the cells must have received at least one break. From the results in Tables II and III, we may conclude that many of the expected breaks, or rather, the cells containing them, have been lost, and the hematopoietic tissue was repopulated largely from clones derived from those cells with no breaks. It therefore seems unlikely that there has been appreciable selection for cells with aberrations, but rather a loss of these cells. Such a loss is expected because only certain classes of first-division aberrations yield abnormal chromosomes that can persist and be seen in later divisions.

Although the origins of many persistent types of aberrations are complex, some are fairly simple. Dicentric chromosomes are a good example. They may arise in

TABLE IV

EXPECTED MINIMUM CHROMOSOME BREAKAGE AND EXCHANGE PRODUCTION CALCULATED FROM THE DOSES RECEIVED BY THE IRRADIATED CASES[a]

Case	*Dose (rem)*	*Breaks per cell*	*Cells with no breaks (%)*	*Asymmetrical exchanges per cell*	*Cells with at least one asymmetrical exchange (%)*
A	461	2.3	10.0	1.1	66.7
B	341	1.7	18.3	0.6	45.1
C	428	2.1	12.3	0.9	59.3
D	413	2.1	12.3	0.9	59.3
E	298	1.5	22.1	0.5	39.4
F	86.5	0.4	67.0	0.04	3.9
G	86.5	0.4	67.0	0.04	3.9
H	28.8	0.1	90.5	0.003	0.3

[a] Calculated from the rates of human cells *in vitro* given by Bender and Wolff (*4*) on the basis of a linear dose curve for breakage and Poisson distribution of breaks and exchanges (see text for details of the assumptions involved).

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two ways: from the dicentric chromosomes produced by asymmetrical chromosome exchange, and from the dicentric chromatid formed in asymmetrical chromatid exchanges. Both are, of course, two-hit aberrations when induced by X-rays. Although the exponent relating yield to dose for neutrons is probably nearer 1 than 2, the neutron doses in the present cases represented only about a third of the total dose (*8*). In view of the other uncertainties involved, we feel justified in treating all exchanges as two-hit effects in the discussion that follows. The yield of exchanges may be expressed as

$$Y = a + bD^2$$

where a is the frequency of spontaneous exchanges, b the coefficient of exchange production, and D the dose. The value of a can be taken to be very nearly zero (*2*, *5*). There is only one estimate of b available, which is for chromatid exchanges induced by hard X-rays (*4*). This value, 5×10^{-6} exchanges per cell per r^2, was measured for the asymmetrical type of exchange only. If, for simplicity, we assume that chromosome exchanges occur with about half the frequency of chromatid exchanges, the expected yield of aberrations that would produce dicentric chromosomes is

$$Y = (5 \times 10^{-6})D^2$$

(since both daughter cells can receive dicentric chromosomes from an asymmetrical chromosome exchange, but only one of the daughter cells can receive a dicentric chromosome from an asymmetrical chromatid exchange, the yield of dicentrics would be equal in both cases). Table IV, column 6, gives the percentage of cells expected to have dicentrics in the first division on the basis of the above assumptions and if the exchanges are Poisson-distributed. Comparison of the numbers of asymmetrical exchanges expected (Table IV, column 5) with the observed values in Table II reveals that many of the dicentrics that must have been induced, if the rates *in vivo* are anywhere near the rates *in vitro*, must have been lost in subsequent divisions. Only one of the patients had a very high frequency of dicentrics ("C"), and even this is still far below the value predicted had none of the dicentrics been lost.

The major mechanism of loss of dicentrics is chromosome bridge production. If the centromeres on a dicentric were distributed randomly to the poles in each mitosis, then half of the time the centromeres on one daughter chromatid would move to the opposite poles, causing a bridge to be formed. The fact that any of the dicentrics in the present cases have persisted for as long as 29 months indicates that there is some selection *for* cells with dicentrics or that distribution of the centromeres is not random. It seems quite possible that, at least where the two centromeres are close together, mechanical resistance to twisting of the chromosome might cause both centromeres on the chromatid to move to the same pole.

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The variation in the number of chromosomes seen in the irradiated cases was much greater than in the controls. This variation was not, however, strictly dose-dependent, probably for the same general reasons that have been given earlier for chromosome aberrations. That the large variation in number was, in fact, caused by the irradiation is shown by the fact that the aberrant numbers were distributed both above and below $2n = 46$, whereas in the controls (Table I) only cells with less than $2n = 46$ were found. These counts could have been caused by breakage of cells during fixation and slide preparation.

Tough *et al.* (7) found a large increase in the frequency of polyploid cells in cultures of leukocytes made shortly after the patient had been given partial-body X-ray therapy. No such increase was found in the present material, which suggests that polyploid cells induced by irradiation do not persist for long periods of time.

There can be no doubt about the usefulness of the peripheral leukocyte culture technique for the study of the radiosensitivity of human chromosomes. Although it is already known that human chromosomes behave both quantitatively and qualitatively the same as those of the more extensively studied plant and animal materials when irradiated (4), no quantitative measure of their sensitivity *in vivo* has been made. For such a study it is mandatory that the blood samples be taken immediately after irradiation and scored in their first postirradiation division. The donor must, of course, have received a single total-body dose of radiation. It is also desirable that the donor be a normal, healthy individual, since a number of diseases, particularly those for which radiation therapy is given, have been shown to be associated with abnormal chromosomes. Although preirradiation control examination of diseased subjects about to undergo total-body therapeutic irradiation would remove one objection to the use of leukocytes for this purpose, there still remains the possibility that the disease state itself might alter the sensitivity of chromosomes, especially where chromosomal abnormalities frequently are part of the disease syndrome. Accidental exposures, such as have occurred in the past, and which will possibly occur in the future, are an obvious source of material.

In addition to the information about human radiosensitivity that such investigations can provide, it is probable that chromosome aberration analysis will become a useful clinical procedure. Once accurate correlations of dose-aberration frequency are available, it should be possible to estimate dosage from such an analysis. The circulating leukocytes become, then, a biological dosimeter that may well be of use in planning the treatment of cases of accidental irradiation.

In spite of the persistent chromosomal abnormalities shown by the cases studied, none had any associated clinical symptoms. Apparently the leukocytes examined by our technique and the tissues responsible for their production are quite capable of normal function, even when they possess chromosomal alterations. Certainly we can make no speculations about the possible effects of the chromosomal aberrations on the future health of the patients. In fact, it seems entirely reasonable to

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assume that the aberrations are being gradually lost and that the patients' circulating leukocytes will eventually become entirely normal. Only the examination of irradiated individuals over much longer periods than in the present study can answer this question.

SUMMARY

The chromosomes of a number of men exposed to whole-body mixed γ-ray and fission neutron irradiation, as well as those of five unirradiated control individuals, have been examined. Cytological preparations were made from short-term cultures of peripheral blood leukocytes approximately 29 months after the irradiation occurred. The doses were estimated to range from 22.8 to 365 rads. Five of the men received doses estimated to exceed 230 rads; three others received lower doses. In the five control individuals no major chromsomal abnormalities were found, other than the expected low frequencies of chromatid-type aberrations and counts other than $2n = 46$. In cells from the irradiated individuals, however, changes in both chromosome number and form were observed. In the five "high-dose" cases, the frequency of cells with counts of other than $2n = 46$ ranged from 7 to 23%. Grossly altered chromosomes, such as rings, dicentrics, and minutes, were found, often in cells with abnormal counts, with a frequency ranging from 2 to 20 per 100 cells. Two of the three "low-dose" individuals also show variations in number and form of their chromosomes, but at a much lower frequency than the "high-dose" cases.

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