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Chromosome Damage Induced by Plasma of X-Rayed Patients: An Indirect Effect of X-Ray* (33295)

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Radiation can damage human chromosomes, both *in vivo* (1–5) and *in vitro* (6–8). This report describes more fully the results of an experiment devised to distinguish between the direct effects of X-rays on leukocyte chromosomes and a possible indirect effect mediated by way of blood plasma. Blood plasma from patients who had received large doses of X-ray enhanced the degree and frequency of chromosomal aberrations of normal nonirradiated lymphocytes in short-term cell culture (9).

Materials and Methods. Six patients with a variety of tumors were chosen because they had received large doses of X-rays through several different ports (see Table I). X-ray integral doses varying from 2.88 to 25.1 × 10^6 g R were given with a 2 meV van de Graaf generator. Six other patients with similar tumors, studied prior to X-ray, served as controls for the first group. Three of the control patients with tumors were studied before and after X-ray. None of the patients received chemotherapeutic agents or had symptoms of viral infections during the studies. Blood for the studies was drawn from the irradiated patients 24–72 hr after irradiation. Subjects who had not received therapeutic X-ray furnished the normal lymphocytes and plasma.

Chromosomes were studied by a modified method of Moorhead *et al.* (10). Plasma from the following sources was added to normal nonirradiated lymphocytes in short-term culture: (i) plasma from irradiated tumor patients ("irradiated tumor plasma"), (ii) plasma from tumor patients before irradiation ("nonirradiated tumor plasma"), (iii) homologous "normal plasma," or (iv) autologous "normal plasma."

The cells for culture were obtained from leukocyte-rich plasma after sedimentation

TABLE I. Summary of Clinical Information and X-Ray Administration in Patients Receiving Therapeutic X-Ray.

Pt.	Tumor	Port	X-ray dosage* Integral (10^6 g R)	X-ray dosage* Midline (R)
FT	Unidentified mediastinal	Upper thorax	15.2	3610
MF	Glioblastoma	Cranium	13.3	4490
HD	Lymphosarcoma of testis	Pelvis & para-aorta	25.1	3200
BB	Trans. cell Ca of bladder	Pelvis	7.46	3350
DM	Squamous cell Ca	Neck	2.88	3200
RZ	Astrocytoma	Cranium	11.8	4460
MR	Trans. cell Ca of bladder	Pelvis	0*	
VK	Astrocytoma	Cranium	0	
JR	Ca of uterus	Pelvis	0	
TG	Ca of lung	Thorax	0→10.8	3000
ET	Ca of cervix	Pelvis	0→ 4.3	1000
MM	Ca of tongue	Neck	0→ 0.4	1000

* Therapeutic X-ray.

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0017101

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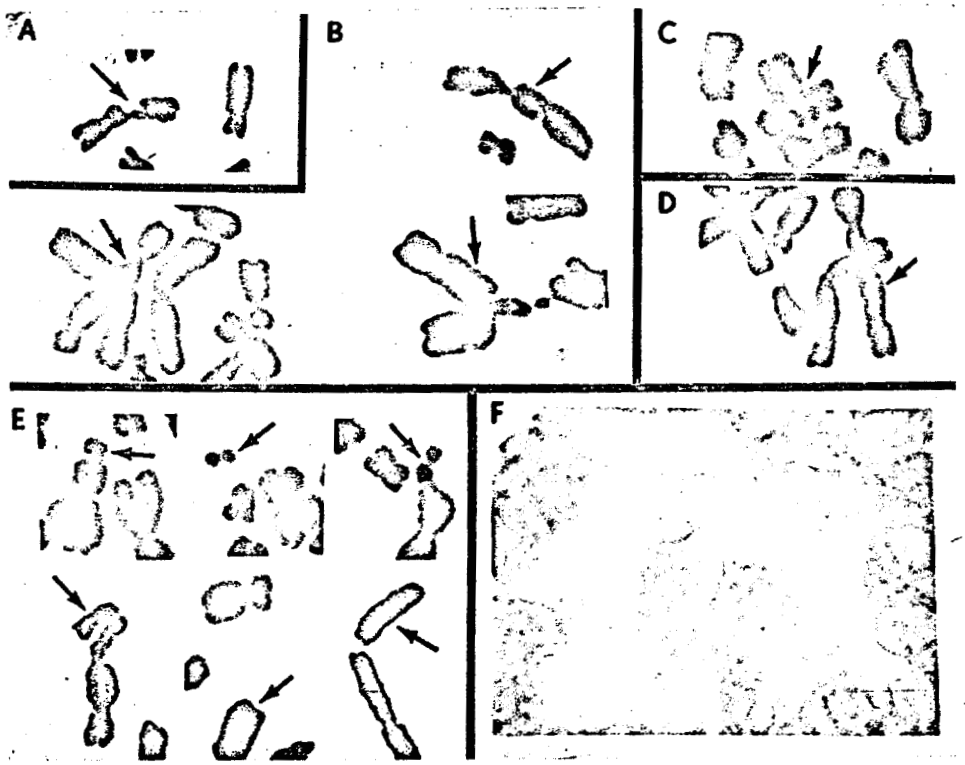

FIG. 1. Chromosomal abnormalities seen in normal cells treated with plasma from irradiated patients. A. Chromatid break. B. Dicentric chromosomes, one with a break. C. Quadriradial chromatid exchange figure. D. Translocated chromosome. E. Chromosome and isochromatid breaks (includes acentric fragments). F. Chromosome clumping and inter-chromosome standing seen in some cells.

of heparinized blood. The leukocyte-containing plasma remaining after removal of erythrocytes, was centrifuged at 2400 rpm (IEC Universal model UV, head no. 240) for 20 min to obtain leukocyte-free plasma. This final plasma was free of erythrocytes and leukocytes, but contained platelets. In all homologous combinations the Y chromosome was used as a marker.

Leukocyte-rich plasma (0.4–0.6 ml) and cell-free plasma (1.4–1.6 ml) were added to culture bottles containing TC 199, phytohemagglutinin and supplemented with 10% fetal calf serum. The total volume of media, cells, and plasma was 10 ml. The cells were harvested, fixed with glacial acetic acid: methanol::1:3 spread on chilled slides, flamed dry, and stained with Giemsa blood stain. Cells were selected for study with the low power objective and, once chosen, were counted and scored. Approximately 50 cells on 3 or 4 slides were scored in each culture.

In scoring the cells, the chromatid was considered broken only if the chromatid fragment distal to an achromatic region was displaced or misaligned. Breaks occurring in the same region of sister chromatids, acentric fragments, and chromosome deletions were classified as chromsome breaks. Chromatid exchange formations, dicentric chromosomes, and ring chromosomes were listed separately (Fig. 1 B, C). Reciprocal translocations giving stable chromosomes were excluded because of the difficulty in the positive identification unless a marker was produced.

To measure mitotic indices, 300 cells from

0017102

TABLE II. Comparison of Chromosomal Damage by Plasma from Irradiated and Nonirradiated Subjects.

Plasma added to normal cells	Total cells (no.)	Chromosome aberrations: Gaps (no. seen; no./100 cells)	C'tid breaks (no. seen; no./100 cells)	C'some breaks (no. seen; no./100 cells)	Complex c'somes: Ring	Di-centric	C'tid exchange fig.	Abnormal cells[a] (no. seen; %)
Normal								
Autologous or homologous	782	33 (4.2)	28 (3.6)	3 (0.4)			1	32 (4.1)
Nonirradiated								
Tumor patient	335	23 (6.8)	14 (4.2)	3 (0.9)				13 (3.9)
Irradiated								
Tumor patient	325	40 (12.2)	35 (10.7)	23 (7.1)	1	3	1	55 (16.9)
Comparison in same patients with tumors before and after X-ray (3 studied)								
Nonirradiated	141	12 (8.5)	6 (4.2)	1 (0.7)				6 (4.2)
Irradiated	151	15 (9.9)	18 (11.9)	13 (8.6)		1		28 (18.9)

[a] Cells with gaps only were not included.

each culture were counted and classified as small lymphocytes, immature forms, or cells in mitosis. The ratio of cells in mitosis to the total number constituted the mitotic index. The data were compared statistically using chi-square test for significance.

Results. Table II summarizes the results of this study. The frequency of chromatid breaks after treatment of cells with "irradiated tumor plasma" (10.7/100 cells) was significantly higher than the frequency of chromatid breaks when using "nonirradiated tumor plasma" (4.2/100 cells) or "normal plasma" (3.6/100 cells). The frequencies of chromatid breaks in the two control groups were not different from each other. Similarly, the frequency of chromosome breaks was significantly higher in normal cells treated with "irradiated tumor plasma" (7.1/100 cells) when compared with "normal plasma" (0.4/100 cells) and "nonirradiated plasma" (0.9/100 cells). There was one quadriradial chromatid exchange figure seen in the control groups; this was seen in normal cells treated with autologous "normal plasma." In normal cells treated with "irradiated tumor plasma" there was one quadriradial chromatid exchange figure. Three dicentric chromosomes and one ring chromosome were also found in this group, whereas none was found in the controls. We were definitely able to identify translocated chromosomes in several cells in the last group; but because some other metaphases contained questionable translocations, this specific aberration was not tabulated. Also, the frequency of gaps was higher in normal cells treated with "irradiated plasma" than in either control group.

Excluding gaps from the comparisons, 16.9% of normal cells treated with "irradiated tumor plasma" contained abnormal chromosomes or chromatids. This was significantly higher than normal cells treated with "nonirradiated tumor plasma" (3.9%) or with "normal plasma" (4.1%) ($p<.001$).

In addition to the definite increase in chromosome breaks, we were impressed with the number of normal cells, treated with "irradiated plasma," that had "sticky" chromosomes, prominent stranding among the chromosomes, and excessive satellite association

0017103

(see Fig. 1 F). This was rarely seen in the control groups. Also, there were many secondary constrictions in the irradiated group.

We were able to study the effects of plasma from three of the patients with tumors both before and after they had received from 0.4 to 10.8 $\times$ 10^6 gm R integral dose of X-ray. There were abnormal chromosomes or chromatids (excluding gaps) in 4.2% of cells treated with plasma from the patients with tumors before irradiation. Cells treated with plasma from these tumor patients after irradiation showed chromosome and chromatid aberrations in 18.9%, a significant increase.

Mycoplasma cultures of plasma from the first six irradiated patients did not show organisms.[1]

The mitotic index showed a wide variation within each group studied, but there were no significant differences between cultures treated with "normal plasma" (6.1%; range, 3.0–8.3%) and with "irradiated tumor plasma" (6.2%; range, 2.3–11.0%).

Discussion. Material that enhanced chromosome breakage in lymphocytes in short-term cell culture ("chromosome-breakage factor") was present in plasma of patients who received large doses of X-ray for tumors. The possibility that this effect was related to the tumors for which the patients were irradiated was excluded when plasma from the patients with tumors obtained before irradiation did not show an effect different from normal. Thus "chromosome-breakage factor," produced in some way by X-ray, could mimic the "direct" effect of X-ray on the chromosomes or cells involved. These results are similar to those of Goh who studied the effect of plasma from patients 7 years after receiving large doses of whole body X-ray on normal cells (11). It is not known at this time whether the "chromosome-breakage factor" produced chromosome breaks, or whether it impeded repair of a usual breakage process during mitosis. Possibly the "chromosome-breakage factor" in plasma was produced by X-ray damage to cells or subcellular elements. This material may be common to that produced after tissue damage from many conditions and account for chromosome damage with viral infections (12,13), certain drugs and chemicals (14–17), myeloproliferative disorders (18), and anemias (19,20). The *in vitro* method used in this experiment for investigating the indirect effects of X-rays can be adequately controlled both methdologically and internally and would be useful in re-investigating other causes of chromosomal damage.

There were chromosome aberrations found that resulted from damage before replication, e.g., chromosome breaks, rings, dicentrics, and translocations. In addition, the finding of chromatid breaks, gaps, and chromatid exchange figures indicates that damage continued into the post replication phase of mitosis.

The identity of the "chromosome-breakage factor" is unknown. There are many possibilities that might explain this phenomenon. The observations are analogous to those of Wald *et al.* in RF mice in which irradiation of the animals produced myelogenous leukemia with a specific marker chromosome. Ultracentrifugates of the mouse plasma transferred the myelogenous leukemia and the marker chromosome to other mice of the same strain. The investigators observed virus particles which, they proposed, had been "activated" by the X-ray and were responsible for the changes found in the animals and chromosomes (21). Allison and Paton found chromosome aberrations when lysosomes of human fibroblasts in tissue culture were selectively damaged by using light-sensitive dyes and irradiation with light of critical wavelength. They did not observe this effect when the nucleus was damaged in a similar way. They concluded that lysosomal enzymes, released from the damaged organelle, produced the chromosomal damage seen (22). Fialkow, who found that extracts from allogeneic lymphocytes produced chromosome aberrations in cultures of fibroblasts, an effect not seen with autologous extracts, explained the chromosome damage by implicating autoimmune mechanisms (23). It is possible that the "chromosome-breakage factor" causes shift in DNA polymerase equilibrium, as suggested by Ahnstrom and Na-

[1] Cultures for mycoplasma were done under the direction of R. A. Reddick, Department of Microbiology, Medical College of Georgia.

0017104

tarajan, so that the nucleotides of DNA are removed by the polymerase leaving damaged chromosomes (24). The mechanism of chromosome breakage and identity of the "chromosome-breakage factor" must await further studies.

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Cytologic Changes in Mouse Peritoneal Fluid Between Birth and Sexual Maturity (33296)

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We have noted the similarity of abdominal peritoneal cytology in pregnant women and pregnant mice. Perhaps a better understanding of the significant phase from birth to sexual maturity in the mouse will aid in our studies on the developing child. The effect, if any, of a sex and aging response may be detected in the cytologic composition of the mouse peritoneal fluid in the normal growth process from birth to maturity. This data obtained would then permit future studies on the influence of metabolic disorders. drugs. hormones, and neoplasia on peritoneal fluid cytology during the normal rapid growth period of the mouse.

Materials and Methods. We obtained cytologic specimens from the peritoneal cavities of male and female newborn (day 1), weanling (day 21) and adult CF-1 mice (>35

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0017105