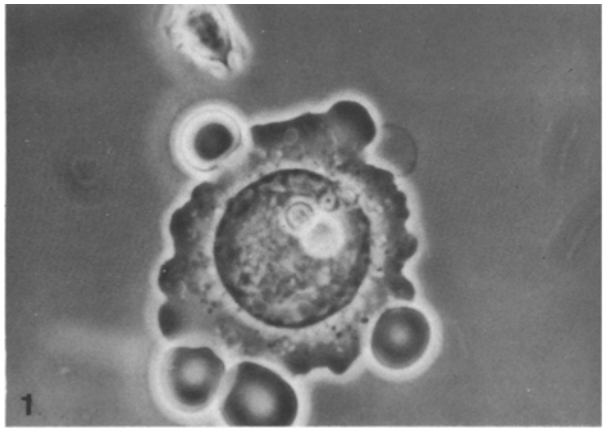

Phase contrast microscopy of rosette formation.
Fig. 1. Ehrlich tumor cells (5×10^5 cells/0.2 ml) were incubated with Con A treated erythrocytes (10^7 cells/1 ml).

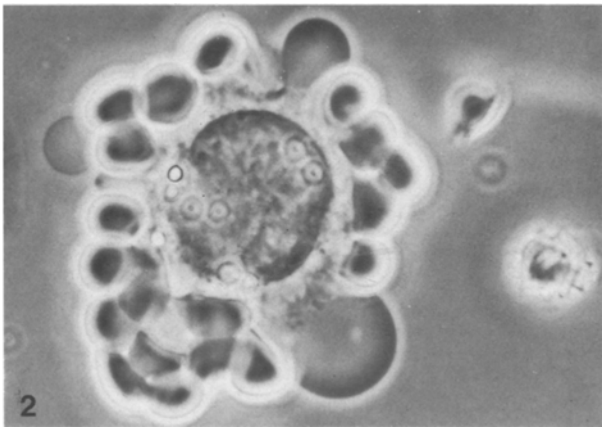

Fig. 2. Ehrlich tumor cells (5×10^5 cells/0.2 ml) were incubated with Con A treated erythrocytes (10^8 cells/1 ml).

Materials and methods. Freshly obtained human blood, with heparin as anticoagulant, was washed 3 times in phosphate buffered saline (PBS) to remove plasma and buffy coat. Erythrocytes were resuspended in PBS at a final concentration of 10^9 cells/ml. The erythrocytes were incubated with trypsin (Sigma Chemical Co. 1 mg/ml) at 37 °C for 30 min with gentle shaking. The suspension was then washed 6 times with PBS to remove trypsin. The erythrocytes, at a concentration of 10^9 cells/ml, were incubated with Con A (Sigma Chemical Co. 50 µg/ml) at 4 °C for 1 h, and the cells were then washed gently twice by centrifugation at $150 \times g$ for 3 min at 4 °C.

Ehrlich ascites tumor cells were collected from a DDY mouse and washed twice with Hanks' balanced salts solution. Rosette formation on mixing the tumor cells with erythrocytes was performed by the following method. In a 10×75 mm glass tube, 0.2 ml of a suspension consisting of 5×10^5 tumor cells was mixed with 1 ml of Con A treated erythrocytes at a concentration of 10^7 or 10^8 cells/ml. The mixture was incubated at 37 °C for 30 min with gentle shaking. The formation of rosettes on mixing the tumor cells with Con A-treated erythrocytes was observed by phase contrast microscope, and rosettes consisting of a minimum of 3 erythrocytes bound to a tumor cell were counted.

Results and discussion. Trypsinized and Con A treated erythrocytes attached to the tumor cells and formed rosettes (figure 1 and 2). 82% of tumor cells formed rosettes with erythrocytes when 5×10^5 tumor cells were incubated with 10^7 erythrocytes and 87% of cells formed rosettes when incubated with 10^8 erythrocytes. A few erythrocytes were agglutinated without rosette formation. At 37 °C a large number of erythrocytes attached to tumor cells, but at 4 °C they did not. α-D-methyl glucoside (Sigma Chemical Co. 10 µg/ml) inhibited rosette formation.

One possible approach for increasing the effectiveness of antitumor drugs would be to find methods of altering their distribution in the body to increase their local concentration at the tumor cell sites. Erythrocytes have been used as a carrier vehicle to enhance the cytotoxic activity of daunomycin against tumor cells[3]. The effectiveness of erythrocytes against tumor cells specifically demands targeting of the erythrocytes to tumor cells. Some success has already been reported in this direction in vivo and in vitro by the use of lectin although the results were not satisfactory[3,5].

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Induction of micronuclei in PHA-stimulated human lymphocyte cultures by therapeutic radiation[1]

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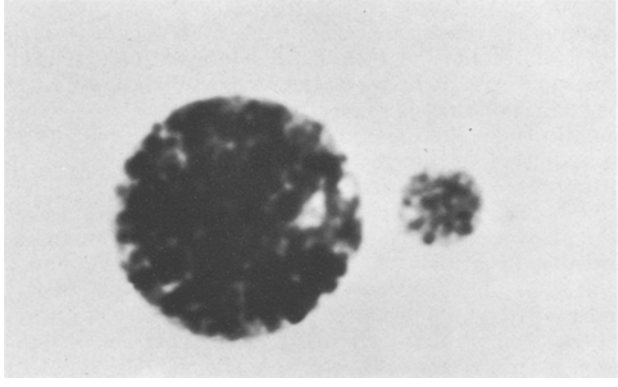
Summary. Micronuclei frequency and percent of chromosome breaks increases significantly in adults whose thymus glands were irradiated in infancy and after irradiation of cancer patients.

Radiation exposure of man produces chromosomal aberrations in human peripheral blood lymphocytes[3]. The micronuclei are formed from chromosomal fragments that are not incorporated into daughter nuclei at mitosis because they lack a centromere. The micronucleus test is a simpler and faster method than chromosome analysis for determining chromosomal fragmentation[4,5]. To determine whether the micronucleus test correlated with chromosome aberrations, we determined the micronuclei frequency in lymphocyte cultures in normal control people, in adults whose thymic glands were irradiated in infancy and in cancer patients before and after radiation treatment.

Effect of radiation on micronuclei frequency and chromosomes

	Micronuclei per 1000 cells (mean ± SE)	% Abnormal metaphases (mean ± SE)	% Chromosome breaks[a] (mean ± SE)
Controls (n = 15)	2.43 ± 0.24	1.62 ± 0.69[b]	2.04 ± 0.97[b]
Thymus radiation in infancy (n = 10)	3.54 ± 0.34	10.12 ± 1.68	15.72 ± 2.36
P-value[c]	< 0.02	< 0.005	< 0.005
Cancer patients before radiation (n = 7)[d]	4.17 ± 0.91	2.91 ± 0.78	4.33 ± 1.09
P-value compared to controls	< 0.025	N.S.[e]	N.S.[e]
Cancer patients after radiation (n = 7)	8.21 ± 1.78	27.25 ± 5.60[f]	65.70 ± 14.67[f]
P-value compared to controls	< 0.001	< 0.005	< 0.005
P-value compared to preradiation	< 0.05	< 0.001	< 0.001
Cancer patients 1-2 months after radiation (n = 4)	5.49 ± 0.40	30.93 ± 6.46	85.53 ± 19.33
P-value compared to controls	< 0.001	< 0.005	< 0.005
Cancer patients 3-4 months after radiation (n = 4)	7.78 ± 3.34	25.80 ± 4.04	63.30 ± 15.29
P-value compared to controls	< 0.005	< 0.001	< 0.005
Cancer patients 1 year after radiation (n = 2)	3.38 ± 1.35	24.85 ± 3.05	79.25 ± 15.85

[a] Breaks per 100 cells. [b] Values obtained from 5 controls published by Goh et al.[6]. [c] According to Student's t-test. [d] % Abnormal metaphases and % chromosome breaks for cancer patients (Reddy et al.[7]). [e] Not significant. [f] Values obtained from 6 patients.



A nucleus and micronucleus observed in a preparation from human lymphocyte culture.

Materials and methods. 15 healthy individuals, 22–45 years old, served as controls. 10 adults, 23–43 years old, who had received 200–768 rads (in air) to the thymic area between 22 days and 559 days of age for alleged thymic enlargement, were studied. 7 cancer patients (2 cancer of prostate, 2 cancer of breast, 1 cancer of lung, 1 cancer of endometrium and 1 cancer of larynx) were studied before and after radiation therapy. They had received between 4000 and 6000 rads.

Lymphocytes were separated and cultured as described previously[6,7]. The micronuclei were counted in slides obtained from 3-day phytohemagglutinin (PHA)-stimulated cultures according to the method of Countryman and Heddle[8]. They have established the following criteria for identifying micronuclei: 1. diameter less than $\frac{1}{3}$ the main nucleus, 2. non-refractility, 3. color same or lighter than that of the nucleus, 4. location within 3 or 4 nuclear diameters of a nucleus but not touching the nucleus. Slides were coded and scored blind at magnification ×400. At least 3000 cells were scored from the 3 slides obtained from each individual.

Results. The Figure shows a nucleus and a micronucleus. The table shows the number of micronuclei and chromosome breaks in the lymphocytes of healthy controls and in individuals with a history of thymus irradiation in infancy. The controls had 2.43 ± 0.24 (mean ± SE) micronuclei per 1000 cells compared to 3.54 ± 0.34 for thymus-irradiated subjects. This difference is statistically significant. The percent of chromosome breaks in controls was 2.04 ± 0.97 compared to 15.72 ± 2.36 in the thymus-irradiated subjects. This difference is also statistically significant.

Cancer patients before radiation had 4.17 ± 0.91 micronuclei per 1000 cells. This is significantly different from the control value. After radiation the micronuclei increased to 8.21 ± 1.78 per 1000 cells which is significantly higher than that before radiation. The percent of chromosome breaks also increased from 4.33 ± 1.09, observed before radiation to 65.70 ± 14.67 after radiation treatment. This difference is also statistically significant.

Discussion. Results of in vitro experiments on the production of micronuclei by radiation in human whole blood cultures showed a quantitative relationship between chromosome aberrations and micronuclei[8]. Micronuclei frequency increased in blood samples from patients after angiocardiography and also after exposure to contrast media[9–11]. Our results indicate thymic irradiation 23–43 years before this study also induced a significantly higher frequency of micronuclei. We observed a significant increase in chromosomal aberrations in these patients. This observation suggests that micronuclei frequency is a good indicator of chromosome damage.

Radiation treatment of cancer patients also increased the frequency of micronuclei and chromosome breaks[7]. The significant increase of micronuclei observed in our cancer patients before radiation could be due to spontaneous chromosome breaks occurring in these patients. The chromosome breaks observed in these patients was twice that of the control values. Although this difference is not statistically significant, it is possible this value could have been had we studied more numbers of patients.

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