DETECTION OF RADIATION EXPOSURE BY FLUORESCENT MICROSCOPY OF HUMAN BLOOD CELLS

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Abstract—Changes in blood and bone marrow cells involving effects of irradiation on nucleic acids can be detected by fluorescence microscopy much prior to any morphological changes. When blood smears are stained with acridine orange, the leucocytes appear mostly as doubly coloured in a fluorescence microscope, the nucleus being green and the cytoplasm red, yellow or orange. The ratio of doubly coloured cells (DCC) to red coloured cells is fixed for each subject but decreases following X-irradiation, as found in the following experiments. Persons exposed to X-ray routine radiography of chest, as well as hospital personnel were taken as subjects. X-ray doses were measured with a Victoreen Condenser R-Meter and Victoreen Ionization chambers, as skin doses, and exposure time was 0.05 to 0.1 sec. Blood samples were taken from a finger tip before exposure and at fixed intervals after exposure. Blood smears were prepared and stained with acridine orange in phosphate buffer (5 mg/50 ml or 10 mg%, pH 6.85). Two hundred cells were counted and classified according to colour. In all cases the number of doubly coloured cells (green and red) before irradiation was above 68%. Following exposure, in most cases the number of DCC decreased while the number of red coloured cells increased. The observed changes began 6 hr after exposure, reached a maximum 24 to 48 hr after irradiation and then began to subside. The extent of the effect increased with dose, giving noticeable results already at a dose of 18 milliroentgen. There was a uniform response among persons exposed in the chest, but a smaller change in persons exposed in other organs. Blood smears of technicians and clerks not directly involved in work near the X-ray unit showed no changes in the ratio of DCC to red coloured cells. Physicians after fluoroscopy, however, showed a drop in percent of doubly coloured cells. Further development of these investigations might lead to a simple method of internal dosimetry for small doses of X-rays.

1. INTRODUCTION

The need for better biological methods of measuring the extent of radiation exposure on humans is evident. Lately some progress was made in the field of biological dosimetry[1] and the evaluation of radiation effects on chromosomes.[2]

Blood cells, and leucocytes in particular, are known to be very sensitive to ionizing radiations. Changes in blood and bone marrow cells involving effects of radiation on nucleic acids and nucleoproteins are found much prior to any morphological changes. It is well established that desoxyribonucleic acids (DNA) are the cell component most sensitive to both ultraviolet[3] and ionizing irradiation.

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The use of fluorescent microscopy for observing early radiobiological effects of irradiation on cells is described in a number of publications.[4-9]

Adsorption of dyes on DNA and RNA is apparently affected by their sensitivity to irradiation. When blood cells are stained with acridine-orange and leucocytes are observed in a fluorescence microscope—the nucleus is coloured green, and the cytoplasm red, yellow or orange.

Leucocytes appear as doubly coloured—green and red under a fluorescence microscope. The ratio of doubly coloured cells to red cells is apparently fixed for each subject. This ratio changes, however, after irradiation. Meisel and Kondrateva,[6] using cell cultures of spontaneous mammary gland cancer of mice, showed

Table 1. *Results of Counts of Fluorescing Blood Cells of Persons exposed to X-ray Chest Radiography. Dose was* 18 *mR. Microscopy performed on Zeiss or Reichert fluorescence microscopes*

	Fluorescence of leucocytes after chest X-ray											
	0 h		3 h		6 h		24 h		48 h		72 h	
Subject	DCC	RCC	DCC	RCC	DCC	RCC	DCC	RCC	DCC	RCC	DCC	RCC
1.	69	27	48	40	40	54	—	—	48	50	67	26
2.	89	5	75	21	—	—	37	67	58	38	69	24
3.	72	24	—	—	—	—	24	72	69	28	80	17
4.	73	21	72	21	—	—	69	27	85	10	—	—
5.	78	17	78	19	—	—	68	24	83	13	62	32
6.	89	10	—	—	—	—	71	6	94	4	—	—
7.	84	13	—	—	—	—	71	23	49	40	81	16
8.	92	7	—	—	—	—	83	1	85	11	—	—
9.	84	13	—	—	—	—	81	16	90	8	—	—
10.	84	14	—	—	—	—	62	26	62	36	88	10
11.	86	12	—	—	—	—	71	8	94	5	—	—
12.	72	25	45	51	28	69	66	27	—	—	—	—
13.	87	11	81	16	—	—	62	33	85	10	—	—
14.	88	8	73	25	—	—	56	38	66	29	84	14
15.	66	30	50	45	38	55	62	29	76	16	—	—
16.	83	15	82	16	—	—	49	50	61	32	63	33
17.	86	12	—	—	66	22	10	86	23	25	80	17
18.	93	6	83	14	73	22	81	18	69	29	90	8
19.	82	17	82	11	—	—	47	45	66	28	72	25
20.	70	29	74	22	66	30	66	31	63	30	81	16
21.	79	17	—	—	—	—	73	21	53	31	75	22
22.	80	18	74	3	51	44	30	56	59	36	68	27
Average	81	15	70	23	51	40	59	33	68	24	75	20

changes by exposure to X-irradiation ranging from 25,000 to 500,000 R.

Kafafova [9] found a similar change with small doses (1 R, partial body irradiation). Kolesar [10] applied the latter method for observing late effects of irradiation on humans, when he observed in radiation workers, that after several years of chronic exposure to low levels of irradiation, the % of green nuclei decreases by about 5%.

2. METHODS AND MATERIALS

Subjects: Persons exposed to X-irradiation of chest at routine X-ray radiography, and hospital personnel, involved and not involved in radiation work.

2.1. *Dosimetry*

An ionization chamber was placed in the exact position of the exposed part of the person's body and was irradiated at the same conditions of electron current, photon energy and exposure time.

The dose was measured with a Victoreen Condenser R-Meter 570 A. The ionization chambers were Victoreen chambers models 188, having a range of 0–0.025 R; models 130 and 576, having a range of 0–0.25 R; model 227 having a range of 0–1 R; and model 633 having a range of 0–2.5 R. All models have a rated accuracy of ± 10%. The readings were corrected by the T factor. Exposure time was $\frac{1}{20}$–$\frac{1}{10}$ sec. All doses are skin doses.

2.2. *Sampling*

Blood samples were taken from a finger tip before and after exposure at fixed intervals and were stained with acridine orange solution prepared in phosphate buffer $\frac{M}{15}$ (5 mg/50ml., pH 6.85), by mixing in a leucocyte pipette for 30 min.

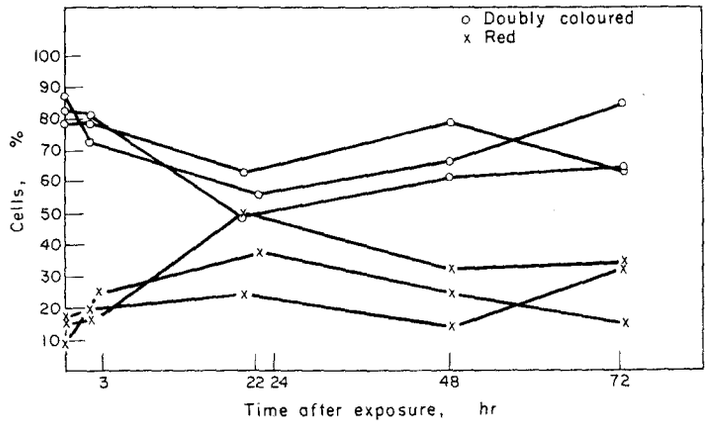

FIG. 1. Graphical description of three cases out of those summarized in Table 1.

Blood smears were prepared and counted in a UV fluorescent microscope.

3. RESULTS AND DISCUSSION

Over 50 cases were included in this investigation. It has been first established that in all cases the number of doubly coloured cells before irradiation is always above 68%.

In the first type of experiment patients were examined before and after X-irradiation of the chest. Peripheral blood smears were prepared and 200 cells were counted and classified by colour.

Table 1 summarizes results of several such counts.

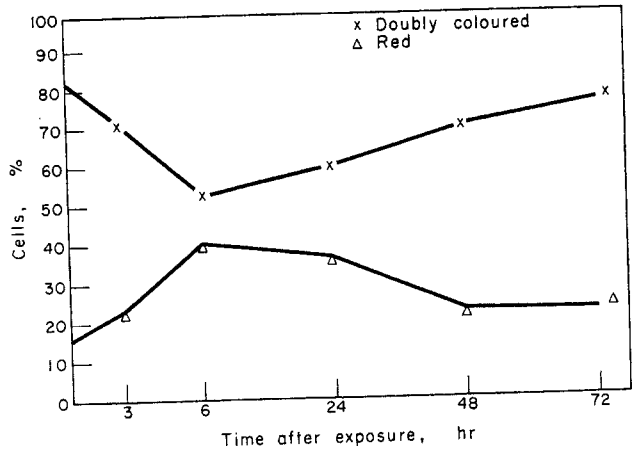

FIG. 2. Average results of 50 cases exposed to chest X-radiography at a dose of 18 mR, and analyzed as described in test.

Pre-irradiated human blood samples showed always over 68% of green–red coloured cells.

Following exposure, the number of DCC decreased while the number of red cells increased.

The changes observed began about 6 hr after irradiation, and usually reached a maximum at a deflection point 24 to 48 hr after irradiation, after which a reversed effect began. Figure 1 shows examples of these results for three cases.

The blood picture returned to the normal percentage of green and red cells after about 96 hr. Figure 2 shows average results of 50 cases.

Two groups of people could be classified as follows:

1. Persons of similar age, sex and weight, exposed on chest.
2. Hospital workers, physicians, technicians and clerks in the vicinity of the exposure area.

Persons of group 1 showed usually a significant drop in ratio of doubly coloured cells.

Blood smears of technicians and clerks, not directly involved in work near the X-ray machine, showed no changes in the ratio of DCC to RC.

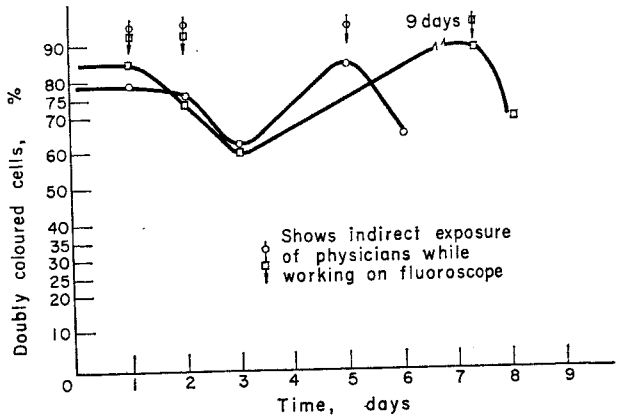

FIG. 3. Results of count of fluorescing blood cells taken from radiologists performing X-ray fluoroscopies. Arrows indicate day of work whereby indirect exposure occurred.

Physicians after performing fluoroscopy showed a significant drop in percent of doubly coloured cells (Fig. 3).

It is rather early to present an explanation of the observed effect. A logical assumption can be made that the radiation damage, whether direct or indirect, is affecting stem cells in such a way that their DNA and RNA content does not stain normally. The affected cells appear in circulation from 6 to 24 hr after irradiation and then are gradually removed from circulation and the blood picture becomes normal again.

4. CONCLUSION

Through a simple procedure, changes affected by small doses of X-irradiation on blood cells can be followed.

Development of this investigation might lead to an internal dosimetry method. The problem of variations in determination of ratios of green to red to doubly coloured cells is yet to be analyzed and the counts given by different observers should be checked independently to overcome subjectiveness.

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