THE EFFECT OF IONIZING RADIATION OF THE AGE SPECTRUM OF ERYTHROCYTES OF RABBIT PERIPHERAL BLOOD

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Although a great number of articles have appeared concerning the effect of irradiation on the integrity of the erythrocytes in the peripheral blood, the kinetics of this phenomenon have not been well studied. In particular, a strict differentiation between the consequences of radiation suppression of erythropoiesis, on the one hand, and the increase in destruction of circulating erythrocytes, on the other, has not been made. Data concerning the dependence of erythrocyte radiosensitivity on cell age are still very scanty. Some light on this question might be shed by investigation of the age spectrum of erythrocytes in the peripheral blood, on the basis of which one might be able to estimate the intensity of destruction of cells in different age groups. As a correlation exists between the age and the size of erythrocytes, the aim of the experiment consists of determining the distribution of erythrocytes by size.

Experiments have been described in the literature in which changes in the size spectrum of erythrocytes in the peripheral blood of irradiated rabbits have been studied [1]. However, the data were not subjected to quantitative analysis and only qualitative conclusions were drawn.

In this paper, we have investigated the changes in the spectrum of distribution of erythrocytes by size after irradiation, using strictly kinetic analysis.

METHODS

Chinchilla rabbits weighing 2200-2500 g were irradiated with gamma-emitting Co^{60}, the dosage being 250 and 500 R. At varying periods after irradiation, blood samples were drawn and determinations made of hemoglobin content, red cell count, and distribution of red cells by size according to the method described previously [2]. All experiments utilized 20 animals with 10 in each series.

Mathematical treatment of the experimental data took the following form. All erythroid cells circulating in the peripheral blood of the animals, were divided into three groups according to cell diameter. The first group included cells with diameter > 10.4 microns, the second group—those with diameter 8.2-10.4 microns, and the third group—with diameter <8.2 microns.

As it is known that the size of the erythrocyte depends on its age, we may establish a relationship between the size distribution of the erythrocytes and their age distribution. Cells with diameter greater than 10.4 microns roughly correspond to the erythrocyte precursor, the reticulocyte; those with diameters of 8.2-10.4 microns correspond with young erythrocytes, and those with diameters of less than 8.2 microns correspond with mature erythrocytes. According to the contemporary view, reticulocytes are released into the blood from the erythropoietic organs and differentiate into mature erythrocytes which in their turn grow old and are destroyed.

When the erythroid population in the peripheral blood is in equilibrium, the number of cells n_{io} in each of i age groups is proportional to the average time the cells remain in the group T_i:

$$\frac{n_{io}}{T_i} = \frac{N_o}{T}; \qquad i = 1,2,3, \tag{1}$$

where the index i = one corresponds to the erythrocyte precursor; i = 2 to the young erythrocytes, i = 3 to the mature

TABLE 1. Effect of Irradiation on the Size Distribution of Erythrocytes and their Hemoglobin Content

Dose of radiation (R)	Time after irradiation (days)	Number of cells of given size per mm³ of peripheral blood × 10^{-6}				Relative hemoglobin concentration in the cell
		10,4 μ	10,4—8,2 μ	8,2 μ	Total	
—	Control	0,17±0,01	1,40±0,07	4,32±0,07	5,89±0,08	1,00±0,01
250	5	0,21±0,01	1,49±0,09	4,17±0,14	5,87±0,15	0,98±0,02
	10	0,23±0,02	1,57±0,08	3,95±0,11	5,75±0,12	1,03±0,02
	19	0,29±0,02	1,69±0,09	3,94±0,17	5,92±0,19	1,04±0,02
	33	0,43±0,02	2,23±0,10	3,41±0,11	6,07±0,11	1,04±0,02
	47	0,24±0,01	1,59±0,05	4,37±0,12	6,20±0,14	1,02±0,02
	68	0,23±0,02	1,56±0,08	4,60±0,13	6,39±0,13	0,96±0,04
—	Control	0,18±0,01	1,25±0,03	4,03±0,05	5,46±0,09	1,00±0,01
500	8	0,16±0,01	0,19±0,05	4,11±0,08	5,46±0,11	0,96±0,02
	10	0,13±0,01	1,03±0,04	3,45±0,20	4,61±0,25	1,00±0,03
	17	0,17±0,02	1,14±0,08	3,09±0,23	4,40±0,32	1,04±0,05
	24	0,28±0,02	1,62±0,11	3,18±0,20	5,08±0,31	0,97±0,02
	38	0,22±0,03	1,41±0,10	4,06±0,18	5,69±0,25	0,93±0,02
	59	0,16±0,02	1,26±0,12	4,23±0,17	5,65±0,12	0,91±0,04

erythrocytes. The value of $N_o = \sum_{i=0}^{3} n_{io}$ signifies the general number of erythroid cells in an equilibrium population in the peripheral blood, and $T = \sum_{i=1}^{3} T_i$ the average lifespan of the cells; The value $1/T_i$ ($i = 1.2$) represents the mean probability of maturation for the reticulocytes and young erythrocytes per unit time, and $1/T_3$ the probability of destruction of the mature erythrocytes during natural aging.

The quantity of cells in i age group after time "t" after irradiation $n_i(t)$ may be distinguished from the equilibrium value n_{io} by changes in the intensity of erythropoiesis, on the one hand, and by the chance destruction of erythrocytes unconnected with the process of cell aging, on the other [6, 8]. If we postulate, that the rate at which natural aging proceeds does not change, then the value $n_i(t)$ may be derived from the following equation:

$$\frac{dn_i(t)}{dt} = \frac{n_{i-1}(t)}{T_{i-1}} - \left(\frac{1}{T_i} + \Omega i\right) n_i(t); \quad i = 2,3, \tag{2}$$

where Ω_i represents the "random" destruction of cells from i age group.

Knowing the average lifespan T of the erythrocytes and the equilibrium spectrum n_{io}, we may, according to Eq. (1), determine the value of T_i. Placing this in Eq. (2) and using the result of changes in the age spectrum at different time periods after irradiation $n_i(t)$, we may find Ω_i.

RESULTS

The results of the experiment are presented in Table 1.

Taking the average lifespan of rabbit erythrocytes T as 68 days [3-5, 7], we may, on the basis of Eq. (1) and the data in Table 1, determine that reticulocytes after 2.08± 0.07 days after entry into the peripheral blood become young erythrocytes which in their turn after 15.90± 0.45 days become mature. The mature erythrocytes exist on an average of 49.90± 0.60 days and are destroyed by the process of natural aging. The mean probability of destruction of a mature erythrocyte per unit time equals 0.020± 0.002 days. Per day, (0.84± 0.03) · 10^5 mature erythrocytes per

TABLE 2. Rate of Destruction of Young and Mature Erythrocytes at Different Periods after Irradiation

Dose of Irradiation (R)	Time interval (days)	Mean probability of destruction of cells per unit time (days)	
		10,4—8,2 μ	8,2 μ
—	Control	0,00	0,020±0,002
250	0—5	0,000±0,030	0,028±0,014
	5—10	0,004±0,016	0,033±0,009
	10—19	0,013±0,010	0,026±0,006
	19—33	0,014±0,006	0,043±0,004
	33—47	0,053±0,005	0,012±0,003
	47—68	0,017±0,005	0,019±0,002
—	Control	0,000	0,020±0,002
500	0—8	0,007±0,023	0,018±0,008
	8—10	0,065±0,029	0,106±0,027
	10—17	0,019±0,030	0,036±0,021
	17—38	0,001±0,010	0,015±0,006
	38—59	0,004±0,008	0,019±0,003

mm^3 of peripheral blood are destroyed and the same number of reticulocytes enter the blood from the erythropoietic organs.

From the first to the eighth day after irradiation with a dose of 500 R no statistically certain decline in the rate of destruction of young and mature erythrocytes from the normal values (zero and 0.020±0.002, respectively) is observed. Further, during this very short period, the process of "random" destruction of cells develops and then rather quickly fades (Table 2). The maximum intensity of random destruction of young erythrocytes reached 0.065±0.029 per day and that of mature erythrocytes 0.086±0.027 per day. The nature of this process indicates that the disintegration of erythrocytes is a short-term result reflecting the effect on the organism as a whole and which develops from the acute radiation injury, rather than a direct radiation injury to the peripherally circulating erythrocytes. The high radio resistance of erythrocytes irradiated in vitro confirms this hypothesis.

The release of reticulocytes in response to a diminished content of erythrocytes in the peripheral blood observed in non-irradiated rabbits [2] in this instance was significantly delayed and offers proof of suppression of erythropoietic function (see Table 1). In parallel with the restoration of erythropoietic organs and the increase in the rate of erythropoiesis the red cell count and the size distribution of erythrocytes in the blood will become normal. The increase in the rate of erythropoiesis at the end of the restoration period is accompanied, as must be expected, by an increase in the content of young cells and a corresponding decrease in the mean hemoglobin concentration per cell.

With irradiation at a dose of 250 R when the acute radiation sickness of irradiated animals does not develop, the process of radiation-destruction of erythrocytes is expressed very weakly and the increase in cell destruction to the 19th day does not appear statistically certain. However, even this weak destructive process slowly evokes and increase in the rate of erythropoiesis, which, gradually building up, reaches $2^1/_2$ times normal by the 33rd day after irradiation (see Table 1).

In order to evoke a similar stimulation of erythropoiesis in a non-irradiated rabbit, it was necessary to take roughly 40% of its blood [2], whereas in the present instance, we did not observe any decrease in the number of erythrocytes. Thus, a very large increase in the rate of erythropoiesis does not appear to be necessary in this case. Moreover, it may appear harmful, since it evokes a "useless" expenditure of the energy resources of the animal. Actually, the cells produced under such a high rate of erythropoiesis possess a diminished viability [2, 7], as a consequence of which by the 19th day after irradiation the mean probability of destruction per unit time both for young and for mature erythrocytes is sharply increased (see Table 2). An analogous situation is observed with massive phlebotomy [2, 7]; however, in this instance sharp stimulation of erythropoiesis is stimulated by tissue hypoxia and is of use.

Further studies are needed to elucidate the reason for the paradoxical reaction of the erythropoietic control system in response to irradiation.

SUMMARY

A study was made of the effect produced by the γ -irradiation with Co^{60} in doses of 250 and 500 R on the hemoglobin content, erythrocyte count and the spectrum of distribution of these cells according to size in the rabbit peripheral blood. On the basis of strict kinetic analysis of the data obtained an estimation was made of the intensity of discharge of erythroid cells into peripheral blood and their disintegration at various periods after the irradiation.

As demonstrated, irradiation caused disintegration of erythrocytes which were in the peripheral blood at the time of irradiation; the intensity of the process grew somewhat with the increase of the cell age. A description is presented of a paradoxic reaction of the erythropoietic control system in response to irradiation.

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