THE REMOTE AFTER - EFFECTS OF RADIATION ON HEMOPOIESIS

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Only a few publications in modern literature discuss the state of the hemopoietic system in animals at later periods after radiation sickness [1, 2, 4]. As far as we know, there can be no doubt that the normalization of the severe morphological changes in the blood picture, changes which develop during the period of manifest clinical symptoms of radiation sickness, is only of relative character [7-10]. This fact deserves particular attention in view of the available data, concerning the development of malignant conditions in the hemopoietic organs as after-effect of radiation. Hence it seems imperative to attract the attention of research workers to the late after-effects of exposure to ionizing radiation.

In the present paper we carried out systematic studies of the peripheral blood and the bone marrow during the acute period of the radiation sickness as well as after the clinical recovery of the animals.

EXPERIMENTAL METHOD

The experiments were carried out on 464 rats which were exposed to x-rays in a dose of 500 r under the following conditions: RUM-3 apparatus, Potential 180 kv; current strnegth 20 ma; filters 0.5 mm Cu and 0.5 mm Al; capacity of dose: 96.3 r/min. Under the experimental conditions used by us exposure of rats to the above doses of x-rays for 30 days caused in various series of experiments the death of 15 - 30% of the animals.

In the experimental rats we studied the white cell count and the differential count before the radiation as well as 3, 5, 7, 9, 12, 16, 21, 30, 90, 180, 270, and 360 days after the exposure to radiation. In addition, we established the weight of the bone marrow in one tibia before and 90, 180, 270, and 360 days after the radiation. At the same periods we estimated the hemoglobin level, and counted the number of red cells in the peripheral blood.

270 days after the exposure to radiation and simultaneously in the control rats of corresponding age we estimated the rate of uptake of labelled radioactive iron (Fe^{59}) by the red cells and also studied the osmotic resistance of

TABLE 1. The Blood Picture and the Bone Marrow in Rats at Various Periods after Irradiation

Index	Time of investigation					
	Before the irradiation		After the irradiation			
	Age of the rats (in months)		90 days	180 days	270 days	360 days
	6-8	18				
White cell count (in thousands)	12450	7878	12230	13600	13012	13000
Red cell count (in millions). . .	6.7	8.0	6.4	7.3	7.4	4.0
Hemoglobin level (in %).	78	82	71	76	79	46
Weight of bone marrow (in mg). .	15.3	17	14.7	22.8	34.7	34.6

the red cells by the microscopical method of Yanovskii [3]. In a special series of experiments we studied the tissue-iron level in the spleen of the animals by the method of Kaldor [6], 90 and 270 days after exposure to radiation.

In rats the non-hemin-iron in this organ mainly consists of hemosiderin iron and, to a lesser degree, of iron in the ferritin fraction. The ferritin fraction was obtained by heating an aqueous homogenate of spleen to 80° C and adjusting the pH of the supernatant to 4.5-5 in order to remove the bulk of ballast proteins. The iron was estimated spectrophotometrically on the basis of the color-reaction with o-fenantrolin.

EXPERIMENTAL RESULTS

The peripheral blood of rats exposed to x-rays in a dose of 500 r shows characteristic changes in the acute period of radiation sickness: marked leucopenia on the third day, by the 9th day followed by a gradual increase in the number of leucocytes and a reversion to the lower limits of the normal range by the 30th day. During this period of radiation sickness, however, a certain increase in the number of mature neutrophiles can be observed in the blood picture. At later stages after the irradiation (90 days) the white cell and the red cell count of the animals became normalized, both from a quantitative and from a qualitative point of view (Table 1).

The weight of the bone marrow and its qualitative composition, as judged by the myelogram, remained the same as in the control animals. This normalization of the peripheral blood picture and of the bone marrow, however,

TABLE 2. Changes in the Peripheral Blood Picture of Rats 360 Days After Exposure to Radiation

No. of rat	White cell count (in thousands)	Hemoglobin level (in %)	Red cell count (in millions)	neutrophiles				Eosinophiles	Monocytes	Basophiles	Lymphocytes	Erythroblasts
				Myelocytes	Juveniles	Staff cells	Segmented					
Rats exposed to radiation												
1	16450	52	5.3	–	1	5	33	3	3	–	51	4
2	16700	51	4.3	–	1	10	37	2	1	–	47	2
3	17000	62	5.9	–	–	5	40	6	5	–	43	1
4	11150	40	3.9	–	1	14	27	–	3	1	54	–
5	4900	30	2.4	1	3	22	32	–	1	–	35	6
6	7850	44	3.9	–	–	7	35	6	1	–	45	6
7	5600	48	3.5	–	–	6	25	1	2	–	52	14
8	8500	49	3.5	–	2	11	42	–	–	–	44	1
9	3600	32	2.9	–	–	16	56	–	1	–	20	7
Control rats of similar age												
1	8950	88	8.4	–	–	–	12	2	–	–	86	–
2	3500	81	8.5	–	–	5	10	3	2	–	80	–
3	6100	81	9.1	–	–	–	14	6	6	–	74	–
4	5400	89	8.3	–	–	–	14	8	5	–	73	–
5	6400	80	7.4	–	–	–	62	–	–	–	38	–
6	10900	71	6.4	–	–	4	25	4	5	–	62	–

proved to be of transient character. As early as 180 days after the irradiation the percentual proportion of staff cells among the neutrophiles increased, and after 9 months juvenile forms appeared, a fact which could never be observed in the control animals. These shifts in the peripheral blood picture were even more marked 360 days after the irradiation (Table 2).

Study of the hemoglobin level and of the red cell count revealed that in the overwhelming majority of animals anemia developed at this late stage after radiation, although earlier, 9 months after the radiation, only 5 out of 20 animals investigated had proved to be anemic. Erythroblasts appeared in the animals' peripheral blood.

Study of the osmotic resistance of the red cells 12 months after the radiation showed that they possessed an increased resistance to hypotonic sodium chloride solutions. This shows that at the time in question the peripheral blood was rich in young cell forms, which forms have a higher osmotic resistance. Similar results were obtained in studies of the uptake of Fe^{59} by the red cells of the rats. It was found that 9 months after exposure to radiation a statistically significant increase ($p = 0.02$) in the uptake of iron by the red cells of the irradiated rats can be observed (102 counts/min) compared to the control rats (61 counts/min).

It is important that the changes in the red cell count and white cell count described above took place against a background of a hyperplastic bone marrow and a marked increase in the size and weight of the spleen (see Table 1 and 3).

Study of the qualitative bone marrow picture at that time revealed changes in the relative proportion of the erythroid and the myeloid series, (as shown by the myelogram) changes consisting in a relative decrease in the number of erythroid elements and a corresponding relative increase in the number of myeloid elements. Due to the increase in the number of nucleated cells per mg and the considerable increase in the weight of the bone marrow, however, the absolute number of erythroid elements also showed at that period after the irradiation an increase compared to the findings in the control animals (84 600 compared to 45 045).

Study of the content of non-hemoglobin iron in the tissues of the spleen revealed a marked increase in the iron content in the animals exposed to radiation compared to the control rats of similar age (see Table 3).

TABLE 3. Tissue Iron Levels in the Spleen of Rats

Time elapsed between irradiation and investigation	No. of animals	Weight of spleen (in mg)	Content of non-hemoglobin iron (in μg)	Iron content in the ferritin fraction (in μg)
90 days.	16	1382±81	292±46	96±10
270 days.	10	1088±49	628±73	105±11
Control rats (not exposed to radiation)	20	863±48	265±32	49±6

In special experiments 10 rats were given intravenous injections of a suspension of red cells labelled with Fe^{59} in a dose of 3 ml 11 months after exposure to x-rays in a dose of 400 r; 10 control rats of similar age received the same quantity of labelled red cells.

Under the conditions of the count carried out by us in a scintillation counter this corresponds to 40000 counts/min. 2 weeks after the injection of the labelled red cells an increase in the radioactivity of the spleen was found in the irradiated animals in addition to the higher content of iron in the spleen. The radioactivity of the tissue iron in the spleen of the irradiated rats constituted 3% of the total activity injected (1236 counts/min) whereas the spleen of the control rats yielded only 0.9% (359 counts/min). The results obtained proved to be statistically significant ($P = 0.05$) and show, in our opinion, that in animals exposed to radiation an increased destruction of red cells takes place at late stages after the exposure.

It seems that the accumulation of excess iron in the hemosiderin fraction of the spleen at late stages after exposure to radiation represents above all the result of an increase in its hemolytic function, and this fact may on the other hand lead to the hyperplasia of the bone marrow and the appearance of immature cell forms in the peripheral blood. Besides, the continuous influence of excess quantities of the products of blood cell destruction may also exert a stimulating effect upon the hemopoietic tissues.

It can be assumed that the changes in the hemopoietic system observed by us at late periods after exposure to radiation are a consequence of the impaired correlation between the spleen and the bone marrow.

SUMMARY

The authors studied the state of the blood system in animals at remote periods after radiation sickness. As shown, normalization of the morphological picture of the peripheral blood and the bone marrow in 2 - 3 months after irradiation was but temporary, since the appearance of young forms was noted in 9 - 12 months against the background of marked bone marrow and spleen hyperplasia. It is suggested that the changes observed are the sequelae of a disturbed correlation in the interaction of the spleen and bone marrow.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
