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FROM THE DEPARTMENT OF RADIATION BIOLOGY, CENTRE D'ÉTUDE DE L'ÉNERGIE NUCLÉAIRE, B-2400 MOL, BELGIUM.

IRON INCORPORATION AFTER SINGLE AND FRACTIONATED IRRADIATION OF INFANT MICE

G. B. GERBER and J. MAES

Fractionation of a radiation dose in general permits partial repair of radiation injury and thus causes less injury than the same dose delivered in a single application. However, certain exceptions to this rule exist. Thus, mice exposed on day 6 and day 9 of life display a higher mortality than when they are given the same dose either on day 6 or on day 9 (BAUMANN & MUTH 1977). The mechanism causing this sensitization is not yet known. It may be related to the adaptation of hemopoiesis to adult life taking place during the neonatal period (METCALF & MOORE 1971), a suggestion which is supported by the fact that the sensitization ceases as the mice reach adulthood. Since such an apparent lack of repair of radiation injury in the infant organism not only is of interest on theoretic grounds but also may have implications for radiation therapy and radiation protection it was considered of interest to elucidate this mechanism in more detail. The present report deals with the normal development of the erythropoiesis during the infancy of mice and the changes caused by a single and a fractionated radiation dose. Incorporation of iron into hemopoietic tissues was utilized to assess the capacity of the erythropoietic system (BELCHER et coll. 1954, BERAN & TRIBUKAIT 1971, cf. also review in GERBER & ALTMAN 1970). Later, GERBER & MAES (1980) reported on the behavior of the stem cells and their sensitivity to radiation as well as the significance of such a sensitization for late effects.

Methods

Infant mice of the C_{57} Bl strain were whole-body irradiated at 250 kV, 1 mm Cu filter, 0.84 Gy/min. The following groups were used (1) Controls, (2) exposed to 1.26 Gy on day 6, (3) exposed to 4.2 Gy on day 6, (4) exposed to 4.2 Gy on day 9, (5) exposed to 1.26 Gy on day 6 followed by 2.94 Gy on day 9. At an age of 7, 8, 10, 14, 17, 20, 25 or 30 days, the mice were injected intraperitoneally with 37 MBq of ^{59}Fe citrate and killed 4 hours later. The activity of serum and erythrocytes was determined by gamma spectrometry after separation by centrifugation in calibrated hematocrit tubes, and the data were related to 0.14 ml of blood. In addition, the dependence on time after injection of serum and erythrocyte activities was followed for selected doses and time intervals after exposure. Activities of both femurs, liver and spleen and bone marrow were also assayed. Practically all activity is bound to the protein fraction at this time. All experimental groups were followed in at least two independent experiments; 4 to 6 animals were used for each time point.

Results

Preliminary experiments demonstrated that the strain used showed about the same sensitization as that of BAUMANN & MUTH. All animals survived a

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dose of 1.26 Gy, about 5 to 10 per cent died before an age of 30 days after a single dose of 4.2 Gy given on day 6 or 9; 50 per cent of the mice given the fractionated irradiation (1.26 Gy on day 6, 2.94 Gy on day 9) had died at an age of 20 days, and 80 per cent had died before an age of 30 days.

The growth of infant mice is retarded by irradiation in a dose dependent manner: Whereas controls had attained a weight of 15 g at an age of 30 days, mice exposed to 1.26 Gy weighed 10 g and those exposed to 4.2 Gy 7.5 g on day 30, no difference being discernible between animals irradiated with a single or a fractionated dose. Similar results were observed with respect to liver or spleen weight. Hematocrit values displayed a dose dependent reduction wich was more marked and longer lasting after a fractionated exposure to 4.2 Gy than after a single one (Fig. 1). ^{59}Fe in serum, i.e. that not incorporated into heme, rapidly diminished with time to low levels in the controls (not shown, half life time less than 30 min). Four hours after injection, only traces of active iron were found in the serum of control mice, whereas much higher activities remained in that of irradiated mice, maximum values being observed 1 to 3 days after exposure (Fig. 2). One week after exposure, serum activity had again returned to normal, but in mice given a fractionated irradiation the serum iron rose again from an age of 20 days until day 30.

In explaining the data on iron incorporation into heme it should be recalled that after 4 hours, many cells with newly synthetized heme have already left the hemopoietic tissues and have entered the blood. The time at which labelled erythrocytes appear in blood is shorter in young than in older mice and also diminishes during regeneration after irradiation. Radioactivity incorporated into heme of bone marrow (Fig. 3) increased in controls between day 15 and day 20. Irradiation with 1.26 Gy on day 6 caused a temporary increase in activity 1 to 3 days later, whereas irradiation with 4.2 Gy on day 6 reduced incorporation in bone marrow during the following week and prevented the age-related rise in non-irradiated mice. This is also observed following exposure to 4.2 Gy on day 9, except that the early decrease is absent. Fractionated exposure caused a marked temporary increase in activity at an age of 15 days and prevented the normal increase during the third week of life.

Iron incorporation into heme of control spleens (Fig. 4) increased slowly up to the third week of life and then declined. This rise and decline seem to be slightly delayed after 1.26 Gy. However, irradiation with higher doses caused an immediate fall in iron incorporation; later the values approached normal.

Iron incorporation into hepatic heme (Fig. 5) appeared to be nearly independent of the age of the animals. After irradiation, this incorporation was increased to a degree which appears about inversely proportional to the fall in incorporation of iron into hemopoietic tissues.

The percentage of active iron appearing in erythrocytes in 0.14 ml of blood decreased with age (Fig. 6). During the initial 4 days after irradiation, ^{59}Fe activity in erythrocytes diminished slightly after 1.26 Gy and markedly after 4.2 Gy; the fall after a fractionated exposure was less marked than after a single one. During the second week after exposure to 4.2 Gy, erythrocyte activity was markedly enhanced above control levels and then returned to normal. The changes after a fractionated exposure, although less marked, were long lasting.

Discussion

The early period after birth is characterized by profound modifications in hemopoietic functions (METCALF & MOORE). Already before birth hemopoiesis in the liver has been replaced by that in the spleen and from the second week of life, i.e. at a time when adult hematocrit levels are attained, hemopoiesis in bone marrow takes more and more precedence to that in the spleen. This is also demonstrated by the data presented. Kinetic investigations on iron incorporation into erythrocytes (not shown in the data) indicate that the time interval until labelled erythrocytes appear after ^{59}Fe injection, i.e. the maturation of heme synthetizing cells, also increases with age during the neonatal period.

In explaining the data after irradiation it should be kept in mind that only mice surviving at a given time are examined. When severely injured mice die and are thus removed from the experimental group, the mean of the surviving animals may suggest an improvement of a given parameter. Moreover, the relations between iron activity of erythrocytes and those in the hemopoietic organs must also be taken into account. The data demonstrate that irradiation during infancy produces a bone marrow syndrome characterized by profound alterations particularly in erythropoiesis. The maturation of the erythropoietic functions of bone marrow appear especially affect-

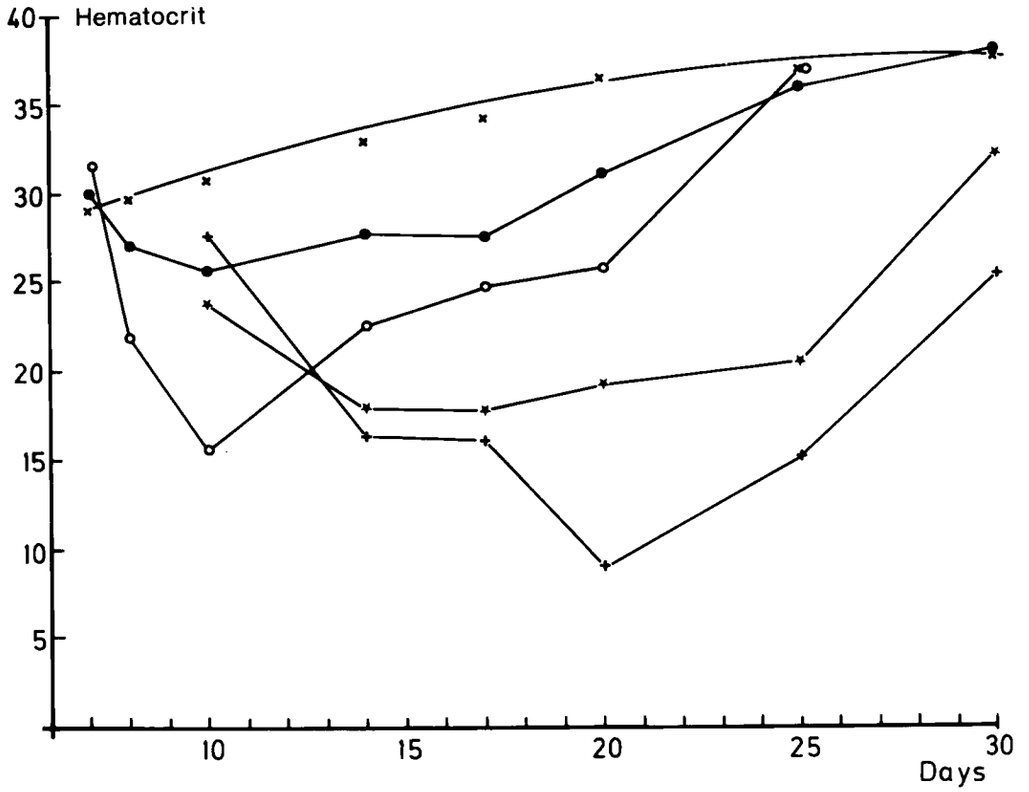

Fig. 1. Hematocrit (in per cent) of control and irradiated infant mice from the age of 7 to 30 days. (× controls, ● 1.26 Gy on day 6, ○ 4.2 Gy on day 6, ★ 4.2 Gy on day 9, † 1.26 Gy on day 6 followed by 2.94 Gy on day 9).

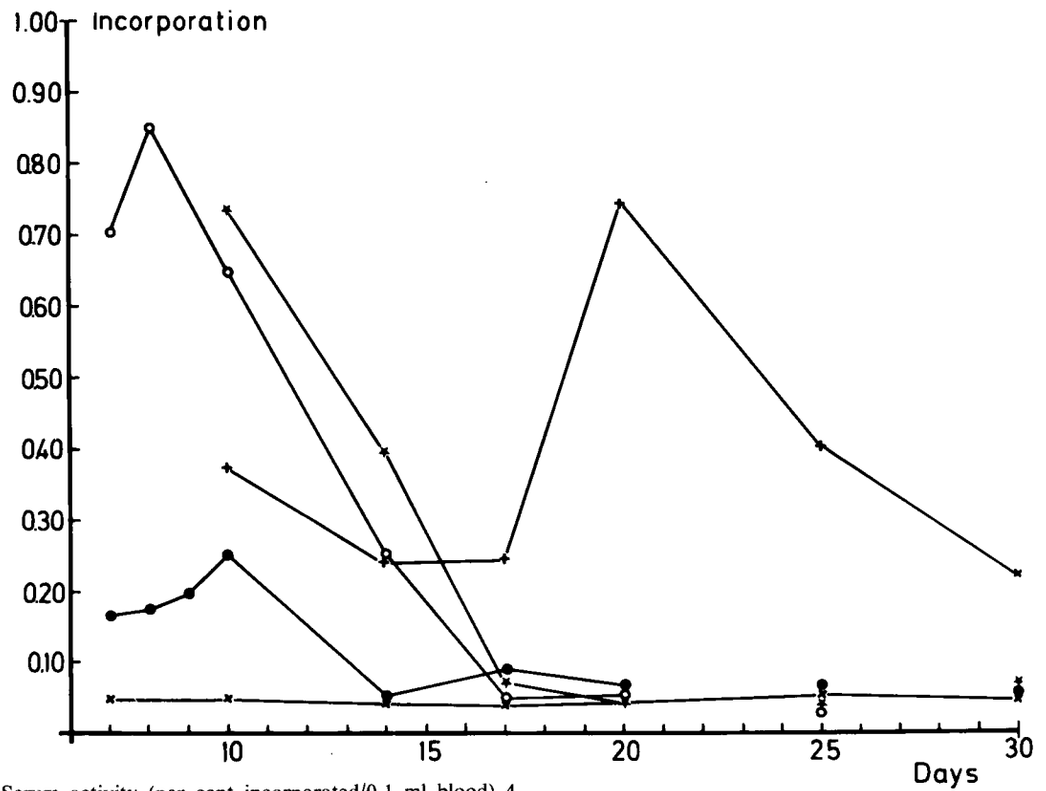

Fig. 2. Serum activity (per cent incorporated/0.1 ml blood) 4 hours after injection of ^{59}Fe into control and irradiated infant mice. Symbols as in Fig. 1.

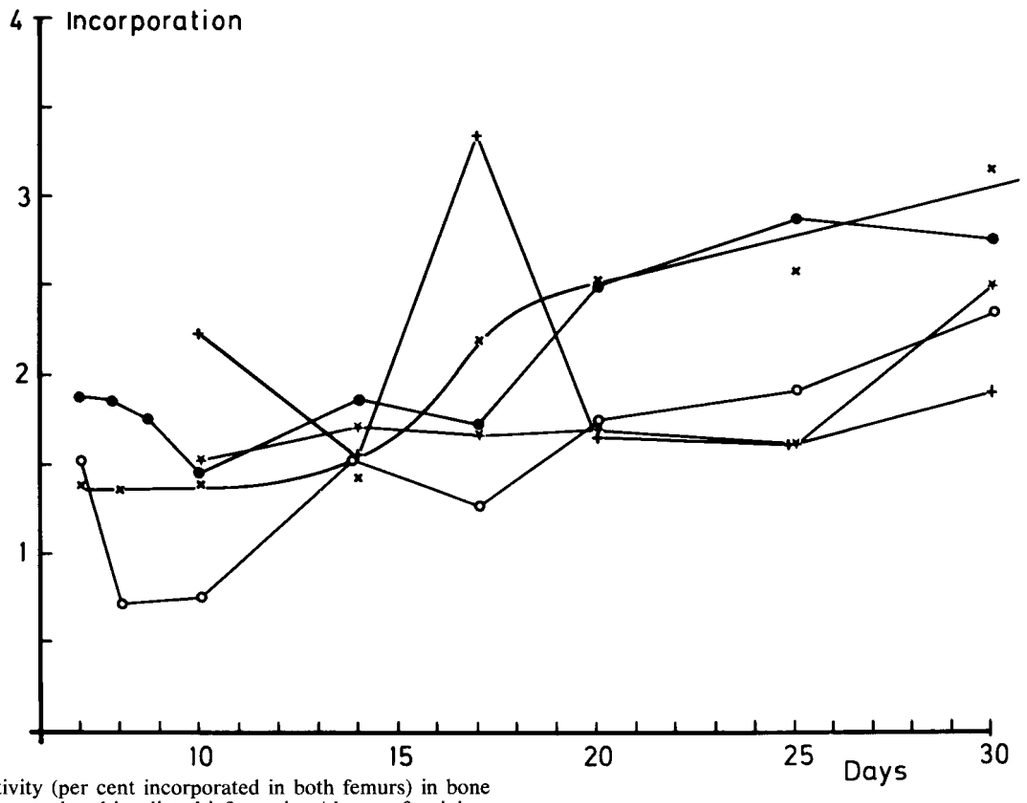

Fig. 3. Activity (per cent incorporated in both femurs) in bone marrow of control and irradiated infant mice 4 hours after injection of ^{59}Fe. Symbols as in Fig. 1.

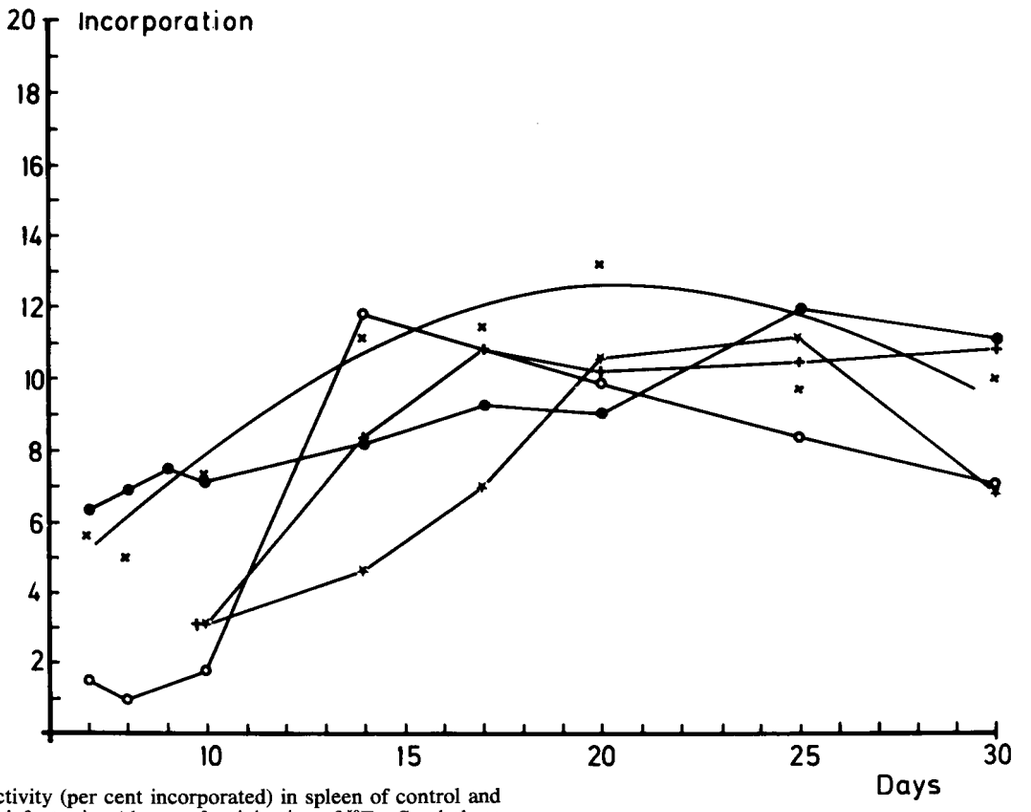

Fig. 4. Activity (per cent incorporated) in spleen of control and irradiated infant mice 4 hours after injection of ^{59}Fe. Symbols as in Fig. 1.

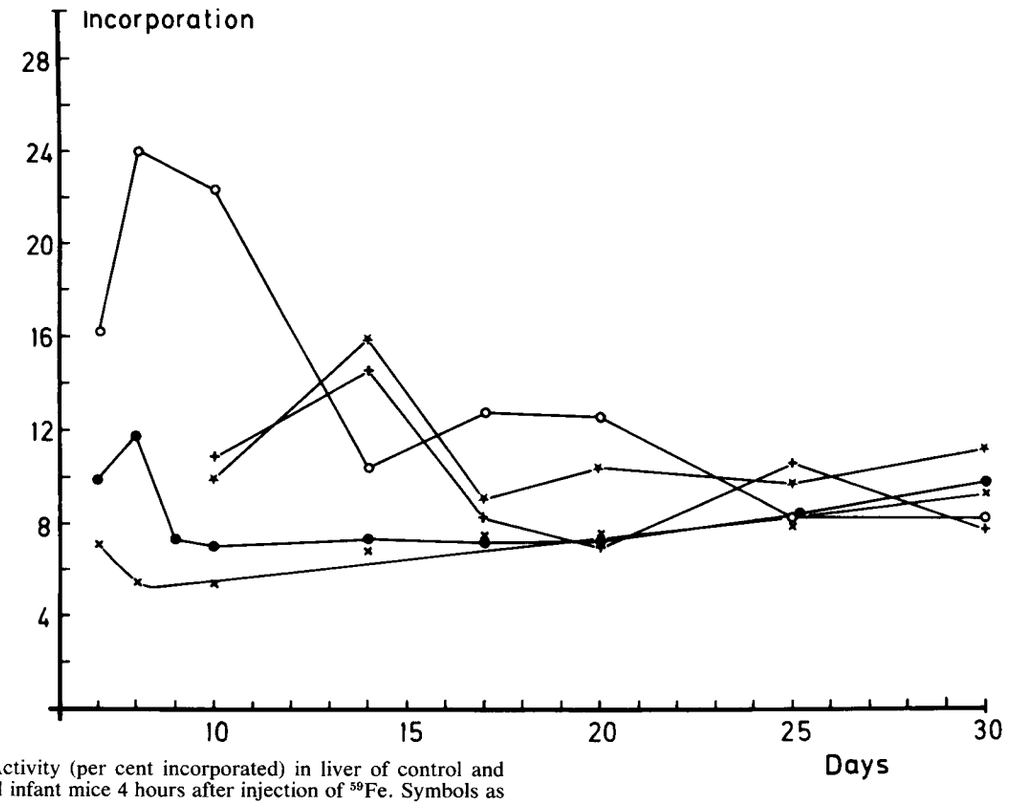

Fig. 5. Activity (per cent incorporated) in liver of control and irradiated infant mice 4 hours after injection of ^{59}Fe. Symbols as in Fig. 1.

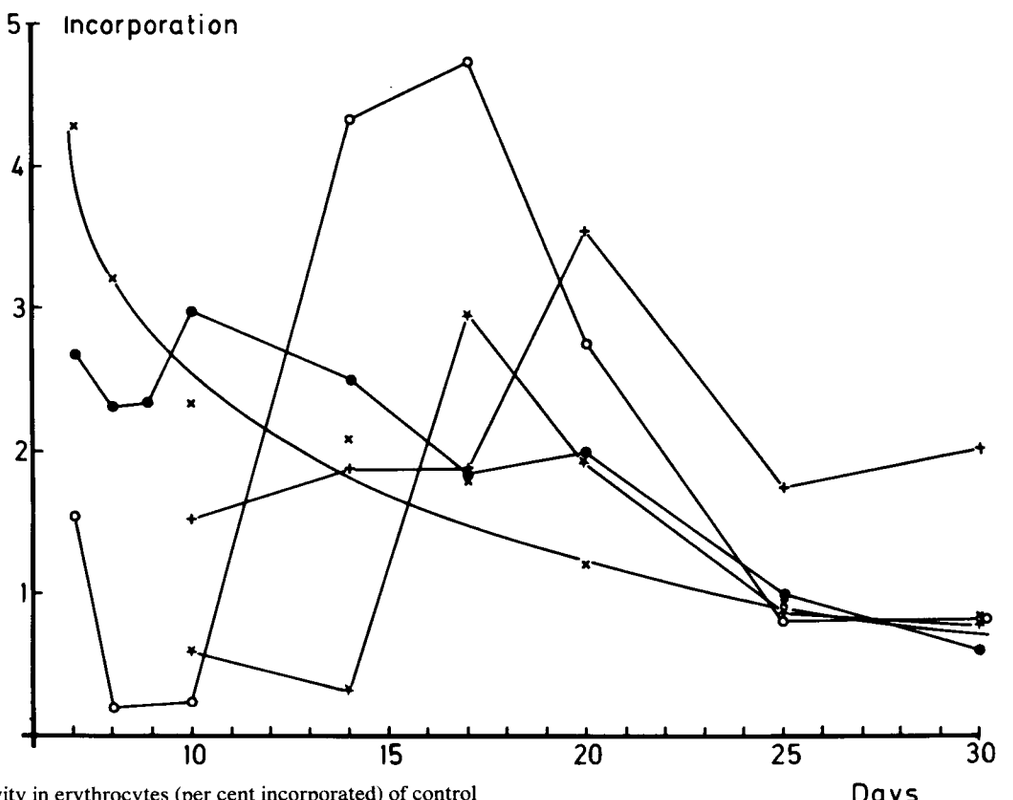

Fig. 6. Activity in erythrocytes (per cent incorporated) of control and irradiated infant mice 4 hours after injection of ^{59}Fe. Symbols as in Fig. 1.

ed, changes in the spleen are less marked. These changes are, in general, somewhat more marked and last longer after a fractionated than after a single exposure to the same dose. An increase in iron incorporation occurring a few days after a dose of 1.26 Gy may be caused by a compensatory mechanism.

The lower turnover of serum iron during the first days after exposure reflect the lack of heme synthetizing cells in hemopoietic organs and allow a greater deposition of iron in the liver.

In conclusion, the observations confirm the higher effectiveness of a fractionated irradiation during infancy compared with a single one and indicate that these animals die from hemopoietic failure mainly due to an impaired maturation of the hemopoietic capacities of the bone marrow. It remains the task of future investigations to better define the cellular basis of these alterations.

SUMMARY

The erythropoiesis in control and irradiated infant mice was investigated on the basis of incorporation of iron into hemopoietic organs and blood. Iron incorporation in bone marrow increased from the second week of life reflecting the maturation of the marrow whereas that in the spleen showed only minor changes. Irradiation of infant mice on day 6 or 9 caused a bone marrow syndrome characterized by an impaired iron incorporation into hemopoietic tissues and blood, by a reduced utilization of serum iron, and particularly by a delayed maturation of the erythropoietic functions of bone marrow. Fractionated irradiation not only had a greater effect on mortality but also caused a more severe and long lasting depression of erythropoiesis than a single dose.

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