Effect of Whole Body X-Irradiation on Plasma Iron Turnover in Rats.* (24667)

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That irradiation depresses mitotic activities is well known. Bone marrow cells are particularly sensitive and many investigations, as reviewed by Jacobson(1), have produced evidence of depression of erythropoiesis following irradiation. Most studies have been made by examination of the erythroid elements of the bone marrow, disappearance of reticulocytes from the circulating blood, or reduction in uptake of injected radioiron by newly formed red cells(1,2). Since Bothwell and co-workers(3) have shown that plasma radioiron turnover is depressed following radiation, and others(4-8) have shown the validity of plasma iron turnover rate as an index of erythropoiesis, we have used this method to quantify the sensitivity of the bone marrow to irradiation and to study the depression and recovery of its activity.

Materials and methods. We have used male CFW rats weighing 325-475 g and Holtzman rats weighing between 250 and 375 g. They were given Wayne lab-blox and tap water *ad libitum*. In preliminary experiments food was withheld 24 hours before determining plasma iron turnover; however, it was found that failure to do so did not affect the results. Thus, the remainder of the experiments were done without withholding food. *Radioiron disappearance* measurements were made by injection of .1 ml of iron citrate solution containing a maximum of 1.5 μg of chemical iron (1-2 microcuries Fe^{59}) into the femoral vein of a nembutalized rat. Four serial blood samples of .3 ml were taken from the opposite femoral vein at 15- to 30-minute intervals after injection. These samples were centrifuged and 100 lambda of plasma was pipetted for determination of radioactivity. Radioactivity measurements were made in a well-type scintillation counter using a sodium iodide thalium-activated crystal. The radioactivity was plotted on semi-logarithmic paper against time and the T ½ value was computed from the disappearance curve. *Radiation* was administered with a Westinghouse duo-condex X-ray machine at 200 KVP, 12.5 ma, ½ mm Cu, and 1 mm Al added filtration. Calibration was done with a 250 r thimble of the Victoreen instrument at 40 cm TSD in air with a dose rate of 58 to 62 r per minute. The animals were restrained during irradiation in individual perforated lucite boxes on a turntable run at 3 rpm. *Plasma iron* determinations were made by the method of Schade *et al.*(9) on samples of plasma drawn after the last sample was taken for determination of radioiron disappearance. All radioiron disappearance and plasma iron measurements were run between 1:00 p.m. and 3:00 p.m., to avoid effects of diurnal variations(8).

Results. I. CFW rats were exposed to irradiation doses of 50, 150, and 300 r, and T ½ values for Fe^{59} disappearance were determined 1, 7, 13, and 21 days following irradiation. The results are shown in Fig. 1. Each observation represents an average of 4-6 animals. Control T ½ values were determined on 6 rats and ranged from 69-93 min. The extended T ½ values indicate that erythropoiesis is depressed at 24 hours after all doses of irradiation. Amount of depression is directly related to dose. At 7 days T ½ values have returned to the control level. This rapid and apparently complete recovery continues through 14 and 21 days.

II. A preliminary experiment using CFW rats was carried out following 450 r. T ½'s were determined at 6, 12, 18, 48, 66, and 96 hours post-irradiation. The results showed that bone marrow depression, as reflected in T ½, was rapid and sufficiently uniform for the period of maximum depression to be de-

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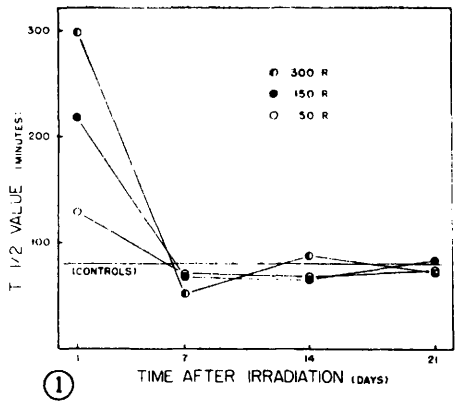

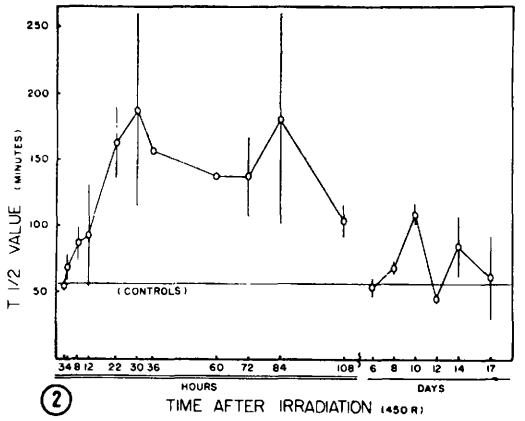

FIG. 1. T ½ value for disappearance of Fe^{59} from plasma of CFW rats at different times following various doses of X-ray. 4-6 rats/datum.

FIG. 2. T ½ value (± S.D.) for disappearance of Fe^{59} from plasma of Holtzman rats at various times following 450 r X-irradiation. 2-5 rats/datum except at 36 and 60 hr, which points represent single animals.

termined. Thus, in a later experiment Holtzman rats, instead of CFW, were exposed to 450 r and examined at frequent intervals from 3 hours to 17 days post-irradiation. Evidence of bone marrow depression appeared within 4-8 hours after irradiation and proceeded rapidly to 24 hours (Fig. 2). Maximal depression occurred between 24 and 48 hours and the largest deviation around mean values was observed during this period. Rapid recovery proceeded from the 3rd and 4th day to the 5th or 6th day. Variations in T ½ were present from the 6th to 17th days.

Discussion. These experiments have confirmed the findings of many others that bone marrow activity is depressed following irradiation(1). We have used a method (disappearance of radioactive iron from the plasma) that is especially sensitive and suitable for carrying out time course observations since disappearance rate reflects bone marrow activity during the one-hour period of sampling. We have shown that a marked diminution in turnover rate of plasma iron is seen 24 hours after irradiation. Furthermore, depression of iron turnover rate is evident by 6 hours post-irradiation. While it has long been known that leukocytes show an early post-irradiation reduction(1), it has been less obvious that bone marrow erythrogenic cells responded so quickly. Due to the long life span of red cells, lack of production of cells for many days is required to cause a conspicuous reduction in red cell count.

Of equal interest is the fact that red cell production rate, as indicated by plasma iron turnover, appears to have returned to normal by the sixth day. A period of anemia is commonly observed, which is prominent during the second post-irradiation week. That hemorrhagic diathesis and plasma volume changes have contributed to this anemia has been shown by Kahn and Furth(10). Our data distinctly show early depression and early functional recovery of bone marrow and thus confirm the conclusion that the anemia may be largely relative to factors other than erythropoiesis.

In other laboratories the importance of determining plasma iron level has been emphasized(3,4,8). We agree with the necessity for realizing that plasma iron turnover is a function both of turnover rate and the size of plasma iron mass. Accordingly, we determined plasma iron chemically and calculated plasma volumes of animals by extrapolation of the disappearance curves(7). Although Chanutin and Ludwig(11) reported hyperferremia in rats following 500 r, the plasma iron mass of the animals in our experiments varied so little that T ½ values remained the substantial reflection of iron usage.

We believe that measurement of the T ½ value in the period 24 to 48 hours post-irradiation is a sensitive and reasonably quantitative indication of bone marrow depression following irradiation. Our experiments sug-

gest that this approach would produce data more reliable than red cell uptake of radioiron, especially during weak or short-lived effects. Hodgson *et al.*(12) have reached the same conclusion following use of this method to study bone marrow stimulation. These experiments are being extended to determine the limits of accuracy and sensitivity of this method.

Summary. We studied the depression of plasma radioiron turnover rates in rats following X-irradiation. The depression is prompt (maximal by 24-30 hours) and, at 24 hours post-irradiation, is directly related to dose between 50 r and 300 r. Recovery occurs by the 6th post irradiation day, indicating that subsequent anemia is more likely due to hemorrhagic diathesis than bone marrow dysfunction. Sensitivity of the radioiron turnover method permitted a study of bone marrow response at frequent short intervals after irradiation.

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Potentiation of Toxicity of Malathion by Triorthotolyl Phosphate.* (24668)

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The demonstration by Frawley and associates(1,2) that potentiation of toxicity results from simultaneous administration of ethyl p - nitrophenyl thionobenzenephosphonate (EPN) and S-(1,2-dicarbethoxyethyl) - O,O-dimethyl phosphorodithioate (malathion) to rats and dogs stimulated other investigations (3,4) to ascertain whether various pairs of insecticidal organic phosphates cause a similar effect. In view of widespread use of these insecticides on food crops, the testing of each organic phosphate in combination with each of the others currently used, is now an established procedure essential from standpoint of protection of public health. Potentiation of toxicity which occurs with EPN and malathion has recently been explained by Cook *et al.*(5) and Murphy and DuBois(6) on the basis of ability of EPN to inhibit detoxification of malathion. Detoxification of malathion consists of hydrolysis of 1,2-dicarbethoxyethyl side chains(5,7) and this reaction is catalyzed by non-specific esterase present in liver and some other tissues(6). Under these circumstances it seemed likely that any chemical agent capable of inhibiting non-specific tissue esterases could potentiate toxicity of malathion. Our attention was attracted to finding of Myers and Mendel(8) that triorthotolyl phosphate (TOTP) inhibits aliesterase activity of livers of rats. This compound has the chemical structure shown on page 484. The present investigation was conducted to ascertain whether this agent, used industrially

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