

The Effect of Radiation on the Diisopropylphosphofluoridate- ^{32}P Red Blood Cell Survival Curve in the Mouse* (33350)

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Considerable effort has been devoted to the study of the effects of ionizing radiation on mammalian hemopoiesis. Very little work, however, has dealt with the effects of radiation on the intravascular survival of red blood cells (RBC). This communication reports the results of experiments designed to measure the influence of X-rays on RBC survival, as measured by the diisopropylphosphofluoridate- ^{32}P (DF ^{32}P) method.

Methods. Mature (120 ± 7 day old, 25–30 gm) white Swiss Webster female mice were used. Before starting the experiment, the animals were randomized into five groups of 50 animals each. One group served as control. Three groups received single total-body doses of 200, 400, and 800 rad. The fifth group received 900 rad given as three 300-rad fractions separated by 2 weeks each.

A Westinghouse 250 kVp X-ray therapy unit was used for the irradiations. The beam had an HVL of 2.0 mm Cu and the doses were delivered at 46 rad/min. All irradiations were made under conditions of full backscatter.

To tag the RBC, each animal received 5 μCi of DF ^{32}P (Volk, 200 $\mu\text{Ci}/\text{ml}$) diluted to a 0.1-ml volume with sterile propylene glycol, immediately before irradiation. The mice of the fractionated dose group received the DF ^{32}P immediately before the last 300-rad fraction. At predetermined intervals after irradiation (see "Results") animals were sacrificed by cervical dislocation and blood collected via aortic puncture. Samples of blood were collected in microhematocrit tubes. After centrifugation the hematocrit was recorded and the tube scored and broken at a level just below the "buffy" coat. The lower portion of the tube (containing the RBC) was weighed, the cells expressed from tube with a

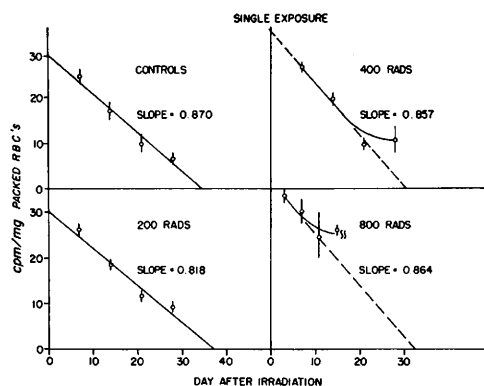


FIG. 1. The DF ^{32}P RBC survival curves for mice irradiated with single doses. The linear curves were fitted by the methods of least squares; the slopes are indicated. In the case of the 400- and 800-rad animals, the values given are the slopes of the linear portions (— — —) of the survival curve; the uncertainties are SE.

syringe onto a planchet, and the tube reweighed. After drying the planchets were counted with a gas flow counter. The data are given in units of cpm/mg RBC. All counts were corrected for radioactive decay.

Results and Discussion. Figure 1 shows the effect of the single doses on the DF ^{32}P curves. The ordinate gives cpm/mg of RBC while the abscissa shows days after irradiation. The points are the means of groups of 4–5 animals; the uncertainties are standard errors (SE). The controls and mice irradiated with 200 and 400 rad were sacrificed on days 7, 14, 21, and 28 after irradiation. Since the mean survival time for mice irradiated with 800 rad is less than 20 days, the animals of this group were sacrificed on days 3, 7, 11, and 14. Linear curves were fitted (with a digital computer) by least squares to the control and 200-rad data. The survival time was 34 days for the controls and 37 days for the 200-rad group. These values agree with results published by Van Putten (1). Where

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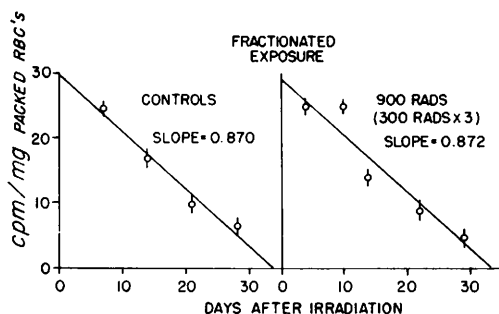


FIG. 2. $DF^{32}P$ RBC survival curves for controls and mice irradiated with 900 rads given as three 300-rad fractions (see text for details); the uncertainties are standard errors.

the control and 200-rad data are well fitted by linear curves, the 400- and 800-rad data are decidedly nonlinear. These curves seem to have two distinct portions. The first portions when extrapolated to the abscissa indicate survival times of 30–32 days. The last points (28 days for the 400-rad group and 15 days for the 800-rad group) fall considerably to the right of the extrapolated curve.

Figure 2 shows the results for the 900-rad fractionated dose group. The control response is included, for comparison. Linear curves were fitted to the data by the method of least squares; the slopes are similar. In both instances the RBC survival times were 34 days. The nonlinearity seen after day 11 in the 800-rad group did not appear in the 900-rad fractionated dose group.

Figure 3 shows the effect of irradiation on the hematocrit measurements. The hatched portion shows the range (mean \pm 2 SE) for the control hematocrits. The means of 800-rad single exposure animals are shown by open circles; the means of the 900-fractionated dose group are given by closed circles. Between days 4 and 15 after irradiation, the hematocrits of the 800-rad animals fell precipitously. The hematocrits of the 900-rad fractionated group, however, remained essentially within the normal range, except for a slight depression during the first 10 postirradiation days. The 200- and 400-rad hematocrits did not vary from normal.

Our results indicate that radiation produces no evidence of accelerated destruction of RBC. If anything, the results would sug-

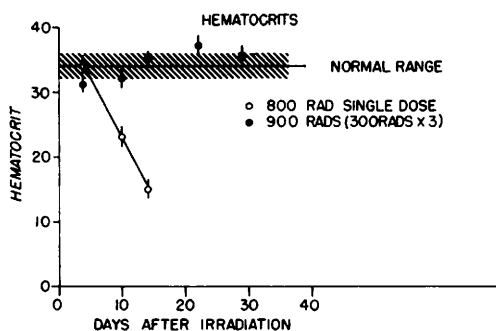


FIG. 3. Hematocrit values after irradiation. The hatched area represents the normal range (mean \pm 2 S); the uncertainties are SE.

gest a lengthening of the RBC survival. We believe this latter possibility to be a consequence of the experimental method, rather than a biologic fact, however.

In the normal nonirradiated animal, destruction of old RBC must be balanced by formation of new ones. If the RBC have been tagged with $DF^{32}P$, the loss of RBC appears as a decrement of the count rate, because of cell destruction *plus* a dilution of the label from new (but nontagged) RBC. Since the process of RBC destruction is age dependent (the oldest cells are destroyed first) (2,3), the $DF^{32}P$ survival curve will be linear. In other words, a certain number of RBC, and consequently a certain amount of label, will be lost per day. To maintain the linear RBC survival curve, the lost cells (tagged) must be completely replaced by new cells (nontagged).

Shortly after irradiation with large doses (such as 800 rad) the bone marrow stops making new RBC. This is seen by the falling hematocrits. Consequently, if normal RBC destruction occurred, but the formation of new RBC stopped we would anticipate a less marked depression of the measured activity (cpm/mg of RBC). The new nontagged RBC would not be present to "dilute" the remaining tagged RBC. We believe this to be the case for the 800-rad group and to a lesser degree for the 400-rad group. The animals which received the 900 rad as 300-rad fractions did not show the nonlinearity. We believe this is a consequence of recovery of the bone marrow between the fractions. Similar findings, based upon mouse mortality,

have been previously reported by one of us (4).

Summary. Our findings support the principle that radiation produces its major effect on hemopoiesis and not on the circulating RBC. The changes found in the DF³²P curves can be explained on the basis of bone marrow depression after irradiation.

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Isolation and Characterization of *Mycoplasma arginini*: spec. nov.* (33351)

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Ten strains of *Mycoplasma* were isolated from brain tissues of a scrapie infected sheep and mouse, joint exudate of an arthritic goat, and cell cultures established from human, chimpanzee, and dog tissues. These strains were related to each other, but were unrelated to known species of *Mycoplasma*. The present report characterizes the new strains, proposes that they be classified as *Mycoplasma arginini* and discusses their ecological significance.

Materials and Methods. *Media for isolation.* Hayflick (1), BYE (2), and BBL (3) broth and agar media were used under aerobic and anaerobic (5% CO₂ in nitrogen) conditions (2). See Table I for media used for isolation of individual strains.

Media for metabolic reactions. **Arginine utilization reaction:** Hayflick and BYE broth media were supplemented with 10 mM arginine, 10 mM glutamine, vitamins (4), 0.002% phenol red, and adjusted to pH 7.1.

Glucose fermentation reaction: Hayflick broth medium was supplemented with 0.5% glucose, 10 mM glutamine, vitamins, 0.004% cresol red, and adjusted to pH 7.5.

Urea metabolic reaction: Hayflick broth medium was supplemented with 0.5% urea,

0.002% phenol red, and adjusted to pH 6.0. Utilization of arginine and urea was indicated by an alkaline shift in pH while fermentation was indicated by an acid shift in pH.

Isolation of strains. Source and origin of specimens from which 10 strains were isolated are given in Table I. Two strains were isolated from brain tissues of two animals infected with scrapie, 1 from a sheep (C506) with naturally acquired scrapie and the other from a mouse (G230) with scrapie experimentally induced by the intracerebral injection of a brain suspension from another sheep with naturally acquired scrapie. The latter suspension was not available for direct culturing for *Mycoplasma*. These 2 *Mycoplasma* strains were isolated by direct culture in both BYE and Hayflick broth and agar media aerobically and anaerobically.

One strain (BBLG119) was isolated during a localized outbreak of suppurative arthritis affecting 15 goats in a herd of 60. Specimens were obtained from two affected goats and cultured for *Mycoplasma*. In one goat, the swollen joint was scrubbed clean, washed with thimerosal, and 4 ml of hemorrhagic exudate were aspirated. *Mycoplasma* strain BBLG119 was isolated in high titer from this exudate which contained 10⁴ colony forming units (cfu)/ml.

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