Studies on the Radiosensitivity of Bone Marrow

II. The Effect of Large, Repeated Whole Body Irradiation Exposure on Hematopoiesis

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During World War II a relatively large amount of research on the hematologic effects of repeated small doses of irradiation in animals was performed.1-3 The results have been reviewed by Jacobson et al.4 and by Lawrence and co-workers.5 For the most part, dosage schedules ranged from 0.1 to 10 r per day although Prosser1 has reported findings with dosage schedules somewhat higher than this. Few data exist on the effects of single large irradiation exposures, repeated at intervals of months or longer. It is the purpose of this report to present data on cats exposed to 200 r to the whole body, repeated four times, over approximately one and one-half years with several months between each exposure. The L.D. 50/30 days radiation dosage has never been exactly determined for the cat. However, a large number of irradiation exposures employing this animal suggest that it is in the neighborhood of 300 to 350 r, and 200 r whole body exposure represents a severe general and hematologic insult. Leukocyte counts consistently fall to 10 to 30 per cent of pre-irradiation levels and fail to return to normal values for a month or more. The animals frequently have anorexia for a few days after irradiation. The platelet counts fall but the dosage was deliberately selected at 200 r since at this level only an occasional animal presents a picture complicated by bleeding. The present report represents a segment of a study on bone marrow physiology as influenced by irradiation in the experimental animal. Detailed data on the hematologic effects of 200 r irradiation exposure are presented in a companion report.6

Materials and Methods

Animals were from the stock colony and all were immune to infectious feline agranulocytosis. In every instance immediately prior to irradiation each animal was subjected to a large, single phlebotomy under light ether anesthesia. The resultant blood loss was sufficient to reduce the erythrocyte and hemoglobin level to approximately 60 per cent of the pre-phlebotomy baseline value. Since the hemorrhage was immediately followed by 200 r to the whole body it was possible in each instance to assess the effect of this amount of irradiation on red cell regeneration as compared with the similarly bled but nonirradiated control animals. It was also possible to determine the effects of repeated phlebotomy and irradiation injury on any given animal, or in the experimental group as a whole as compared with the effect of the initial phlebotomy and injury. The ability of the damaged leukopoietic tissue to recover could be similarly assessed for the initial and subsequent exposures of the same animal. The details as to the phlebotomy technics, the characteristics of the radiation em-
ployed and the hematologic follow-up of irradiated animals are discussed in Part I of this study. Six animals were followed through four separate phlebotomies and whole body irradiation exposures over a period of one and one-half years. The first phlebotomy and irradiation occurred between April and August of 1949, the second in January 1950, the third in July of 1950 and the fourth in late November of 1950. In November and December of 1949 all animals received substantial supplements of ferrous sulfate. During the remainder of the study they were maintained on a diet high in iron. This consisted of commercial canned food with a horsemeat base, weekly supplements of fresh horsemeat, and a generous admixture of Rockland Rat Chow containing 37 mg. of iron per 100 Gm. Body weight of animals employed was slightly less than 3 Kg. on the average. All animals ate well and appeared healthy in the intervals between phlebotomy and irradiation.

**PRESENTATION OF DATA**

The data are presented in figures 1, 2 and 3 and table 1. Figure 1 indicates the regeneration of erythrocytes following the production of a standard anemia by phlebotomy immediately prior to each irradiation. It is evident that there is little difference in the regeneration patterns irrespective of the number of irradiation injuries previously sustained. There is a slight tendency for erythrocyte regeneration to lag after the fourth exposure. As will be discussed below, this may be related to the development of iron deficiency at the time of the fourth phlebotomy. Virtually complete marrow recovery has apparently occurred between each insult such that marrow reserve capacity capable of mobilization in response to the stress of sharply induced anemia is relatively little impaired.

Figure 2 indicates the regeneration of hemoglobin under the same circumstances. Here there is no evidence of a significantly different response to any of the first three exposures. However, regeneration of hemoglobin was distinctly slower after the fourth phlebotomy and irradiation. This may represent beginning encroachment on marrow reserve function by multiple severe radiation insults. However, it may be an artifact in that a degree of iron deficiency may
have developed in the wake of the multiple phlebotomies despite the relatively abundant iron of the diet and the intervals allowed for replenishment of iron stores. That this may be the case is further suggested by serum iron determinations done on 4 of the 6 experimental animals at the conclusion of the experiment. Serum iron values in 11 normal cats were found to range from 67 to 147 μg. per 100 ml. of blood with a mean of 93 μg. per cent. In the four experimental animals studied, the serum iron values were respectively 104, 49, 56, and 85 μg. per cent. The mean was 73 μg. per cent. These data suggest an element of
iron deficiency in some of the experimental group after the fourth phlebotomy.

Figure 3 shows the injury and recovery pattern for leukocytes after each irradiation. It has been pointed out in Part I of this study\(^4\) that there is a tendency for average leukocyte counts to fall somewhat, probably as a result of long continued training of the animals and lessening excitement. This is noted in nonirradiated controls and the failure of the leukocyte count to return to baseline values after the first exposure is no different from the findings in non-irradiated animals. In subsequent exposures, training has apparently exerted its maximal effect and baseline values are more closely approximated during recovery. It is apparent that multiple exposures failed to bring any noticeable change in the leukocyte regeneration curves. Capacity for regeneration appears nearly identical after all four exposures.

Table 1 indicates the relative number of mononuclear and granulocytic cells prior to any irradiation exposure as compared to that subsequent to apparent hematologic recovery from the fourth whole body irradiation.

The figures prior to the first irradiation represent the averages for the 6 animals based on four counts per animal; those after recovery from the fourth irradiation likewise represent the averages for 6 animals based on four counts per animal made on the forty-fourth, forty-sixth, fifty-first, and fifty-ninth days after the fourth irradiation. Despite the fact that total leukocyte counts did not vary remarkably from study to study, the four radiation exposures appear to have produced some diminution in the relative numbers of lymphocytes. At postmortem examination\(^*\) on the seventy-third day after the fourth hemorrhage and irradiation, slight adenitis with well filled follicles was seen in the lymph nodes. In the bone marrow, mild hyperplasia of myeloid elements was seen.

**Table 1.—Per Cent Mononuclear and Myeloid Cells before First Irradiation and after Fourth**

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<th>% Mononuclear</th>
<th>% Myeloid</th>
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<tbody>
<tr>
<td>Before first irradiation</td>
<td>24.2</td>
<td>75.8</td>
</tr>
<tr>
<td>After fourth irradiation</td>
<td>16.0</td>
<td>84.0</td>
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Despite the fact that whole body irradiation in the amount of 200 r is a profound insult to the hematologic structures of the cat and consistently depresses the leukocyte count to 10 to 30 per cent of baseline values for about a month, four repeated exposures show little evidence that complete functional recovery failed to occur in the intervals between irradiations. It can hardly be considered that radiation exposures of this degree fail to exact a toll on bone marrow reserve or that marrow potential is not in some way reduced. Certainly it would be foolhardy to assume that such insults could be indefinitely repeated. However, it is of some interest that the toll is so subtle as to be virtually undetected by test of marrow function. It may be argued that the bone marrow, as is the case

\(^*\) Courtesy of Dr. Alvin E. Lewis.
with the kidneys, lungs and many other organs, possesses a large functional reserve not utilized in meeting the needs of everyday existence. However, in the present experiments an attempt was made to evaluate encroachment on marrow reserves by artificially inducing a severe stress on the erythropoietic tissue. Not only was the marrow required to carry out its usual task of replacing the daily loss of circulating erythrocytes, but, also, it was required to utilize its reserve powers in generating erythrocytes to overcome a relatively severe acute loss of blood. It is therefore remarkable that only questionable impairment of the ability to respond maximally and normally to a severe stress resulted after four major irradiations. In the case of the myelopoietic tissue, despite prolonged and marked depression of activity by the radiation in the amount employed, regeneration patterns after repeated injuries appear unchanged. While one cannot accept the fact of complete recovery without permanent injury, the fact remains that further studies with more numerous acute exposures would be required to demonstrate residual damage. It may also be true, of course, that over a period of a lifetime hematologic abnormalities, though virtually undetectable now except by some reduction in the percentage of mononuclear leukocytes, might become evident.

**Summary**

1. Six animals (cats) were subjected to four whole body irradiation exposures over a period of one and one-half years. Irradiation dosage was 200 r to the whole body, an amount producing severe hematopoietic abnormalities in the cat. Irradiation in each case was immediately preceded by a large phlebotomy, so that functional impairment of erythropoiesis resulting from irradiation could be assessed in terms of a defective regeneration of erythrocytes. The effect of multiple radiation exposures could also be evaluated for each animal in terms of its prior response to phlebotomy and irradiation. The effects of repeated exposures on the leukocytes could likewise be assessed by means of the comparative leukocyte regeneration curves.

2. Little evidence of permanent functional damage of erythropoietic tissue by repeated exposures under the conditions of this experiment were observed. Erythrocyte and hemoglobin regeneration patterns were very similar from one exposure to another. However, hemoglobin regeneration was slightly slower after the fourth and last irradiation. Whether this represents encroachment on marrow reserve by multiple irradiations or whether it was an artifact resulting from iron deficiency cannot be said with certainty. Multiple exposures exerted little apparent effect on the leukocyte regeneration curves.

3. After recovery from the fourth irradiation, the percentage of mononuclear leukocytes was somewhat less than that prior to the first.

4. It is concluded that while repeated irradiation insults under the conditions of our experiment probably result in hidden damage even in the presence of apparent complete recovery, this damage is so subtle that it is difficult to detect by the tests of marrow reserve employed in this study.

**REFERENCES**

W. N. VALENTINE, M. L. PEARCE AND J. S. LAWRENCE


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