Long-term Effects of Local Irradiation of the Marrow on Erythron and Red Cell Function

By WIL B. NELP, MAHENDRA N. GOHIL, STEVEN M. LARSON and RAE ELLEN BOWER

Changes in erythron and RE cell function were examined in the marrow of the rabbit after 250–5000 R of localized irradiation by comparing the amount of $^{59}$Fe and $^{99m}$Tc sulfur colloid concentrated in the irradiated tibiofibula to that in the unirradiated control. At all levels of irradiation, there was immediate and severe loss of erythron function while RE cell activity remained nearly intact. Erythron function showed prompt partial recovery to a maximum level at approximately 8 days but with doses greater than 1000 R there was a secondary decline to 20 per cent of normal during the next 8 weeks. After 15 days, RE cell function had decreased to the same level as the erythron and subsequently fell in parallel with it. After 15 months, the marrow showed a secondary recovery of both RE cell and erythron function to 50 and 66 per cent of normal. The results of these experiments suggest that radiocolloid photoscans of the marrow showing decreased or absent RE cell activity will reflect a similar degree of erythropoietic damage if the studies are obtained weeks or months following radiation therapy.

CURRENTLY IT IS POSSIBLE to safely and conveniently visualize the bone marrow by photoscanning the spatial distribution of radiocolloids within the reticuloendothelial cells of the marrow organ. Radioactive $^{99m}$technetium colloid is the scanning agent most often used. $^{99m}$Technetium is readily available and is conveniently converted to colloidal form for intravenous administration. Its short 6-hour half-life and lack of beta irradiation result in acceptably low radiation exposure to the liver, spleen and marrow. The erythropoietic marrow may also be visualized by imaging the deposition of $^{52}$iron (a positron emitter with an 8-hour half-life); however, few laboratories have the facilities for its production (cyclotron) or optimum detection. $^{59}$Iron can be used, but its high gamma energy prohibits detailed imaging of the marrow.

Because of the potential clinical usefulness of radiocolloid marrow scanning it is important to establish the relationships between the reticuloendothelial and hematopoietic components of the marrow during various functional or...
pathological changes. In normal animals and man, the quantitative distribution of RE cell is essentially the same as the hematopoietic marrow. In diseases with expanded erythropoietic marrow, such as chronic hemolytic anemia or polycythemia vera, the RE cells shows concomitant expansion. Likewise, with decreased hematopoiesis, RE cell marrow scans usually reflect this decrease, although VanDyke has observed certain cases of aplastic anemia where RE cell function may be present when erythron function appears to be decreased to absent.

Radiation of various portions of the bone marrow in patients having radiation therapy for lymphoma or Hodgkin's disease may be fairly extensive and repetitive. Prior to the initiation of additional therapy in these patients, it would be helpful to assess the degree of bone marrow damage produced by radiation. Radiocolloid scans of the marrow after radiation may show extensive loss of marrow function; still it is uncertain what degree of loss of hematopoietic function occurred in the same area.

The purpose of this paper is to present the results of experiments designed to compare the effect of irradiation on the RE cell and the erythron of the marrow of the rabbit. Animals were studied for up to 15 months after exposing the rabbit tibiofibula to various doses of X ray. Initially, there was transient dissociation of RE cell and erythron function with the erythron showing immediate and severe depression. Within 2 weeks, erythron function had partially recovered and thereafter the quantitative change in function of the RES and erythron remained similar.

**Materials and Methods**

Studies were performed on 2.2 Kg. New Zealand rabbits. After light pentobarbital anesthesia, the left tibiofibula was irradiated with a single dose of 250 keV X ray and the remainder of the body was carefully shielded with lead. Doses of 250 to 5000 R were

<table>
<thead>
<tr>
<th>Radiation Dose (R)</th>
<th>Days After Irradiation</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>250 RE</td>
<td>101</td>
</tr>
<tr>
<td>E</td>
<td>65</td>
</tr>
<tr>
<td>500 RE</td>
<td>88</td>
</tr>
<tr>
<td>E</td>
<td>23</td>
</tr>
<tr>
<td>1000 RE</td>
<td>98</td>
</tr>
<tr>
<td>E</td>
<td>20</td>
</tr>
<tr>
<td>2000 RE</td>
<td>97</td>
</tr>
<tr>
<td>E</td>
<td>18</td>
</tr>
<tr>
<td>5000 RE</td>
<td>110</td>
</tr>
<tr>
<td>E</td>
<td>18</td>
</tr>
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</table>

RE, RE cell activity; E, erythron activity. Values are expressed as per cent of contralateral limb.

* Mean of five rabbits, all other numbers represent the average of two rabbits at each time interval.
delivered during periods lasting from 3 to 20 minutes. Dosimeters placed adjacent to the
unirradiated leg (control) indicated that it received less than 0.3 per cent of the dose.
Following irradiation, pairs of rabbits were sacrificed at selected times up to 60 days for
all doses except 250 R. Five rabbits who received 2000 R were studied 15 months later
(Table 1).

Techniques to measure RE cell and erythron activity were similar to those reported
earlier.6 Six hours prior to sacrifice, animals were injected intravenously with approximately
10 μCi of 59Fe chloride. Thirty minutes prior to sacrifice each animal received 100–1000
μCi of 99mTc sulfur colloid (approximately 1 mg. of colloid per Kg. body weight). At
these times following injection of the isotopes, insignificant radioactivity appeared in the
plasma or circulating red cells in the marrow space.

At sacrifice, both tibiofibulae were removed and the total 59Fe and 99mTc radioactivity in
each bone was determined. When the total marrow of the shaft was removed, 99 per cent
of the total bone radioactivity was in the marrow space. The degree of function of the
erythron or RE cell in the irradiated leg was expressed as the per cent of activity of 59Fe
and 99mTc in the left compared with the right tibiofibula. The term erythron or RE cell
"function" is used in this limited sense. It is referring only to the functioning ability of
the RE cell to phagocytize a trace quantity of radiocolloid or the ability of the erythron to
concentrate iron. The initial deposition of iron in the erythron usually results in its eventual
release into the circulation as a mature erythrocyte; however, the maturation function of
the erythron is not examined in these experiments.

Histologic sections of the marrow were prepared with hematoxylin and eosin stains after
decalification of the bone.

RESULTS

Figure 1 is a series of lines representing RE cell or erythron function for 60
days after the various radiation doses. The smooth lines are derived from the
data in Table 1. All levels of radiation produced an immediate decrease in
erythron function. The maximum depression in 59Fe uptake occurred 48 hours
following the dose. Thereafter erythron function improved to a maximum
approximately between the seventh and tenth days. With lower doses (250–
500 R) there was a little further change in erythron function. With doses of
1000 R or greater, there was a secondary decrease in erythron function during
the subsequent 60 days.
In contrast, RE cell function showed little change during the first 48 hours but by 15 days had gradually decreased to a level similar to that of the erythron. After 15 days there was little change at lower doses (250–500 R) or a continued gradual decline in function (doses of 1000 R or greater). After the 15th day, the loss and rate of change of RE cell function was remarkably similar to that occurring in the erythron. After 60 days, the net effect of the higher doses of irradiation was a reduction in both RE cell and erythron function to 10–25 per cent of normal, the RE cell function being consistently slightly less.

After observation of the marrow response during the first 60 days, an additional group of five rabbits received a single dose of 2000 R to the left tibiofibula and were studied after 15 months. Figure 2 is a composite representation of the change in erythron and RE cell function for the 15 months following 2000 R. It shows the characteristic loss of erythron function at 48 hours, followed by a partial recovery and then a secondary decline paralleling that of the loss of RE cell function. After 15 months there was secondary recovery in both cell systems. The five rabbits studied at 15 months showed average RE cell function of 50 per cent ± 12 (SD), range 30–68, and erythron function of 66 per cent ± 14 (SD), range 52–87.

For rabbits receiving 2000 R, other observations listed in Table 2 were made at 48 hours, 8 days, 60 days and 15 months. These times were chosen to correspond to the periods of suppression and recovery of the erythron seen in Fig. 2. Of interest is the reduction in the weight of the irradiated marrow with time, so that after 15 months the marrow in the tibial shaft was reduced to about one-half of the control marrow. This is essentially the same as the reduction in RE cell and erythron function in the irradiated marrow. The irradiated tibiofibula was obviously smaller at 15 months and total weight (bone plus marrow) was reduced to 82 and 75 per cent of the control in these two animals.

Histologic examinations of the marrow were made at 2, 8 and 60 days and at 15 months after 2000 R. In these animals, carbon particles were injected 60 minutes prior to sacrifice in order to visualize the RE cell pattern. All
Table 2.—Changes in Marrow Weight Following 2000 R Irradiation

<table>
<thead>
<tr>
<th>No. of Rabbits</th>
<th>Time After 2000 R</th>
<th>Average Body Weight (Gm.)</th>
<th>Marrow Wt. (Gm.) of Control Tibia</th>
<th>Ratio Irradiated Marrow Wt.: Control Tibia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean Range of Body Wt.</td>
<td>Per Cent of Body Wt.</td>
</tr>
<tr>
<td>4</td>
<td>2 days</td>
<td>2200</td>
<td>1.58 1.24-1.90</td>
<td>0.072 0.97</td>
</tr>
<tr>
<td>4</td>
<td>8 days</td>
<td>2640</td>
<td>2.0 1.86-2.23</td>
<td>0.076 0.97</td>
</tr>
<tr>
<td>3</td>
<td>60 days</td>
<td>3440</td>
<td>2.13 1.84-2.48</td>
<td>0.062 0.81</td>
</tr>
<tr>
<td>2</td>
<td>15 mos.</td>
<td>3900</td>
<td>2.04 1.84-2.48</td>
<td>0.052 0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3900</td>
<td>1.00 1.00-1.00</td>
<td>0.027 0.62</td>
</tr>
</tbody>
</table>

sections were taken from the proximal end of the tibial shaft. At 48 hours there was almost complete obliteration of hematopoietic elements with prominence of the RE cells containing carbon. The overall structural pattern of reticulum cells and sinusoids was still intact, but certain sinusoids show slight extravasation of mature red cells beyond their endothelial walls. At 8 days, RE cells were reduced in number and new areas of hematopoietic cells were present. The marrow architecture was still easily recognized but marrow fat was prominent. At 2 months, the sinusoids showed irregularities of the endothelium and RE cells and hematopoietic elements were sparse. There was no scarring. At 15 months, the marrow architecture was entirely disorganized with an irregular and indistinct sinusoidal pattern. RE cells were difficult to identify and most hematopoietic elements were located peripherally. Much of the central marrow space was replaced by a homogenous eosinophilic staining material interspersed with a few pleomorphic cells. These changes are very similar to those previously described by Knospe et al. after 2000 R of local marrow irradiation in the rat.8

DISCUSSION

The response of the rabbit marrow to acute irradiation showed several consistent and interesting patterns. At all doses there was a marked dissociation of RE cell and erythron function during the first 48 hours. RE cell function changed only slightly while the erythron was severely depressed. Subsequently, there was a temporary recovery of erythron function to a maximum between the seventh and tenth days. This initial recovery could be interpreted as a period of repopulation of the marrow by circulating stem cells. After this time, however, a secondary decrease in erythron function was evident (at 1000 R or greater) as if the internal milieu of the marrow was no longer capable of supporting continued erythron growth. By 15 days, RE cell function had gradually decreased to the same general level as that of the erythron. Thereafter RE cell and erythron loss were parallel and similar.

In the rabbits receiving 2000 R there was a secondary recovery of 50–66 per cent of the control leg (RE and erythron) after 15 months. At this time, organization of the marrow architecture was distinctly different and marrow mass of the irradiated tibial shaft was also reduced to 50–66 per cent of the control leg. Thus some regenerative stimulus permitted fairly good functional return.

Knospe, Blom and Crosby described comparable histologic changes in the
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marrow of the rat during the year following 2000 R of local irradiation. They deduced that there was an immediate loss, a secondary recovery of elements, very closely resembling the pattern measured in Fig. 2. They were most impressed that changes in the microcirculation of the sinusoids might be the dominant factor controlling the rate and degree of hematopoietic response. Certainly, the marked radiosensitivity of the erythron would be responsible for its immediate and severe depression. If marrow circulation is a major factor in governing the functional loss or regeneration of the marrow, it may be related either to the amount of blood flow per unit mass of marrow or to the functional impairment of sinusoidal flow. We have no direct observations on this point but have had some opportunity to observe indirect assessment of marrow flow in a few rabbits following 2000 R of irradiation of the tibia. In these rabbits, the washout of a 1/20 ml bolus of \( {\textsuperscript{99m}}\text{TcO}_4\)-introduced into the center of the marrow space was observed. The rate and character of the washout curve was the same as that of the controls at 2 and 8 days after irradiation but was definitely slower and less complete at 60 days. In addition, the amount of circulating blood (determined by \( ^{51}\text{Cr} \) labeling of circulating red cells) in the irradiated marrow was consistently greater by 20 to 40 per cent 48 hours after irradiation (four rabbits) and consistently less (40-60%) at 60 days (three rabbits).

In terms of radiocolloid photoscanning, these experiments suggest that the scan pattern of spatial distribution of colloids in the marrow will indicate the pattern of erythropoietic marrow activity if the studies are performed several weeks after completion of irradiation.

REFERENCES

Long-term Effects of Local Irradiation of the Marrow on Erythron and Red Cell Function

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