Studies on medullary and extramedullary erythropoiesis in the adult mouse

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Bozzini, C. E., M. E. Barrio Rendo, F. C. H. Devoto, and C. E. Epper. Studies on medullary and extramedullary erythropoiesis in the adult mouse. Am. J. Physiol. 219(3): 724-728. 1970.—Splenic and marrow erythropoiesis was compared in the mouse following stimulation by hypoxia, bleeding, or injections of erythropoietin or depression of erythropoiesis by transfusion. The effect of splenectomy on both total circulating red cell volume and erythroid response to hypoxia or erythropoietin administration was also determined. It was found that bone marrow erythropoiesis is almost twice splenic erythropoiesis in the normal CFW/ep mouse. The fractional depression of $^5$Fe uptake produced by transfusion was more marked in the spleen than in bone marrow. Bleeding or exposure to hypoxia produced a marked increase in splenic radioiron incorporation. Radioiron uptake by the bone marrow was not affected. The number of nucleated erythroid precursors in femoral marrow was not affected by bleeding. Splenectomy produced a mild anemia, total red cell mass being decreased by 15%. Red cell production was observed in both liver and axillary lymph nodes in splenectomized mice exposed to hypoxia. These data strongly suggest that red cell production in bone marrow is not sufficient in the adult mouse to meet the normal demand for erythrocytes, and, therefore, requires the assistance of extramedullary erythropoiesis.

Bleeding or exposure to hypoxia; axillary lymph nodes; anemia; splenectomy; erythrocytes

The bone marrow has been considered to be the primary site of red cell production in mammals throughout postnatal life. However, the presence of erythroid cells in smears or sections of the mouse spleen and ferrokinetics studies indicate that the normal mouse spleen, in contrast to the human or dog spleen, is an active erythropoietic organ (2, 4, 7, 8, 15). The present investigation was undertaken to compare splenic and marrow erythropoiesis in the mouse following stimulation by hypoxia, bleeding, or injections of erythropoietin or depression of erythropoiesis by transfusion. In addition, the effect of splenectomy on both the total circulating red cell volume and the erythroid response to hypoxia or erythropoietin administration was determined, since evidence has been presented which indicates that the spleen may function as a primary organ of erythropoietic homeostasis in this species (7, 8).

Materials and Methods

Male CFW/ep mice (Atomic Energy Commission, Argentina) weighing 24–28 g were housed individually and were fed a standard laboratory diet. Drinking water was provided ad libitum. The effects of hypoxia were studied in groups which were exposed 19 hr/day to a simulated altitude of 23,000 ft in a decompression chamber. Erythropoietin was prepared from the urine of anemic patients by the collodion-adsorption method (16) and assayed by $^59$Fe incorporation in red cells of polycythemic mice according to the method of DeGowin et al. (6). A laboratory standard calibrated in terms of the International Reference Preparation A (1) was used in each assay. The red cell mass was measured by the $^59$Fe labeled red cell dilution technique. Iron $^59$-labeled red cells were obtained from donor mice injected with 3 μc of $^59$Fe 10 days before and injected intravenously into the experimental animals. They were bled out via cardiac puncture 10 min later. Total nucleated cell and differential counts on the right femur were done according to an isotopic dilution technique previously reported (3). Imprints of fresh spleen, liver, and lymph nodes, and brush preparations of bone marrow were prepared and stained with either a Wright-Giemsa mixture or a benzidine method for the detection of hemoglobin (12). The benzidine stained slides were also counterstained with Harris hematoxylin. The liver samples were fixed in an alcohol-formalin-acetic acid mixture, embedded in paraffin, sectioned at 8 μ, and stained with hematoxylin and eosin. For the determination of radioactive iron incorporation in vivo the experimental animals were injected intravenously with 0.8 μc of $^{59}$Fe as ferric citrate and radioiron uptake determined 3 hr later on the right femur and spleen using a well-type scintillation counter. In other experiments liver and axillary lymph nodes radioiron uptakes were also determined in a similar manner. Radioactivity was expressed as percentage of injected dose incorporated using an appropriate $^{59}$Fe standard. Quantitative measurements of the fraction of the total erythropoietic marrow present in the right femur was done as previously reported for rats (3). It was observed that the right femur contained 7.2% of the total erythroid marrow. This value was used to calculate total marrow $^{59}$Fe uptake. Marrow erythropoiesis-splenic erythropoiesis ratio was calculated as the ratio between the 3-hr $^{59}$Fe uptake into total erythropoietic marrow and the 3-hr $^{59}$Fe uptake into spleen.

Results

Marrow versus splenic erythropoiesis as a function of hematocrit. The erythropoietic response of the spleen and bone marrow

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to changes in the hematocrit value was quantitated by ferrokinetic studies. A decrease in the hematocrit value was obtained by means of a single bleeding by cardiac puncture and immediate replacement of the same volume of blood withdrawn with dextran. An increase in the hematocrit value was produced by a single intravenous injection of an 80% suspension of washed mouse red blood cells. Radioiron was injected 72 hr after the above procedures and percent uptake determined 3 hr later. The results obtained are shown in Fig. 1. In the normal mice, with a mean hematocrit of 44%, bone marrow erythropoiesis was almost twice splenic erythropoiesis. The increase in hematocrit caused a depression in both splenic and marrow ⁵⁹Fe uptake. However, the fractional depression was clearly more marked in the spleen. As a consequence the marrow-splenic erythropoiesis ratio increased from 2.2 to 13.0 with hematocrits of 44 and 71%, respectively. When the hematocrit value was decreased two important events were worth noting: 1) a lack of an increase in radioiron uptake by the marrow, and 2) a marked increase in splenic ⁵⁹Fe uptake, which exceeded that seen in bone marrow at the lowest hematocrit value. Therefore, the marrow-splenic erythropoiesis ratio was reduced from 2.2 to 0.9 with hematocrits of 44 and 20%, respectively.

Erythropoietic responses of spleen and bone marrow to bleeding and to exposure to hypoxia. The differences observed in the erythropoietic responses of bone marrow and spleen to a decrease in red cell mass prompted us to investigate further this phenomenon. The oxygen carrying capacity of blood was reduced by either bleeding or exposure to hypoxia. The percent ⁵⁹Fe incorporation (times normal) into bone marrow and spleen is shown in Fig. 2 either as a function of the amount of blood removed or as number of days of exposure to hypoxia. Both bleeding and exposure to hypoxia resulted in a marked increase in the incorporation of radioiron into spleen. On the other hand, radioiron uptake by the marrow showed a slight fall at the time of maximal uptake by the spleen.

In order to confirm that the values obtained by ⁵⁹Fe uptakes in response to anemic or anoxic hypoxia really reflect changes in erythropoiesis, the number of nucleated erythroid precursors in femoral marrow, and the percent nucleated erythroid cells in spleen were determined. As seen in Table 1 bleeding (40% of the blood volume) did not affect the number of nucleated erythroid cells in marrow. On the other hand, imprint preparations of spleens of bled

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**FIG. 1.** Marrow-splenic erythropoiesis ratio (Δ—Δ) and percent ⁵⁹Fe uptake into spleen (○—○) and bone marrow (■—■) as a function of hematocrit value. Each point represents mean ± SEM for 10 animals.

**FIG. 2.** Iron ⁵⁹ uptake into spleen (○—○) and bone marrow (■—■) as a function of either amount of blood drawn (upper figure) or numbers of days of exposure to hypoxia (lower figure). Each point represents mean ± SEM for 6 animals.

**TABLE 1.** Nucleated erythroid cells in right femur and spleen of normal and bled mice

<table>
<thead>
<tr>
<th></th>
<th>Normal Mice</th>
<th>Bled Mice</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
<tr>
<td>Nucleated cells in femur per gram body wt (X 10⁸)</td>
<td>1.13 ± 0.1</td>
<td>1.05 ± 0.3</td>
</tr>
<tr>
<td>% Nucleated erythroid cells in femur</td>
<td>17.4 ± 1.4</td>
<td>16.9 ± 2.3</td>
</tr>
<tr>
<td>Nucleated erythroid cells in femur per gram body wt (X 10⁸)</td>
<td>0.19 ± 0.02</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td>% Nucleated erythroid cells in spleen</td>
<td>7.08 ± 2.1</td>
<td>16.12 ± 3.8</td>
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</tbody>
</table>

Values are means ± SEM.
mice revealed an increase in the number of erythroid precursors following a similar bleeding stimulus.

Effect of splenectomy on total circulating red cell volume (TCRCV) in mouse. Since the spleen contributes to erythropoiesis in the normal mouse and the primary erythropoietic response to anemic or hypoxic hypoxia is splenic, it seemed important to determine the effect of splenectomy on TCRCV in this species. Mice were splenectomized and their TCRCV determined 30 and 60 days later. As indicated in Table 2, ablation of the spleen produced a slight but significant decrease in the TCRCV, which was reduced by approximately 15%.

**TABLE 2. Effect of splenectomy on total circulating red cell volume in the mouse**

<table>
<thead>
<tr>
<th></th>
<th>Hct</th>
<th>RCV</th>
<th>PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mice, n = 14</td>
<td>45.0 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Splenectomized mice (30 days) n = 9</td>
<td>41.0 ± 0.1</td>
<td>2.4 ± 0.1*</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Splenectomized mice (60 days) n = 10</td>
<td>45.0 ± 0.1</td>
<td>2.5 ± 0.1†</td>
<td>3.1 ± 0.1</td>
</tr>
</tbody>
</table>

Hct = hematocrit value; RCV = red cell volume; PV = plasma volume. Values are means ± SEM. n = number of observations. The significance of differences between means was obtained by the Student t test. * P < 0.001 when compared to normal mice. † P < 0.005 when compared to normal mice.

Red cell production in liver and lymph nodes in splenectomized mice exposed to hypoxia. It is of interest that splenectomized mice exposed to hypoxia or injected with erythropoietin are capable of increasing their TCRCV in spite of the fact that the bone marrow does not increase its rate of erythropoiesis. Obviously, enhanced red cell production is occurring at another site under these conditions. To test this possibility, mice were exposed to hypoxia 30 days after splenectomy for 20–25 days and both imprint preparations and sections of both liver and axillary lymph nodes were made at the time of sacrifice. Examination of imprints of both tissues revealed significant numbers of nucleated erythroid cells. The type of erythroid cells found in the lymph nodes and livers of splenectomized mice exposed to hypoxia are shown in Figs. 4 to 7. No erythroid cells were seen in lymph nodes or livers from normal mice which were exposed to hypoxia, or from splenectomized mice not subjected to hypoxia. If these organs replace the spleen as erythropoietic organs, there may be a detectable increase of $^{59}$Fe uptake by them in splenectomized mice subjected to hypoxia. The 3-hr $^{59}$Fe uptake into both liver and axillary lymph nodes of mice treated as stated above is presented in Table 3. Iron 59 incorporation increased considerably in both organs, which confirms their participation in the erythropoietic process under these conditions of stimulated red cell production.

**DISCUSSION**

Red blood cells are produced under normal conditions in mammals throughout postnatal life exclusively in the bone marrow. The mouse is an exception in that the spleen also functions as an erythropoietic organ (2, 4, 7, 8, 15).
Splenic erythropoiesis in the mouse does not appear to be identical to bone marrow erythropoiesis if we consider their different responses to conditions in which red cell production is either stimulated or depressed. When erythrogenesis is stimulated the response is primarily splenic, as indicated by splenic uptake of $^{59}$Fe and morphological evaluation of the spleen. Marrow erythropoiesis does not appear to be affected to a considerable extent under these conditions. As a consequence, splenic erythropoiesis overshadows marrow erythropoiesis when the erythropoietic stimulus is great enough.


FIG. 6. Portal area of liver showing nucleated erythroid elements. Hematoxylin-eosin stain. $\times$100.


**TABLE 3. Three-hour $^{59}$Fe uptake into liver and axillary lymph nodes of mice exposed to hypoxia**

<table>
<thead>
<tr>
<th></th>
<th>$%$ $^{59}$Fe Uptake</th>
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<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Normal mice, $n = 10$</td>
<td>7.4 ± 0.7</td>
</tr>
<tr>
<td>Splenectomized mice exposed to hypoxia, $n = 10$</td>
<td>13.1 ± 1.8*</td>
</tr>
</tbody>
</table>

Mice were splenectomized 30 days before. Values are means ± sem. $n =$ number of mice. * $P < 0.02$ when compared to normal mice. † $P < 0.001$ when compared to normal mice.

The present findings that the spleen increases its rate of erythropoiesis following a marked erythropoietic stimulus confirm earlier reports by other workers (2, 5, 11, 15). When erythrogenesis is suppressed by transfusion or starvation (7, 15) a depression in both splenic and marrow erythropoiesis occurs. However, the changes observed in these two organs are not parallel, since the fractional depression is higher in the spleen than in the marrow.

Splenectomy produces in mice the appearance of a mild anemia, which is apparent 30 and 60 days after the ablation of the organ. However, splenectomized mice respond to hypoxia by increasing their total red cell mass, this response being lesser than that seen in normal mice in the same conditions. A possible explanation for this difference could be that splenectomized mice produce less erythropoietin than...
normal mice when exposed to hypoxia. This possibility was not tested since others have found that splenectomy does not alter the production of erythropoietin following erythropoietic stimulation (9, 13, 14). It seems more likely that ablation of the spleen, by removing an important site in the mouse for red cell production, produces an inability in the animals to fully respond to the hormone. The lesser increase in the total red cell volume observed in splenectomized mice chronically injected with erythropoietin in the present experiments than in normal mice tend to support this explanation.

Since the bone marrow of splenectomized mice exposed to hypoxia failed to increase its rate of erythropoiesis, other tissues were studied which might be able to produce erythrocytes following hypoxia. Extramedullary red cell production is known to occur under various pathologic conditions in adult animals, including man. The tissues most frequently involved are lymph nodes, spleen, and liver (10). Cytologic examination and ferrokinetic determinations in both lymph nodes and liver in our studies showed evidence of erythropoiesis in these tissues. This probably explains the erythropoietic response of our splenectomized mice to both hypoxia and erythropoietin administration despite the inability of the bone marrow to increase its rate of red cell production. No erythroid cells were seen in the lymph nodes and liver of normal mice exposed to hypoxia or injected with erythropoietin. This may be related to the ability of the spleen to meet this accelerated demand for erythrocytes. The reason for the failure of the bone marrow in both normal and splenectomized mice to participate in the response to hypoxia is not known. It is quite possible that the bone marrow in this species is producing erythrocytes at a maximal rate which is not sufficient in the normal animal to meet the normal demand for erythrocytes and, therefore, requires the assistance of the spleen. When the demand for erythrocytes increases, the spleen is the organ which is called upon to meet this need. When the spleen has been extirpated, both liver and lymph nodes replace it as hematoipoietic organs.

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REFERENCES