Hemopoietic Support Capacity of Adult Mouse Liver. Studies in $^{90}$Sr Marrow-ablated Mice

By Solomon S. Adler and Frank E. Trobaugh, Jr.

The capacity of normal livers in adult mice to support proliferation of pluripotent hemopoietic stem cells (CFU-S) was studied. We assayed CFU-S of the blood and livers of mice with intact marrows and of mice whose marrows had been ablated with $^{90}$Sr (4 μCi/g) either before or after removal of their spleens, the major hemopoietic organ in marrow-ablated mice. Splenectomy alone resulted in an increase in the numbers of blood and hepatic CFU-S; since the spleen is an efficient trapper of CFU-S released from the marrow, in the splenectomized mice more CFU-S were available for trapping by the liver. Mice splenectomized 3 days prior to $^{90}$Sr injection had virtually no blood or liver CFU-S by the tenth day after $^{90}$Sr injection. Fourteen days after injection of $^{90}$Sr there were supranormal numbers of CFU-S in both blood and liver of intact mice. One week after such mice were splenectomized, however, CFU-S were virtually absent from both blood and liver. This study suggests that normal livers in adult mice cannot support detectable proliferation of normal CFU-S even if the animal is subjected to severe and relatively prolonged hemopoietic stress. In addition, the results of our studies demonstrate that normal livers of adult mice have the capacity to trap large numbers of CFU-S.

Although the proliferation of pluripotent hemopoietic stem cells (CFU-S) and the interaction of these cells with various hemopoietic microenvironments have been the subjects of enormous investigative efforts, there has been no definitive study determining the capacity of normal livers in adult mice to support proliferation of normal CFU-S, even though the liver functions as the main hemopoietic organ during much of fetal life.

Some investigators have suggested that the livers of normal adult mice house dormant CFU-S that can be induced to proliferate following the stimulus of partial hepatectomy, while others have suggested that the environment of postfetal mouse liver precludes even sequestration of CFU-S from the blood. It has been shown that the number of hepatic CFU-S increases after repeated sublethal whole body irradiation or after injections of methylcellulose or cyclophosphamide; these findings have been interpreted as providing evidence that under certain conditions livers of adult mice can support pluripotent stem cell proliferation. In addition, it has been suggested that the liver’s contribution to hemopoiesis should be taken into account when evaluating the total hemopoietic effort of the animal.

The result of these studies could be explained by at least two mechanisms: (1) the livers of the studied mice supported CFU-S proliferation, or (2) the...
livers of these mice trapped more circulating CFU-S than the livers of normal mice. Increased hepatic trapping of CFU-S may result from: (1) an increase in the number of circulating CFU-S and thus an increase in those available for trapping, or (2) an increase in the efficiency of the studied livers to trap blood CFU-S as compared to normal livers. Both conditions are known to exist. It has been shown that increased numbers of CFU-S are released into the circulating blood in states of stress, and Varon and Cole showed that the livers of carbon tetrachloride-treated mice trapped more CFU-S from the blood than did normal livers because of fibrin deposition associated with local blood coagulation. Local coagulation, no doubt, also occurs after partial hepatectomy and could account for the increase in hepatic CFU-S in that circumstance.

It is important to know if normal livers of adult mice can support normal hemopoiesis, including the proliferation of normal CFU-S. If livers support the proliferation of CFU-S, the number of hepatic CFU-S would have to be taken into account when studies of CFU-S kinetics in the mice are analyzed. If, on the other hand, normal CFU-S do not proliferate in the livers of normal adult mice, the presence of CFU-S proliferation in that organ would indicate that there is either an abnormality of the CFU-S or a modification of the hemopoietic support capacity of the liver. To resolve this issue we exploited the observation that the radionuclide \(^{89}\text{Sr}\) selectively ablates marrow hemopoiesis. We assayed blood and liver CFU-S in \(^{89}\text{Sr}\) marrow-ablated mice before and after removal of their spleens, the primary hemopoietic organ in such mice.

\(^{89}\text{Sr}\) is a \(\beta\)-emitting isotope with a 55-day half-life and 1.3 meV energy. This isotope can be used to ablate selectively marrow hemopoiesis because a large part of an intraperitoneally administered dose rapidly localizes in and is bound to bone; only minimal amounts are found in other tissues after the first 24 hr. When administered to mice, \(^{89}\text{Sr}\) rapidly causes extensive bone marrow hypoplasia. By the tenth day after mice are given a dose of 4 \(\mu\)Ci/g body weight of \(^{89}\text{Sr}\) their femurs contain less than 2\% of the normal number of CFU-S. Because spleens of mice support marked compensatory hemopoiesis, \(^{89}\text{Sr}\)-treated mice develop only minimal anemia; such mice, however, do develop a substantial leukopenia. Klassen et al. showed that marrow hemopoietic activity was insufficient to sustain the life of mice splenectomized even 37 days after treatment with \(^{89}\text{Sr}\) (4-6 \(\mu\)Ci/g body weight). With the passage of time, hemopoiesis gradually is restored in the marrow of non-splenectomized \(^{89}\text{Sr}\)-treated mice; we have provided evidence indicating that the CFU-S responsible for this restoration are predominantly of splenic origin. In addition, it is highly likely that the CFU-S in the blood and the few CFU-S in the marrow of mice soon after \(^{89}\text{Sr}\) treatment are also of splenic origin.

**MATERIALS AND METHODS**

*Strontium.* \(^{89}\text{SrCl}\) was purchased from Oak Ridge National Laboratories, Oak Ridge, Tenn., and adjusted to pH 5-6 before injection. Each animal received 4 \(\mu\)Ci \(^{89}\text{Sr}/g\) body weight by intraperitoneal injection in a volume of about 0.25 ml buffered solution. Control mice received equivalent amounts of "cold" strontium chloride (\(^{88}\text{Sr}\)).

*Mice.* Female CAF1 (Balb/C x A/He) mice, 12-16 wk old, each weighing about 25 g, were used throughout (Cumberland View Farms, Clinton, Tenn.). Ten mice were housed as a unit in
cages with disposable plastic bottoms and were given food ad libitum. The pH of their drinking water was adjusted to 3.2 to minimize early postirradiation deaths in assay animals.

**Splenectomy procedure.** Lateral abdominal wall incisions were made under sodium pentobarbital anesthesia, and splenic pedicles were ligated prior to splenic extirpation; for sham splenectomies, the spleens were exteriorized briefly. Skin and muscle incisions were closed with stainless steel wound clips.

**Procedure for harvesting tissues for CFU-S assays.**

**Blood.** Under sodium pentobarbital anesthesia, animals were bled into heparinized syringes from cardiac punctures. Blood from the mice of each group was pooled.

**Liver.** The livers used for CFU-S determinations were weighed, and weighed fragments of about 0.2 g from the left lobe of each liver were pooled. A single-cell suspension was prepared by forcing a scissors-prepared liver mash thru a series of three stainless steel wire meshes, the finest of which had 200 openings/linear inch. All cell suspensions were kept in an ice bath from harvest until injection.

**Cell quantitation.** Blood samples for hematocrits and cell counts were obtained by orbital plexus bleeding. Microhematocrits were performed on blood drawn into heparinized capillary tubes. All nucleated cell counts were performed on a Model F Coulter Counter after erythrocyte lysis with ZAP-isoton.

**CFU-S assay procedure.** The technique of Till and McCullough was employed using female CAF1 mice injected with test cell suspensions within 2 hr after exposure to 955 rads lethal whole body irradiation delivered from a Co source at a rate of 70.76 rads/min to a field 24 sq cm in size at a distance of 82.5 cm from the source or after exposure to 850 rads whole body -y-irradiation delivered from a 137Cs Gamma-Cell 40 irradiation unit at a rate of 127.2 rads/min. The volumes of blood and weights of liver tissue used for the assays depended on the group assayed and were selected to yield countable numbers of spleen surface colonies; for blood, the volume range was 0.04-0.5 ml, and for liver tissue the weight range was 1-20 mg. The cell suspensions assayed were pooled as noted above and diluted in Hanks' balanced salt solution so that each animal received the test cells in a volume of 0.5 ml injected into a lateral tail vein. Prior to the injection of liver tissue, the mice were injected intravenously with 65 units of sodium heparin to prevent acute pulmonary insufficiency. Surface spleen colony counts were performed with the aid of a 2.5 x magnifying lens 8 or 9 days after irradiation and transplantation after fixation of the spleens in Bouin's solution.

**Experiment I.**

Groups of mice were splenectomized or sham splenectomized and 3 days later injected with "cold" 90Sr or radio-90Sr. Ten days after injection, blood counts and CFU-S content of the blood and livers of these mice were determined. Twelve splenectomized and twelve sham splenectomized 90Sr-treated mice were housed to determine 30 day survival.

**Experiment 2.**

Groups of mice were injected on day 0 with "cold" 90Sr or with the 90Sr radionuclide and 14 days after injection were splenectomized or sham splenectomized. Assays for blood and liver CFU-S were performed on the following days: on day 0, prior to injection; on day 14, prior to surgery; and on days 21, 28, and 35. Blood counts were performed on the postoperative assay days (i.e., 21, 28, and 35).

Three or four replications of each of the experiments were performed except for the survival study in experiment I, which was performed only once. For each CFU-S determination, blood or liver tissue was pooled from 8-15 treatment animals. Hematocrits and white cell counts with differentials were performed at each time interval on individual samples of blood from 5 experimental and 5 control animals for each of the replications. The results of the several replications were pooled and analyzed by the unpaired Student's t test.

**RESULTS**

**Experiment 1 (Table 1, Fig. 1)**

Compared to the intact 90Sr-treated mice, the splenectomized 90Sr-treated mice had only a mild anemia and no granulocytopenia (Table 1). The splenectomized 90Sr-treated mice, however, were severely anemic and granulocytopenic compared to their intact counterparts (Table 1).

**TABLE 2. Hormonal regulation of glycogen synthase**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Glycogen Synthase Activity</th>
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<tbody>
<tr>
<td>Insulin</td>
<td>Increased</td>
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<tr>
<td>Glucagon</td>
<td>Decreased</td>
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<tr>
<td>Cortisol</td>
<td>Decreased</td>
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Values were obtained 7, 14, and 21 days after surgery, i.e., on days 21, 28, and 35 after the Sr injections.

In the splenectomized mice, 10 days after treatment with $^{89}$Sr both blood and liver CFU-S were strikingly elevated, whereas in the splenectomized mice 10 days after $^{89}$Sr treatment CFU-S were virtually undetectable in either blood or liver (Fig. 1).

In the study designed to evaluate the 30-day survival of $^{89}$Sr-treated mice, all 12 of the intact $^{89}$Sr-treated mice survived for the 30-day period, whereas the

Table 1. Blood Hematocrit and Granulocyte/mm$^3$ Values (Mean ± SE) of Mice 10 Days After Injection of $^{89}$Sr or $^{88}$Sr

<table>
<thead>
<tr>
<th></th>
<th>$^{88}$Sr</th>
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<th>$^{89}$Sr</th>
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<tbody>
<tr>
<td></td>
<td>Hematocrit (%)</td>
<td>Granulocytes/mm$^3$</td>
<td>Hematocrit (%)</td>
<td>Granulocytes/mm$^3$</td>
</tr>
<tr>
<td>Sham splenectomized</td>
<td>48.5 ± 0.7</td>
<td>2329 ± 147</td>
<td>44.5 ± 1.3</td>
<td>1745 ± 174</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>46.0 ± 0.4</td>
<td>2605 ± 201</td>
<td>29.4 ± 1.2</td>
<td>265 ± 20</td>
</tr>
</tbody>
</table>

Sr injections were administered 3 days after sham splenectomy or splenectomy.

Table 2. Blood Hematocrit (Hct) and Granulocyte/mm$^3$ (PMN/mm$^3$) Values (Mean ± SE) of Mice Injected With $^{89}$Sr or $^{88}$Sr and Sham Splenectomized or Splenectomized 14 Days Later.

<table>
<thead>
<tr>
<th></th>
<th>Day 21</th>
<th></th>
<th></th>
<th>Day 28</th>
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<th></th>
<th>Day 35</th>
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<tbody>
<tr>
<td></td>
<td>Hct (%)</td>
<td>PMN/mm$^3$</td>
<td>Hct (%)</td>
<td>PMN/mm$^3$</td>
<td>Hct (%)</td>
<td>PMN/mm$^3$</td>
<td>Hct (%)</td>
<td>PMN/mm$^3$</td>
<td>Hct (%)</td>
</tr>
<tr>
<td>Sham splenectomized</td>
<td>$^{88}$Sr</td>
<td>45.8 ± 0.1</td>
<td>2540 ± 180</td>
<td>27.6 ± 0.6</td>
<td>3220 ± 230</td>
<td>47.6 ± 0.5</td>
<td>2153 ± 359</td>
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<tr>
<td>Splenectomized</td>
<td>44.6 ± 0.5</td>
<td>2650 ± 114</td>
<td>45.2 ± 0.7</td>
<td>2955 ± 190</td>
<td>44.2 ± 0.4</td>
<td>2535 ± 581</td>
<td></td>
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</tr>
<tr>
<td>$^{89}$Sr</td>
<td>Sham splenectomized</td>
<td>47.2 ± 0.5</td>
<td>2139 ± 272</td>
<td>46.2 ± 0.6</td>
<td>1299 ± 167</td>
<td>45.0 ± 0.8</td>
<td>1680 ± 168</td>
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</tr>
<tr>
<td>Splenectomized</td>
<td>40.4 ± 0.9</td>
<td>1163 ± 182</td>
<td>31.3 ± 1.7</td>
<td>490 ± 82</td>
<td>24.9 ± 1.7</td>
<td>210 ± 19</td>
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</tbody>
</table>

Values were obtained 7, 14, and 21 days after surgery, i.e., on days 21, 28, and 35 after the Sr injections.
Fig. 2: Numbers of CFU-S per ml blood (A) or liver (B) of mice sham-splenectomized ("with spleen") or splenectomized ("without spleen") 14 days after "cold" 88Sr or radioactive 89Sr injection. Day-0 values from normal mice that received no treatment at all, day-14 values from control mice injected with 88Sr (----) or from marrow-ablated mice injected with 89Sr (-----). By day 14 marrow-ablated 89Sr-injected mice had a significant increase in their liver and blood CFU-S (p < 0.001). Day-21, -28, and -35 values from mice injected with either 88Sr or 89Sr on day 0, sham splenectomized or splenectomized on day 14, and evaluated 7, 14, or 21 days after surgery. Splenectomy of 89Sr-injected mice (-----) resulted in an increase in the number of blood and liver CFU-S when compared to levels in sham-splenectomized mice; (----) this increase persisted throughout the observation period. Sham-splenectomized marrow-ablated 89Sr-injected mice had supranormal levels of CFU-S in their blood and liver on day 14 (-----), and in these mice splenectomy (-----) resulted in a dramatic decline in the numbers of blood and liver CFU-S to levels virtually undetectable. 89Sr marrow-ablated splenectomized mice not killed for assay of their CFU-S died with severe pancytopenia. Mean ± SE of three or four replications. Statistical analyses compared data from sham-splenectomized or splenectomized mice within each group. ***p < 0.001.
median survival of the splenectomized mice treated with $^{89}$Sr was 9 days; the first death occurred on day 6, and all mice were dead by day 18.

**Experiment 2 (Table 2, Fig. 2)**

The mice splenectomized 2 wk after $^{88}$Sr injection had only a mild anemia compared to their intact littermates; there was no leukopenia at any of the three times studied (Table 2). The $^{89}$Sr-treated mice, however, developed progressively severe anemia and leukopenia after splenectomy (Table 2).

During the 14 days after treatment with $^{89}$Sr, the numbers of CFU-S in the blood of these mice increased about threefold (Fig. 2 A); this increase was accompanied by a substantial increase in the numbers of CFU-S in their livers (Fig. 2 B). However, when these mice were splenectomized, the numbers of CFU-S in their livers and blood declined dramatically and did not recover (Fig. 2).

**DISCUSSION**

In experiment I we studied mice splenectomized prior to $^{89}$Sr treatment. The results of these studies indicate that the livers of adult mice are much poorer hematopoietic organs than are spleens. The spleens of $^{89}$Sr-treated mice weigh less than 0.2 g, and yet enable $^{89}$Sr-treated mice to maintain respectable values of formed elements in the blood, whereas their livers, which weigh more than 6 times as much as their spleens, are incapable of sustaining hematopoietic function in splenectomized mice treated with $^{89}$Sr. In sham-splenectomized mice, 10 days after treatment with $^{89}$Sr both the blood and the liver CFU-S were strikingly elevated, whereas in splenectomized mice 10 days after $^{89}$Sr treatment CFU-S were virtually undetectable in either blood or liver (Fig. 1). Thus even under severe and sustained hematopoietic stress the liver does not support any detectable proliferation of CFU-S. The CFU-S that we detected in the livers of intact mice treated with $^{89}$Sr were likely trapped from the large numbers of CFU-S in the circulation; these were most probably of splenic origin.

There is the possibility that the reason for the failure to detect proliferation of CFU-S in the livers of the splenectomized $^{89}$Sr-treated animals is that CFU-S are not normally present in the livers of adult mice, but if the livers were first seeded with CFU-S, they could support CFU-S proliferation. To study this possibility, we performed a second experiment, in which we first treated mice with either "cold" $^{88}$Sr or the $^{89}$Sr radionuclide and 14 days later either sham-splenectomized or splenectomized them. The 14-day interval was provided to permit the livers to be seeded by the increased number of circulating CFU-S in the marrow-ablated, spleen-supported, $^{89}$Sr-treated mice. The hepatic seeding by CFU-S was quite striking (Fig. 2 B), yet after splenectomy there was an abrupt fall in the number of hepatic CFU-S (Fig. 2 B). This decline convincingly shows that the normal mouse liver cannot support substantial CFU-S proliferation even after being seeded with CFU-S and in the face of great need for hemopoiesis.

It may be argued that for CFU-S to proliferate in the livers of mice, splenic conditioning of CFU-S is required, hence the marked decline in CFU-S of $^{89}$Sr-treated mice after splenectomy. The increase in hepatic CFU-S in "cold" $^{88}$Sr-treated splenectomized mice would mitigate against this (Fig. 2 B).
The most likely explanation for the increase in the numbers of CFU-S in the livers of the splenectomized mice treated with "cold" \textsuperscript{89}Sr (Fig. 2 B) is that there are more circulating CFU-S (Fig. 2 A) available for liver trapping because the more efficient trapper, the spleen, has been removed.

Testa and Hendry\textsuperscript{4} found an increase in the numbers of hepatic but not blood CFU-S in mice that had received 450 rads whole body irradiation on four separate occasions at 6-wk intervals and concluded that "repeated whole-body irradiation of adult mice induced hemopoiesis in livers as shown by the presence of stem cells (CFU-S) . . . ," findings that seem to conflict with ours. However, the studies of Testa and Hendry do not necessarily support the conclusion that normal livers of adult mice can support the proliferation of normal CFU-S since in their repeatedly irradiated mice the marrow compartments contained about 20 times as many CFU-S as were contained in the livers; the marrow therefore could well have seeded the livers with CFU-S. The absence of an increase in blood CFU-S in their mice may indicate that the livers of their mice successfully cleared the blood of the modest numbers of CFU-S supplied by the irradiated hemopoietic tissues. It should be noted that the maximal numbers of CFU-S in the livers of the mice studied by Testa and Hendry were substantially smaller than those in ours (700 versus 3000); thus the livers of their mice probably could have accommodated many more CFU-S from the circulation. In addition, the repeated near-lethal whole body irradiation may have induced changes in the livers' microenvironment or may have altered the CFU-S themselves (e.g., by inducing mutations).

These are the first reported studies of the capacity of normal livers of mice to support the proliferation of CFU-S in which both the marrow and spleen have been rendered ineffective as producers of CFU-S, the spleen by splenectomy and the marrow by treatment with \textsuperscript{89}Sr. The results of our studies indicate that normal livers of adult mice cannot support detectable amounts of CFU-S proliferation even in mice subjected to severe hemopoietic stress and even if the livers are preseeded with large numbers of CFU-S. In addition, these studies underscore the hazards of evaluating the hemopoietic capacity of a given organ in the face of ongoing hemopoiesis in other organs capable of seeding the former.

**ACKNOWLEDGMENT**

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