Hemopoietic Regeneration in Control and Recovered Heavily Irradiated Dogs Following Severe Hemorrhage

By Victor Perman, Dale K. Sorensen, Edward A. Usenik, Victor P. Bond and Eugene P. Cronkite

Regeneration of the bone marrow following single or repeated doses of radiation appears to be complete as evidenced by a return of blood counts to the normal range. In the present studies, dogs given lethal doses of radiation and successfully treated with maintenance therapy one to two years previously were stressed by severe bleeding, and the regenerative capacity of hemopoietic tissue as evidenced by the rate of recovery of blood counts was compared with control nonirradiated dogs.

Methods

Fifteen recovered irradiated dogs that received either 400, 420 or 460 r of whole body radiation at approximately 27 r/min. along with control dogs were observed for a one to two year period (see refs. 3 and 4 for radiation factors used). Baseline values (separate determinations for each animal) including hemoglobin, hematocrit, red blood cells, reticulocyte, white blood cells, platelets and differential cell counts were established on all dogs three successive days prior to stressing by bleeding. Hemoglobin was determined by the cyanomethemoglobin method; hematocrit values were obtained by the use of a microhematocrit centrifuge; red blood cell and white blood cell counts were made with an electronic cell counter; reticulocytes were stained by the new methylene blue method, and platelet counts were made by the phase contrast method. Blood studies were done daily for the first week and then at intervals during the recovery period.

The dogs were placed under pentothal sodium anesthesia and bled via the femoral artery on three successive days. Twenty per cent of the calculated blood volume was withdrawn at each of the first two bleedings and variable amounts (12 per cent to 20 per cent in most dogs) at the last bleeding.

The diet consisted of two parts Purina Dog Chow and one part canned beef. No supplements were added to the diet during the recovery period.

Results

All dogs tolerated the first two bleedings with only minor changes in clinical condition. During the last withdrawal of blood it was necessary to discontinue bleeding due to severe hypotension in three dogs, which was corrected by the administration of autologous plasma. All dogs were active and alert 24 hours after the last bleeding.

The drop in hematocrit for all dogs ranged from 30 per cent to 52 per cent (table 1) with a mean drop of 37 to 40 per cent for the various groups (fig. 1). The red blood cell and hemoglobin drop (fig. 1) was of the same order as the drop in hematocrit. The lowest red blood cell values were ob-

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Table 1.—Summary of Data Pertaining to Control and Irradiated Groups of Dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Dogs</th>
<th>Weight of Dogs in Pounds</th>
<th>Time in Days Between Irradiation and Bleeding</th>
<th>% Drop in Hematocrit After Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>60.1</td>
<td>49.5-70.5</td>
<td>-</td>
</tr>
<tr>
<td>400 r</td>
<td>9</td>
<td>45.6</td>
<td>26.5-75.5</td>
<td>551-637</td>
</tr>
<tr>
<td>420 r</td>
<td>2</td>
<td>46.0</td>
<td>41.0-51.0</td>
<td>487-496</td>
</tr>
<tr>
<td>460 r</td>
<td>4</td>
<td>42.0</td>
<td>40.5-45.0</td>
<td>331-359</td>
</tr>
</tbody>
</table>

tained on the 3rd day, 24 hours after the last bleeding in most dogs. A rise in red blood cell values on the 4th, 5th and 6th days was followed by a drop on the 7th day, then a steady return to normal values by the 37th day. The first significant rise in reticulocytes was noted in all groups on the 3rd day after the initial bleeding with a peak reticulocyte response occurring on the 6th day.

Platelet and leukocyte responses to bleeding are depicted in figure 2. A rise in the platelet counts in all dogs occurred by the 9th to 11th day, following which the counts gradually returned to near baseline values by the 37th day. The mean platelet values for each group of dogs was nearly double the baseline value during this period. The peak of the leukocyte rise occurred on the 6th day with a return to baseline levels by the 9th day.

DISCUSSION

The clinical response to hemorrhage by bleeding, although apparent in most dogs, was of short duration. All dogs were in good physical condition within 24 hours after the last bleeding. The classical early response to hemorrhage, of a rise in platelets in the first few hours followed by the transitory neutrophilia, was not detected with the sampling schedule employed in this study.

A slight drop in red cell values on the 7th day may be associated with a redistribution of body fluids and is evident in all dogs. The reticulocyte response by the 6th day with a linear return to normal values for red blood cells by the 37th day is consistent with the normal response to external hemorrhage. No difference between control dogs and recovered lethally irradiated dogs was found.

The rise in platelets following bleeding in control and irradiated dogs with peak values by the 9th to 11th day were similar to the results obtained by a platelet depletion technic, and those that occur postoperatively in man. This response is apparently associated with the loss of platelets and the resultant marrow stimulation that occurs.

The rise in leukocytes was significant by the second day and reached peak values for all groups on the 3rd to 5th day after the initial bleeding. The stimulation of the leukocyte count can not be ascribed to depletion, if a large storage compartment is considered, and is similar to the response following leukopheresis. The response appears to be confined uniformly to the neutrophil cells. Although a rise in lymphocytes occurs in some dogs, it is not a uniform response in all dogs and the magnitude of the response does not appear to be significant.
Fig. 1.—Summary of the red blood cell changes in control and recovered heavily irradiated dogs after bleeding.

It appears, then, that the hemopoietic response in control and recovered irradiated dogs to stress by severe bleeding is similar. As measured by erythrocyte, leukocyte and platelet response after bleeding, no difference was detected between control nonirradiated dogs and recovered lethally irradiated dogs.

The question raised is why the simultaneous regeneration of all myelocytic and erythrocytic elements occurs following bleeding, if the response is due to depletion. With erythrocytic and platelet elements, a true depletion or reduction occurs with bleeding; however, with leukocytes, if the storage compartment is considered to be large, the number of leukocytes removed by bleeding may be insignificant. It would appear that the stimulation of all elements occurs simultaneously and that the response to bleeding is a reflection of the time necessary for normal proliferation and production of the various formed elements. This response to bleeding would indicate that a total stimulus to all bone marrow elements occurs following stress by severe bleeding. It would appear that this response might be fruitful in the investigations of factors controlling hemopoiesis in the normal individual.

Conclusion

Dogs which had been heavily irradiated one to two years previously were severely bled. Both control and recovered dogs responded to the bleeding in
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Fig. 2.—Summary of the white blood cell and platelet changes in control and recovered heavily irradiated dogs after bleeding.

a similar manner, indicating complete functional recovery of the previously irradiated marrow.

**SUMMARIO IN INTERLINGUA**

Canes que habeva essite subjicite a forte irradiation un o duo annos previamente esseva subjicite a sever grados de sanguination. Un grupo de canes de controlo e le canes experimental respondeva al sanguination in un simile maniera, lo que indica un complete restablimento functional in le previemente irradiate medulla.

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Victor Bond, D.V.M., Instructor, School of Veterinary Medicine, University of Minnesota, St. Paul, Minn. Research Collaborator, Medical Research Center, Brookhaven National Laboratory, Upton, N. Y.

Dale K. Sorensen, D.V.M., Ph.D., Associate Professor, Division of Veterinary Medicine and Clinics, University of Minnesota, St. Paul, Minn. Research Collaborator, Medical Research Center, Brookhaven National Laboratory, Upton, N. Y.

Edward A. Usenik, D.V.M., Ph.D., Veterinary Clinic Building, University of Minnesota, St. Paul, Minn. Research Collaborator, Medical Research Center, Brookhaven National Laboratory, Upton, N. Y.

Victor P. Bond, M.D., Ph.D., Assistant Department Chairman, Medical Research Center, and Head, Division of Microbiology, Brookhaven National Laboratory, Upton, N. Y.

Eugene P. Cronkite, M.D., Head, Division of Experimental Pathology, Medical Research Center, Brookhaven National Laboratory, Upton, N. Y.
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