BONE MARROW REGENERATION IN EXPERIMENTAL BENZENE INTOXICATION

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BENZENE intoxication is known to affect the hemopoietic system of man and of lower animals. Depending upon the duration of exposure, concentration of the chemical, frequency of administration and the age of the animal, changes of the bone marrow vary from complete aplasia to selective hypoplasia. Since little is known of the regulating mechanism of production and distribution of leukocytes, the action of hemopoietic intoxicants may be utilized for the study of this problem. This work was undertaken from that point of view.

The mechanism by which benzene induces bone marrow changes has not been determined. Two possibilities appear plausible in the present state of our knowledge. The chemical may inhibit cell division or interfere with hypothetic active principles concerned with marrow activity. Cell division may be inhibited either by damage to the nucleus or by the alteration of the lipid-protein medium of the marrow in which the cells are contained. The latter process presupposes that the cell metabolism requires the integrity of the medium.

EXPERIMENTAL PROCEDURE

This study is a morphologic investigation of the effect of benzene on cell division. The following experimental procedures were employed for the evaluation: (1) Marrow of one or more of the long bones was extirpated in living rabbits according to a procedure described previously.1 (2) Various degrees of benzene intoxication were induced in rabbits. (3) The animals were killed at intervals after varying periods of benzene administration. (4) Studies were made of the comparative changes between the extirpated and the controlateral unextirpated marrows. (5) Comparative changes were studied between extirpated marrow of normal animals and those with benzene intoxication. The steps in regeneration of extirpated marrow in normal animals were presented in a previous publication.2

Exirpation of marrow was done by incising the soft tissues at each end of the long bone. In the removal of tibial marrow, the two incisions are preferable. At the narrow end of the bone, the tendons were retracted. At the broad end a cross incision was made to the periosteum. In the case of the femur, humerus, radius and ulna, a single incision from the proximal to the distal end is sufficient. The muscles were separated along fascial lines and were retracted. Muscle injury is the common cause of death of the animals. A single opening was made at the narrow end and four openings at the broad end with a Rake drill. The piece of bone outlined by the four openings was lifted out. A tight-fitting flexible silver cannula was inserted into the single opening. A syringe filled with sterile liquid petrolatum was attached to the cannula. The pressure of the oil separated the marrow and expressed it out of the bone cavity through the larger opening. Occasionally it was necessary to cut each end of the marrow before it could be expressed. The marrow from the epiphyses was removed with a sharp curet and packed first with soft bone wax followed by strips of gauze saturated with wax. The marrow cavity was then cleaned with a pipe cleaner and flushed out with saline (fig. 1). The animal of choice is the rabbit. It is the largest of the animals with a tubular marrow and lends itself for hematologic studies. The animals were anesthetized with pentobarbital sodium. The hair was removed with a depilatory preparation and the leg was wrapped with cotton saturated with an antiseptic. It is essential that the surgical procedure be carried out under strict aseptic precautions.

Forty marrows were extirpated in 30 rabbits in this study. The age of the animals ranged from 3 to 11

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FIG. 1.—Removal of Marrow from a Long Bone of a Living Rabbit

Holes are drilled in the bone with a Ralk nail drill. At one end of the bone a single hole is made (a in A). At the opposite end, four holes are made, and the central bone spicule is removed, leaving a large opening (b in A). A flexible silver cannula is inserted into the single hole (a in B), and with a syringe containing oil or water the marrow is expressed through the larger opening (b in B). Both openings are then sealed with bone wax (a and b in C).
months. The animals were given 1 cc. of a mixture of 5 parts of benzene to 1 part of olive oil subcutaneously one to two times daily. The number of injections was varied for each animal (see table 1). Peripheral leukocyte counts were done one or more times daily. The animals were killed at intervals of 3 to 81 days after extirpation of marrow. Studies were made of the comparative changes of the regenerated marrow after fixation in formaldehyde or Bouin's solutions and staining with hemotoxylin-eosin or Giemsa preparations.

RESULTS

Regeneration of Normal Marrow

For a clearer understanding of the comparative changes, the steps in the regeneration of normal marrow are restated. The earliest significant manifestation, which appears in about nine days, is a sprouting of sheets of primitive reticular cells and bone trabeculae from the endosteum. The next step is the formation of fat cells. This process takes place probably by a coalescence of two or more primitive reticular cells after their cytoplasm is replaced by lipids. Fat cells continue to form for sixty days, but their formation is most active and profuse in the first twenty days after extirpation. Islands of myeloid tissue begin to appear in about nine days and increase progressively in number. Regeneration does not proceed uniformly throughout the bone marrow. In sixty days, most of the marrow has returned to a normal number and distribution of myeloid tissue (fig. 2).

Regeneration of Marrow in Benzene Intoxication

The quantity of benzene and the number of injections were varied. Some animals received relatively little of the chemical over a period of a few or many days. Other rabbits were injected almost daily and received a total large quantity of benzene (see table 1). There was a distinct correlation between the degree of intoxication and the appearance of the bone marrow. In severe poisoning, regeneration did not proceed further than the stage of sprouting of primitive reticular cells. There was some attempt to form fat cells, but they were few and atrophic or rudimentary. Whenever an occasional fat cell did develop, it was followed first by proliferation of a few megakaryocytes and then by an infrequent small focus of erythroblasts.

Even after a period of eighty-one days, those animals which received benzene continuously showed a state of marrow response comparable only to the first phase of normal marrow regeneration. Granulocytes did not make their appearance unless a considerable number of fat cells developed and not until both megakaryocytes and cells of the erythrocytic series were present in moderate numbers. A decreasing degree of intoxication was associated with formation of fat cells and myeloid activity. With a relatively small quantity of benzene, fat cells and myeloid tissue was in considerable evidence in twenty-one days after extirpation of the marrow. When benzene administration was stopped, the marrow in the extirpated bone proceeded to develop fat cells, whereas in the intact contralateral marrow, myeloid hemopoiesis would set in.

The significant changes in these experiments consist in the inability of the marrow to regenerate past the primitive reticular cells and the apparent dependence of myeloid activity upon presence of fat cells.
Fig. 2.—A, B AND C: REGENERATION OF EXIRPAD MARROW IN NORMAL RABBITS

A: 9 days after extirpation. Bone trabeculae and sheets of primitive reticular cells apparently derived from the endosteal layer of bone and from the trabeculae. X 100

B: 20 days after extirpation. Formation of fat cells. Intermediate forms of primitive reticular cells and erythroblasts are in the field. X 720

C: 30 days after extirpation. Islands of myeloid tissue composed of granulocytes and erythroblasts. An occasional area of primitive reticular cells. X 720

D: Normal active marrow of a rabbit for comparison. X 540
Fig. 3.—A and B: Regeneration of Extirpated Marrow in Severe Benzene Intoxication

A: 54 days after extirpation. There are indistinct sheets of primitive reticular cells, rudimentary fat cells and megakaryoblasts. ×720

B: 81 days after extirpation. There are sheets of primitive reticular cells, bone trabeculae and rudimentary fat cells but no myeloid activity. ×720

C: Intact marrow of same animal as in A. There are atrophied fat cells and erythroblastic activity. ×540

D: Intact marrow of same animal as in B. The fat cells are atrophic. There is no myeloid activity. ×540
Effects of Benzene Intoxication on Intact Bone Marrow

The intact bone marrow in benzene intoxication will be described only in so far as it helps to clarify the picture of regeneration. Whenever possible, comparisons were made with marrow from controlateral extirpated bones. Marrow from

TABLE I.—Relationship of Degree of Benzene Intoxication to Bone Marrow Regeneration

<table>
<thead>
<tr>
<th>Period of marrow regeneration</th>
<th>Extent of benzene administration</th>
<th>Range of WBC per cu. mm. of blood during benzene administration</th>
<th>State of the regenerated bone marrow in benzene intoxication</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>9 cc. of benzene for 6 days</td>
<td>1800 to 422</td>
<td>No myeloid cells; few fat cells; sheets of primitive reticular cells and bone trabeculae.</td>
</tr>
<tr>
<td>19</td>
<td>21 cc. of benzene for 11 days</td>
<td>2600 to 420</td>
<td>No myeloid cells; no fat cells; sheets of primitive reticular cells and bone trabeculae.</td>
</tr>
<tr>
<td>21</td>
<td>10 cc. of benzene for 7 days</td>
<td>3900 to 1110</td>
<td>Considerable myeloid activity; many fat cells and an occasional sheet of primitive reticular cells.</td>
</tr>
<tr>
<td>30</td>
<td>34 cc. of benzene for 19 days</td>
<td>8900 to 1300</td>
<td>Few areas of myeloid regeneration, largely erythroblastic; few fat cells; extensive sheets of primitive reticular cells and bone trabeculae.</td>
</tr>
<tr>
<td>30</td>
<td>10 cc. of benzene for 7 days</td>
<td>8160 to 1100</td>
<td>Considerable myeloid activity; many fat cells; few areas of primitive reticular cells.</td>
</tr>
<tr>
<td>30</td>
<td>46 cc. of benzene for 30 days</td>
<td>5700 to 1000</td>
<td>No myeloid regeneration; infrequent rudimentary fat cell; extensive sheets of primitive reticular cells and bone trabeculae.</td>
</tr>
<tr>
<td>35</td>
<td>30 cc. of benzene for 15 days. No benzene for 10 days prior to extirpation, 5 days during experiment and 5 days before death</td>
<td>6600 to 1086</td>
<td>No myeloid cells; few rudimentary fat cells; extensive sheets of primitive reticular cells and bone trabeculae.</td>
</tr>
<tr>
<td>54</td>
<td>93 cc. of benzene for 51 days</td>
<td>7900 to 1850</td>
<td>An occasional megakaryoblast and erythroblast; small number of poorly formed fat cells; sheets of primitive reticular cells; and bone trabeculae.</td>
</tr>
<tr>
<td>81</td>
<td>157 cc. of benzene for 70 days</td>
<td>9950 to 3100</td>
<td>Few areas of megakaryocytes; few rudimentary fat cells; sheets of primitive reticular cells and bone trabeculae.</td>
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the humerus, femur, radius, ulna and occasionally the ribs was studied. Sheets of primitive reticular cells were not found in any of the marrow even after eighty-one days of benzene administration. The fat cells remained intact in most instances up to fifty-four days. They became atrophic and the nuclei migrated from the periphery to the center of the fat cell in eighty-one days. In some instances of severe in-
toxication in very young animals, these changes were observed at the end of thirty days. Myeloid activity became progressively reduced. The erythrocytic series persisted the longest. In twenty days, a megakaryocytic hyperplasia frequently took place. In fifty-four days, islands of erythroblastic activity, a few myeloblasts and megakaryocytes persisted in many instances. The aplasia was complete in eighty-one days in all animals with fairly persistent administration of benzene.

Summary

Benzene intoxication was induced in a number of rabbits in order to study the factors which regulate production of leukocytes. One or more of the marrows of long bones was completely extirpated. After varying intervals, the animals were killed. The regenerated marrow was studied. Comparisons were made with regeneration in normal animals and with the intact marrow of animals treated with benzene. The appearance of regenerated and intact marrow was correlated with the degree of benzene intoxication.

In normal animals, regeneration of extirpated marrow proceeded in a more or less sequential order. At first there was formation of sheets of primitive reticular cells and bone trabeculae, followed by fat cells and then by myeloid tissue. In severe benzene intoxication, the extirpated marrow regenerated only to the point of formation of primitive reticular cells. Further regeneration was halted at the point of formation of fat cells. Myeloid activity appeared to be predicated upon the presence of fat cells. At least, one part of the mechanism by which benzene induces aplasia of bone marrow is probably the inhibition of cell division and maturation past the level of the primitive reticular cell.

REFERENCES

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