Induction of Sustained Hemopoiesis in Fatty Marrow

By Mehdi Tavassoli, Alice Maniatis, and William H. Crosby

Evacuation of the fatty marrow cavity results in a sequence of regeneration which is associated with a phase of hemopoiesis. Hemopoietic activity is, however, transient, being replaced by adipose tissue, so that 6 mo after evacuation the marrow is once again fatty. This phase of transient hemopoiesis can be sustained by phenylhydrazine-induced hemolytic stress. Hemolysis, per se does not induce significant hemopoietic activity in nonevacuated totally fatty marrow.

STEINBERG AND HUFFORD\(^1\) demonstrated that hemopoiesis was reestablished after hemopoietically active marrow cavities were evacuated. Their observation has been confirmed by others.\(^2\)-\(^4\) Several investigators have attempted to exploit this finding by curetting the marrow cavities in patients with marrow failure in an effort to induce hemopoiesis.\(^5\)-\(^7\) Most of these studies involved the evacuation of marrow cavities known to be hemopoietically active under normal conditions (red marrow). This approach must take into account the fact that in man, as well as many other species, the marrow contained within the readily accessible long bone cavities undergoes transformation into hemopoietically inactive fatty marrow shortly after birth. If evacuation of fatty marrow was followed by the establishment of active hemopoiesis, a new approach to the treatment of some forms of aplastic anemia would be opened. The present study was undertaken to examine this approach by studying the sequence of events that follows the evacuation of fatty marrow in rabbits.

MATERIALS AND METHODS

Twenty-eight New Zealand albino rabbits, approximately 6 mo of age, were used. The animals were maintained under normal laboratory conditions (ambient temperature 25°C). Intravenous sodium pentobarbital was used for anesthesia, and all operations were carried out in an operating room under sterile conditions.

Evacuation of the Bone Marrow Cavity

The anterior aspect of one tibia was exposed through a skin incision. Using a low-speed dental drill, an opening was made into the marrow cavity through which a polyethylene tube was inserted. The free end of the tube was then clamped and the tube, now containing marrow tissue, was withdrawn. This procedure was repeated several times until all the marrow tissue was removed. In some animals the endosteum was then curetted, using a dental broach, and the marrow cavity was irrigated with normal saline to ensure complete evacuation of the marrow cavity. The marrow tissue, thus removed, was used to prepare smears and a portion fixed for histologic sections. Animals were killed at 2, 7, and 10 days after operation (two animals on each occasion), others at

From L. C. Jacobson Blood Center, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037.


Supported by USPHS Grant AM 16501-01 from the National Institute of Arthritis and Metabolic Diseases and Atomic Energy Commission Contract AT (04-3) 899.

Mehdi Tavassoli, M.D.: L. C. Jacobson Blood Center, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037. Alice Maniatis, M.D.: Director, Blood Bank, St. Luke's Hospital Center; Assistant Professor of Medicine, Columbia University College of Physicians and Surgeons, New York, N.Y. 10025. William H. Crosby, M.D.: Director, L. C. Jacobson Blood Center, Head, Division of Hematology, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037.

© 1974 by Grune & Stratton, Inc.
weekly intervals for 6 wk (two animals at each interval, apart from a 2-wk interval when six animals were studied) and the remainder at 2, 3, and 6 mo (two animals on each occasion). Both the evacuated and the normal tibias were removed and fixed in 10% buffered formalin. After decalcification, longitudinal sections through the marrow cavities were obtained and stained with hematoxylin and eosin.

Induction of Hemolysis

To stimulate hemopoiesis, hemolysis was induced in two animals by subcutaneous injection of phenylhydrazine hydrochloride (5 mg/kg body weight) three times a week for the duration of the study. The first dose was given 2 wk after evacuation of marrow cavity. Initially the animals became slightly anemic (hematocrit 32%-35%) but within a week, the hematocrit returned to baseline and a reticulocytosis of 15%-20% persisted.

RESULTS

The marrow removed from the tibial cavity was primarily adipose tissue, easily recognizable on gross examination by its yellowish color. Touch imprints stained with Wright's and Giemsa showed no hemopoietic cells. Sections of the tissue consistently showed fatty marrow devoid of hemopoietic cells (Figs. 1A and 2A).

Regeneration of Marrow After Evacuation

Two days after evacuation, the content of the marrow cavity consisted mainly of blood clot (red cells enmeshed within strands of fibrin). Seven days after evacuation, clot was still present in the central part of the tibia, but in the peripheral parts (adjacent to cortical bone) primitive mesenchymal tissue (fibroblasts) was seen penetrating the clot. The tissue contained many small blood vessels having the appearance of granulation tissue. New bone formation was occasionally observed within the fibroblastic tissue. By day 10 postoperatively, the central clot was smaller and most of the cavity was occupied by fibroblastic tissue. Osteoid bone was now more evident, particularly at the periphery of the tibial cavity, adjacent to the tubular bone. At 2 wk, the clot was virtually replaced by fibroblastic tissue and the peripheral area occupied by osteoid bone interspersed by loose connective tissue. After 3-4 wk, most of the marrow cavity was occupied by osteoid bone, and within its interstices hemopoietic tissue was evident, including erythropoietic and granulopoietic cells as well as megakaryocytes (Fig. 1B). In some sections, developing adipose cells could also be seen, apparently replacing hemopoietic tissue. By 5-6 wk, some osteoid bone was still present, but most of the marrow cavity now consisted of mature or developing adipose cells (Fig. 1C). In some areas, it now appeared similar to the control tibia from the opposite side. Two to six months after evacuation, no hemopoietic tissue could be seen, the content of the tibia again being adipose tissue (Fig. 1D) with an occasional area of dense fibrous tissue.

The Effect of Hemolysis

The two animals, subjected to phenylhydrazine-induced hemolysis, were explored at 5 wk and 6 mo, respectively, after evacuation of the marrow cavity. Five weeks after operation, the marrow consisted of hemopoietic tissue (erythropoietic and granulopoietic cells as well as megakaryocytes) interspersed with areas of osteoid bone (Fig. 2B). No mature or developing adipose cells were seen. Hemopoiesis was decidedly more intense than in comparable specimens from animals not subjected to hemolytic stress. The control tibia in this phenylhydrazine-treated animal showed no
Fig. 1. The sequence of regenerating events following evacuation of fatty marrow cavity. (A) Normal tibial cavity in the rabbit contains adipose tissue; no hemopoiesis is seen. (B) Four weeks after evacuation, osteoid bone is interspersed with loose connective tissue, the latter containing large vascular channels and some hemopoietic cells. Hemopoiesis is transient, however, and 6 wk after evacuation (C) is replaced by developing adipose cells, so that 6 mo after evacuation (D) fatty marrow is again established (all figures X 100).
Fig. 2. This sequence of regenerating events in the fatty marrow cavity is taken from rabbits subjected to phenylhydrazine-induced hemolytic stress. (A) Normal tibial marrow, prior to evacuation and phenylhydrazine treatment, primarily contains adipose tissue. (B) Five weeks after evacuation, osteoid bone is seen interspersed with hemopoietic tissue. Hemopoiesis is more intense than that observed in comparable experiments in which phenylhydrazine was not given. (C) Six months after evacuation, intense hemopoiesis is sustained, only a few adipose cells are seen. (D) The opposite (nonevacuated) tibia from the same animal shows only minimal hemopoietic activity. (A and D, X 320; B and C, X 100.)
increase in hemopoietic activity but contained adipose tissue similar to that seen in the control tibias of untreated animals. After 6 mo, the second animal showed persistence of intense hemopoiesis (all three cell lines included) in the evacuated tibia (Fig. 2C), and the opposite tibia showed occasional clumps of hemopoietic cells interspersed amongst adipose tissue (Fig. 2D).

**DISCUSSION**

The sequence of histogenetic events culminating in the reestablishment of hemopoiesis after evacuation of hemopoietic marrow cavity is essentially the same sequence that leads to regeneration of marrow tissue after autotransplantation to ectopic sites. Furthermore, both processes are similar to the embryogenesis of the marrow tissue: Primitive mesenchymal tissue leads to the formation of osteoid bone in whose interstices the marrow microvasculature develops and in turn supports hemopoiesis which expands leading to the resorption of osteoid bone. When extramedullary autotransplants of fatty marrow are made, this process goes one step further—replacement of hemopoietic tissue by developing adipose cells resulting in the establishment of an extramedullary fatty marrow nodule. The transient nature of the hemopoiesis observed after evacuation of fatty marrow in this study was anticipated in the light of previous observations on the fate of extramedullary autotransplants of fatty marrow. Intramedullary regeneration of fatty marrow is, however, a slower process, and the transient hemopoiesis observed is less intense than that seen in extramedullary implants of fatty marrow.

We have previously shown that phenylhydrazine-induced hemolysis, per se, is not capable of inducing significant hemopoiesis in totally aplastic (fatty) marrow in rats, even when normal marrow becomes strikingly hypercellular. The present study confirms this observation in rabbits, as the nonevacuated tibias did not show significant hemopoiesis. The combination, however, of evacuation and phenylhydrazine-induced hemolysis did lead to sustained active hemopoiesis in the evacuated fatty marrow cavities and demonstrated the ability of the newly regenerated marrow to respond to increased demand for hemopoiesis as does normal hemopoietic marrow.

**ACKNOWLEDGMENT**

We are indebted to Dr. Peter Sacks for his help during the preparation of this manuscript and to Mrs. Bonnie Winger for expert secretarial assistance.

**REFERENCES**

Induction of Sustained Hemopoiesis in Fatty Marrow

Mehdi Tavassoli, Alice Maniatis and William H. Crosby