Bone Formation and Resorption as a Requirement for Marrow Development[1] (36426)

HARVEY M. PATT AND MARY A. MALONEY
Laboratory of Radiobiology, University of California, San Francisco, California 94122

Is the connection between bone and marrow merely a matter of proximity or common ancestry, or is it a functional necessity? It is known that hematopoiesis occurs independently of bone in lower vertebrates, in fetal liver of higher vertebrates, and in various pathologic states. Yet, in higher vertebrates, osteogenesis is apparently a prelude to the development of a new sinusoidal marrow when marrow is disrupted (1), removed precipitously from a medullary cavity (2–5), or implanted in an extramedullary locale (6). Indeed, this seems to be a frequent phenomenon; wherever true bone is formed irrespective of the initiating mechanism, it generally leads to a new bone marrow (7). In the course of recent studies of bone marrow regeneration, we have had an opportunity to examine the evolution of bone and marrow in heterotopic autologous implants. Our findings are consistent with the thesis that bone can play a necessary role in the *de novo* formation of marrow in a mammalian organism, irrespective of whether there is an obligatory functional relationship between the two under normal conditions.

Materials and Methods. Marrow was obtained under sterile conditions from the surgically exposed femur of an anesthetized young adult rabbit. The medullary tissue was removed by a curette inserted into the shaft through a 3 mm opening made by a bone punch. It was then implanted without dispersal into a subcutaneous pocket in the groin of the same animal. The marrow implant, which ranged from 75 to 150 mm^3, was devoid of obvious bone as shown by histologic examination. The animals were sacrificed 35 to 40 days after implantation. If growth had occurred, the tissue in the implantation site was removed and sectioned after neutral formalin fixation. Sections were treated with either Harris' hematoxylin and eosin or Mallory's aniline blue collagen stain. Microscopic analysis consisted of: (a) determination of the presence of bone and marrow and their extent, and (b) estimation of ossicle and medullary cavity volume. A calibrated ocular micrometer was used to determine the long and short axes of clearly delineated osseous tissue and any associated medullary cavity. In the few instances where more than one ossicle or island of bone was seen in a section, only the largest was scored. Similarly, only the largest cavity, usually centrally located, was recorded in the few cases where several cavities appeared in a given ossicle. For volume calculation, it was assumed that the bone and its major cavity approximated an ellipse.

Results and Discussion. Osseous tissue and marrow elements were seen in 92 and 82%, respectively, of 50 implantation sites. These frequencies are consistent with earlier results (8). Of the 46 implantation sites with bone, 11% revealed no hemic elements, 19% presented only hemocytoblasts, *i.e.*, undifferentiated round cells, 11% hemocytoblasts and scattered hematopoietic islands, 20% a clearly defined but hypocellular sinusoidal marrow, and 39% normal appearing sinusoidal marrow. Significantly, marrow elements were never detected in the absence of bone. Rather, such elements were always located within a developing cavity toward the center of the osseous tissue.

The volume of discrete bone foci inclusive of any cavity as measured in the microscopic sections ranged from 10^{-3} to 10^2 mm^3. This wide range far exceeds the approximately

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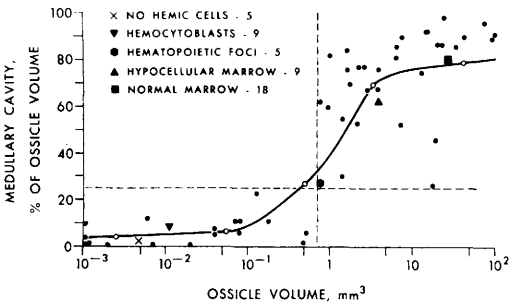

FIG. 1. Relationship of medullary cavity and ossicle volume to marrow development. The sigmoidal curve is derived from the mean volume of osseous tissue over each decade and the corresponding mean medullary cavity percentage. Individual cases (●) and means are designated by (○). Horizontal and vertical broken lines represent minimal medullary cavity and ossicle volumes, respectively, which are associated with a sinusoidal marrow. The various stages of marrow development and number of cases are shown in figure inset.

twofold range in size of the initial marrow implant. Fortunately, it provides a basis for ascertaining possible relationships between development of the ossicle and its medullary cavity and the development of new marrow, even though all determinations were made at one time after heterotopic implantation. Figure 1 depicts the evolution of a medullary cavity as a function of ossicle volume, which is shown on a log scale for convenience. When the mean relative medullary cavity size is plotted against the mean ossicle volume for each decade (*i.e.*, 10^{-3} to 10^{-2} mm³, *etc.*), the overall pattern is sigmoidal. Apparently, a critical volume of bone must be laid down before bone resorption, *i.e.*, cavity formation, greatly exceeds bone formation. This volume corresponds to about 10^{-1} mm³. It is noteworthy that the medullary cavity represents approximately 70% of the ossicle when its volume is 3 to 4 mm³, after which there is comparatively little further increase even with ossicles of 50 to 100 mm³.

An organized hematopoietic tissue was not seen in 17 cases where the medullary cavity volume represented less than 25% of the developing ossicle. But it was seen in 27 of 29 cases when the cavity volume was 25% or more of the total. The corresponding limiting ossicle volume from the calculated sigmoidal curve was about 0.5 mm³, which is in fairly close agreement with the actual limiting volume of 0.7 mm³. A more striking correlation between medullary cavity growth and the genesis of marrow is also shown in Fig. 1. It is noteworthy that the mean relative medullary cavity size and ossicle volume for each stage of marrow development from scattered hemocytoblasts to a sinusoidal structure conform closely to the sigmoidal curve. Thus, hematopoietic foci become evident during the period of rapid bone resorption and a sinusoidal marrow appears as medullary cavity volume approaches an asymptote relative to ossicle volume. It should be emphasized that the salient factor in this relationship is the fraction of an ossicle which is represented by a medullary cavity. A fully developed marrow tissue may occur in a small or large ossicle provided that its main cavity constitutes a substantial proportion of the total volume.

Apropos of the cellular interplay in the growth of a heterotopic implant, it appears that the bone which forms in the implantation site is donor-type, that is, derived from cells present in the initial marrow implant (9). Chromosome marker studies indicate that the hematopoietic cells associated with newly formed bone can have a similar origin (10). It is also known that the regeneration of marrow in a mechanically depleted medullary cavity is basically a local phenomenon, dependent presumably on mesenchymal elements derived from the adjacent bone (11, 12). Viewed in developmental perspective, it is not surprising that bone contains cells capable of hematopoiesis and that marrow contains cells capable of osteogenesis (13).

It is of interest to recall that during embryogenesis there is a rapid formation of primitive marrow in bone resorption centers which give rise to a haversian system and ultimately to a medullary cavity. Thus, it is also not surprising that new marrow should evolve in intimate association with the phase of rapid bone resorption in a heterotopic implant or for that matter in an evacuated medullary cavity. Although the reason for this connection is unknown, there are two

obvious possibilities which are not mutually exclusive: (a) processes of bone resorption contribute to the formation of an appropriate microenvironment, and (b) the onset of rapid bone resorption provides a source of appropriately modulated progenitor cells. Branemark *et al.* (4) and Amsel *et al.* (1) have suggested that short-lived trabecular bone plays a role in the reconstruction of marrow sinuses and reticulum. Support for the latter may be adduced from our current studies with tritiated thymidine labeling, the results of which are also consistent with the hypothesis that cells freed from bone lacunae as trabeculae resorb can serve as hematopoietic progenitors. Although bone formation and resorption can lead to the *de novo* formation of marrow, such intermediation is not always a necessary condition for hematopoiesis. Hematopoietic foci can develop independently of bone in various tissues through colonization or in a tissue such as spleen which already contains the requisite progenitors.

Summary. The formation of a new sinusoidal marrow in a heterotopic autologous marrow implant requires the intermediation of bone. This conclusion follows from the intimate association of developing marrow tissue and newly formed bone in the implantation site. It has been shown that there is a sigmoidal relationship between the evolution of a medullary cavity and ossicle volume. Moreover, the mean relative medullary cavity size and ossicle volume for each stage of marrow development from hemocytoblasts to a sinusoidal structure conforms to this relationship. Hematopoietic foci emerge during the period of rapid bone resorption. A fully developed marrow may occur in a small or large ossicle provided that the medullary cavity represents a critical fraction of the total volume. It is suggested that processes of bone resorption may contribute to the formation of an appropriate microenvironment and/or provide a source of appropriately modulated progenitor cells. Significantly, bone plays a necessary role in the genesis of an organized marrow in an evacuated medullary cavity as well as in a heterotopic marrow implant.

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