Increased Mast Cells in Regenerating Marrow of Normal and Hypophysectomized Rats[1] (35029)

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Since the classic studies of Röhlich, it has been known that mechanical removal of hematopoietic marrow is followed by a series of events in the medullary cavity leading to complete regeneration of hematopoietic marrow containing all elements and morphologically indistinguishable from normal marrow (1). The role of bone (2), circulatory anatomy and vascular regeneration (3), and the source of contributing cellular elements (4) in the regenerative process have been the source of several recent investigations.

In the course of studies of factors influencing the rate of regeneration of hematopoietic marrow following mechanical removal from one femur, it was observed that in both normal and hypophysectomized rats the proportion of mast cells in the regenerating marrow was 8 times (av) that of the unoperated contralateral femur. Because the nature of these ubiquitous cells remains mysterious, their behavior under this experimentally induced condition seemed worthy of reporting.

Materials and Methods. Experimental animals consisted of two groups of female rats of the Long-Evans strain. One group was hypophysectomized at 28 days of age. The other group were normal rats of the same age. At 31 days of age both groups were subjected to endosteal curettage of the left femur, entering from the distal end with a No. 54 twist drill. This size drill just fit the cavity of the shaft, completely disrupting and removing marrow as well as curetting the inner surface of the bone. At the wider proximal and distal ends some hematopoietic marrow remained. The unoperated contralateral femurs served as controls.

On various days up to 7 months after curettage rats were killed to determine the extent of regeneration. Both femurs were removed, the midshaft segments were fixed in Bouin's, and decalcified in DECAL (Decal Chemical Corporation, Pomona, New York). Paraffin sections were cut at 6 μ and stained with alum hematoxylin and eosin. Alternate sections were stained with toluidine blue for identification of mast cells. Completeness of hypophysectomy was determined at autopsy by examination of the pituitary site. The extent of regeneration was estimated qualitatively from the microsocpic sections on the basis of recovery of cellularity and disappearance of fibrous tissue and bone callus.

In the sections stained with toluidine blue the number of mast cells per 400 $\times$ field were counted in 12 fields. The number of mast cells was expressed as percentage of total marrow cells based on an estimated 800 total cells/field, and 270 for marrow in the early post operative period containing extensive intramedullary bone callus. The estimates of total cellularity were obtained from hematoxylin stained adjacent sections.

Electron microscopy was done to confirm the histochemical (toluidine blue) identification by morphological criteria. One hypophysectomized rat was killed for this purpose 3.5 months after curettage. The marrow was extruded from the central segment of the operated femur and prefixed in 4% glutaraldehyde. Half of the marrow was then fixed in acetic acid containing alcian blue and the rest in osmium tetroxide. The fixed tissue was embedded in epon and cut at 600–800 Å.

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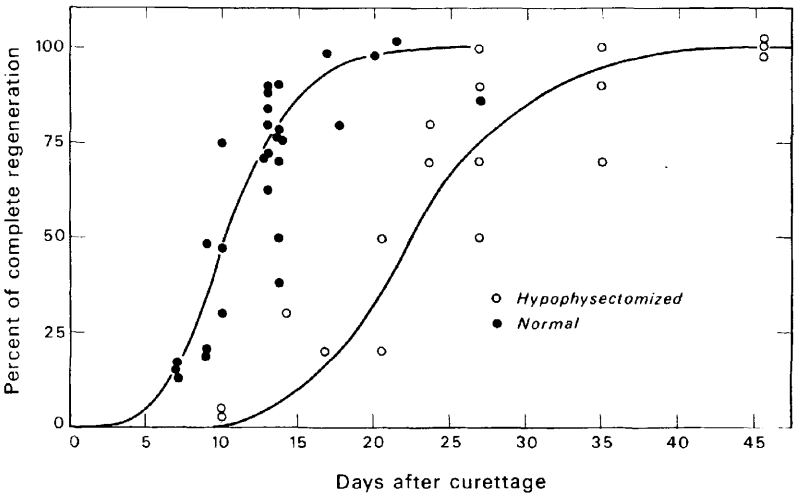

FIG. 1. Rates of marrow regeneration after mechanical removal by endosteal curettage; comparison of hypophysectomized and normal rats.

Some sections were poststained with uranyl acetate and lead citrate. Thick epon sections (1–2 μ) of this tissue were also cut and poststained with toluidine blue for examination by light microscopy to confirm that the number of mast cells in this animal was representative.

Autoradiographs were made using tritiated thymidine to determine whether the mast cell population was rapidly proliferating at the operative site. 71 μCi of tritiated thymidine were given intravenously to one hypophysectomized rat 6.5 months after curettage. One hr later the animal was killed and midsegments from both operated and unoperated femurs, as well as sections of small intestine and thymus, were prepared for paraffin sections. Paraffin was removed from the sections, and they were dipped in Kodak nuclear track emulsion type NTB2. After exposure for 2 weeks they were developed and stained with toluidine blue.

Results. Marrow regeneration followed the pattern first observed by Röhlich (1) and since, by many others (2, 5–7). The clot was replaced by connective tissue and a syncytium of new bone. This bone was gradually resorbed and simultaneously replaced with morphologically normal marrow. Recovery, based on qualitative estimates of cellularity, was 100% at 20 days for normal rats and at 40 days for hypophysectomized rats (Fig. 1). The change in mast cell numbers with time, relationship between curetted and unoperated femurs, and extent of marrow regeneration are summarized in Table I.

In marrow of the unoperated femurs of normal rats mast cells were scarce (av 0.06% of total marrow cells), and showed no change with time. In regenerating marrow of the curetted femurs of these rats they increased to 0.65% 21 days after curettage which was as long as the normal rats were followed.

In hypophysectomized rats, in contrast to the normals, the relative numbers of mast cells in both regenerating and undistrubed marrow increased with time. During the first 3 months mast cells in marrow of the unoperated femurs varied between 0.01–0.49%; after the third month, between 0.28–1.60%. In regenerating marrow of the curetted femurs the increase was much greater, and by the middle of the second month after curettage mast cells constituted nearly 5% of the marrow cells. From 3–7 months mast cells gradually decreased but were still more numerous than in undisturbed marrow.

The frequency of appearance of morphologically typical mast cells in electron micro-

TABLE I. Mast Cell Numbers and Rate of Marrow Regeneration.

Rats	Days post-curettage	Estimated % recovery on the basis of cellularity	Mast cells as % of total marrow cells	
			Unoperated side	Operated side
Normal	7	15	0.05	0.10
	9	30	0.09	0.41
	13	83	0.04	0.47
	21	100	—	0.65
Overall av			0.06	0.48
Hypophysectomized	10	5	0.06	0.40
	14	30	0.03	1.1
	17	20	0.13	2.5
	21	35	0.20	2.9
	27	66	0.01	2.2
	35	87	0.26	4.5
	46	100	0.49	4.8
	83	100	0.02	3.4
	133	100	1.60	2.9
	137	100	0.19	2.2
	186	100	0.88	3.0
	229	100	0.28	0.59
Overall av			0.35	2.7

graphs confirmed their abundance as observed by light microscopy (toluidine blue stained sections).

Labeled mast cells were not observed 1 hr after administration of tritiated thymidine. Many other marrow cells and also cells of the gut and thymus were labeled.

Discussion. Our data indicate that in normal rats, during regeneration of marrow following mechanical disruption, the proportion of mast cells in the recovering marrow is increased 8 times (av). An even greater increase takes place in hypophysectomized rats and persists longer than 7 months. This is a percentage increase in mast cells, but it also reflects an absolute increase in mast cell numbers. The proportions of mast cells in regenerating marrow are higher than can be accounted for by decreased total cellularity, and are still higher than in untouched marrow when cellularity reaches 100% (Table I).

The significance of these observations is not known, and the relationship of mast cells to marrow regeneration and hypophysectomy remains merely a numerical correlation without interpretation.

One interesting possibility is that mast cells may be involved in the bone resorption that is a part of marrow regeneration. Recent evidence from tissue culture experiments (8–10) and studies of patients receiving heparin therapy (9) indicate that heparin potentiates bone resorption; and mast cells are known to contain heparin (11).

The possibility of such a role for mast cells has been suggested by Frame and Nixon (12). In a study of osteoporosis of aging they correlated marrow mast cell numbers with bone density of patients and found increased marrow mast cells in 68% of patients with osteoporosis but in only 11% of those with normal bone density. They postulate that heparin produced by marrow mast cells may be involved in the bone resorption in certain kinds of osteoporosis (12).

Another interesting association of bone resorption and mast cell increase was found by Urist and McLean (13) during a study on calcium deficient rats. Young rats maintained on a diet deficient in calcium and vitamin D developed a combination of rickets

osteoporosis and osteitis fibrosa; large numbers of mast cells accumulated in the endosteum (13).

This report describes one more situation in which remodeling of bone is accompanied by increased mast cells.

The failure of mast cells to become labeled with tritiated thymidine was interpreted to mean that the mast cell population was not rapidly proliferating at that time (6.5 months postcurettage). It suggests that the increase was due to migration, but a burst of rapid *in situ* proliferation at some earlier postoperative time is also a possibility. The work of Padawer (14), Walker (15), and Allen (16) indicates that mast cells can be labeled with tritiated thymidine (14, 15), and that they do undergo mitosis in adult animals but infrequently (15, 16). Our observation is consistent with this normally slow turnover.

Summary. In both normal and hypophysectomized rats femoral marrow regenerating after mechanical disruption was found to contain a proportion of mast cells 8 times as high as marrow in the unoperated contralateral femur. In hypophysectomized rats this increase resulted in peak mast cell densities of 4.8%, and persisted for over 7 months.

1. Röhlich, K., Z. Mikrosk.-Anat. Forsch. **49**, 425 (1941).

2. Mital, M., and Cohen, J., Clin. Orthop. Relat. Res. **61**, 129 (1968).

3. Knospe, W. H., Blom, J., and Crosby, W. H., Blood **28**, 399 (1966).

4. Friedenstein, A. J., Petrakova, K. B., Kurolesova, A. I., and Frolova, G. P., Transplantation **6**, 230 (1968).

5. Van Dyke, D., and Harris, N., Blood **34**, 257 (1969).

6. Maloney, M. A., and Patt, H. M., Cell Tissue Kinet. **2**, 29 (1969).

7. Steinberg, B., and Hufford, V., Arch. Pathol. **43**, 117 (1947).

8. Asher, J. D., and Nichols, G., Jr., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **24**, 211 (1965).

9. Griffith, G. C., Nichols, G., Jr., Asher, J. D., and Flanagan, G., J. Amer. Med. Ass. **193**, 91 (1965).

10. Goldhaber, P., Science **147**, 407 (1965).

11. Benditt, E. P., and Lagunoff, D., Progr. Allergy **8**, 195 (1964).

12. Frame, B., and Nixon, R. K., N. Engl. J. Med. **279**, 626 (1968).

13. Urist, M. R., and McLean, F. C., Arch. Pathol. **63**, 239 (1957).

14. Padawer, J., Angiology **12**, 538 (1961).

15. Walker, B. M., Nature (London) **192**, 980 (1961).

16. Allen, A. M., J. Nat. Cancer Inst. **28**, 1125 (1962).

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