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Age-Related Osteopenia: Evidence for an Intrinsic Defect of Bone Resorbing Cells and a Possible Treatment[1] (42093)

MARVIN L. TYAN

Medical and Research Services, Veterans Administration, Wadsworth VA Medical Center, Los Angeles, California 90073, and Department of Medicine, UCLA School of Medicine, Los Angeles, California 90024

Abstract. B6D2F$_1$ and B6AF$_1$ mice of various ages were given sublethal or lethal doses of X radiation and injected with marrow and/or spleen cells from young, mature, or old syngeneic donors. Four to five months later they were killed and ash weights were determined on femurs, sacrum, and ilium. It was found that (1) large numbers of marrow cells (i.e., $>25 \times 10^6$) and/or spleen cells ($>50 \times 10^6$) from old mice retarded the growth of bone in young hosts and induce loss of bone mass in mature recipients, (2) spleen cells from young donors consistently prevented the loss of bone mass normally seen in aging mice, and (3) the thymus and T cells did not appear to play a significant role in bone resorption and remodeling. These observations suggested that in aging mice loss of bone mass is caused by an intrinsic defect in a hematopoietic cell population, perhaps the macrophage/osteoclast or their common precursor, which results directly or indirectly in increased bone resorption. On this basis, promethazine HCl, an inhibitor of macrophage metabolism and phagocytosis, was added to the drinking water (1.0 to 4.0 mg/dl) of aging mice. Four to five months later it was found that bone mass was significantly greater in the groups given promethazine than in the age and weight matched controls.

Previous studies from this Laboratory showed that when spleen and/or marrow cells from old mice are transferred to lethally irradiated hosts, bone growth is retarded in young animals and bone mass is decreased in mature mice. Preliminary experiments suggested that the bone loss normally seen in aging mice could be minimized by the transplantation of hematopoietic cells from young donors (1). Taken together, these observations suggested that in aging mice loss of bone mass is caused by an intrinsic defect in a hematopoietic cell population which results directly or indirectly in increased bone resorption.

In this report, data will be presented which support the original observations and suggest that the thymus and T cells do not play a significant role in bone resorption or remodeling. In addition, it will be shown that promethazine HCl which among its several pharmacological effects inhibits macrophage metabolism and phagocytosis is able to prevent much of the loss of bone mass normally seen in aging mice.

Materials and Methods. B6D2F$_1$ and B6AF$_1$ male and female mice were obtained at 6 to 8 weeks of age from the Jackson Laboratory, Bar Harbor, Maine, and they were maintained on Purina Laboratory Chow and acidified tap water *ad lib.* In normative studies, mice were killed at various ages; their femurs were removed intact, autoclaved, defleshed, defatted, ashed at 800°C, and weighed. In these and in all other experiments only healthy mice were selected and only mice found to be free of disease at autopsy were evaluated. Experimental and control mice were of one sex, and they were age and weight matched at the beginning of the studies.

Young, mature, and old mice were given sublethal or lethal doses of X radiation (500, 600, or 900 rad; 300 kVp, 20 mA, HVL 0.1 mm Cu) and within 2 hr they were injected intravenously with $5–25 \times 10^6$ marrow cells or $21–80 \times 10^6$ spleen cells from healthy syngeneic donors of various ages. From 4 to 15 months later they were killed and the ash weights of the femurs and, in some experi-

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ments, the sacrum and ilium were determined.

In experiments designed to study the role of the thymus and T cells in bone growth and remodeling certain of the hosts were thymectomized 4 weeks before irradiation, and marrow and spleen cells were treated with AKR anti-C3H thymus serum (anti-Θ) or mock anti-Θ serum (Bionetics, Kensington, Md.) and guinea pig complement as described previously (2).

In five experiments a total of 101 old mice (26 to 30 months old) were given promethazine HCl (Sigma Chemical Co., St. Louis, Mo.) in the drinking water (0.1, 1.0, 2.0, or 4.0 mg/dl) and 101 control mice of the same sex, age, and weight were given acidified tap water. Four to five months later the survivors were killed and bone mass determined as described above.

On average a cage of five mature female mice will consume approximately 210 ml of water per week (five males will consume approximately 50% more). It is not certain how much of this volume is wasted secondary to leaking sipper tubes or to mice which play with the nipple. However, if one assumes that no wastage occurs the average female mouse at maximum will ingest the following amounts of promethazine daily (males will ingest approximately 50% more).

0.1 mg/dl:	0.006 mg,	0.24 mg/kg/day
1.0 mg/dl:	0.06 mg,	2.4 mg/kg/day
2.0 mg/dl:	0.12 mg,	4.8 mg/kg/day
4.0 mg/dl:	0.24 mg,	9.6 mg/kg/day

Results. *Normative studies.* The femur ash weights of $B6D2F_1$ female mice peak at about 12 months and begin to decline at 20 months. At 35 months of age, femur ash weight has decreased a mean of 30% from peak values (Fig. 1). Femur ash weights of $B6AF_1$ female mice peak between 10 and 13 months, and by 37 months of age ash weight has decreased a mean of 25% (Fig. 2). Also shown in Fig. 1 are data which have been reported previously on the ratio of total calcium to dry femur weight (1). The results do not show a significant change with age, and this is consistent with the type of bone loss seen in human osteoporosis.

Cell transfer studies. Spleen cells from old mice alone or mixed with old marrow cells reduced bone growth in young hosts (Tables I and II). Old spleen and marrow cells significantly reduced the femur mass of mature 12-month-old hosts (Table II). Both marrow and spleen cells from old donors were effective in reducing bone mass in lethally irradiated mature mice (Table III). Spleen cells but not marrow cells from young donors prevented the loss of femur mass found in 29.5-month-

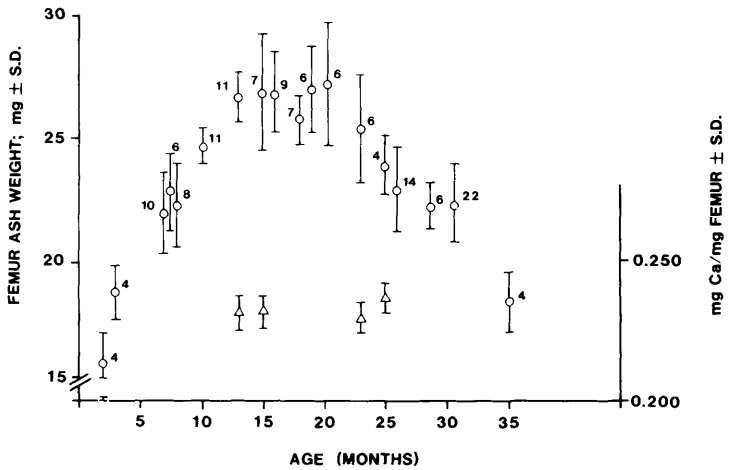

FIG. 1. Femur ash weight (open circle, *n*, ±SD) and mg Ca/mg dry femur (△) of $B6D2F_1$ mice at various ages.

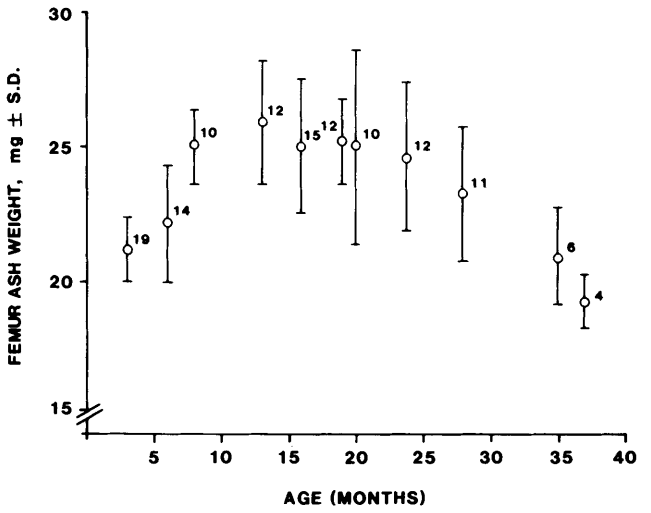

FIG. 2. Femur ash weight (open circle, *n*, ±SD) of B6AF$_1$ female mice at various ages.

old mice followed for 9.5 months after cell transfer [Table IV; fewer than 25 × 10^6 marrow cells were transferred. Larger numbers may be required as reported (3)].

In sublethally irradiated hosts marrow and spleen cells from old donors alone or mixed retarded bone growth in young hosts (Table V). Spleen cells from young donors prevented bone loss in old sublethally irradiated hosts, and marrow cells prevented bone loss in old hosts in two experiments but the results were statistically significant in only one.

Role of thymus and T cells. Normal and thymectomized young hosts were lethally irradiated and given anti-Θ or mock anti-Θ and complement-treated marrow or spleen cells from young or old donors (Table VI). It was found again that old marrow and spleen cells retard the growth of bone in young hosts but with one exception neither thymectomy of the host nor treatment of the transferred cells with anti-Θ serum had an effect on bone mass. In one experiment thymectomized hosts given mock anti-Θ-treated marrow cells from young donors had femur weights significantly lower than noted in the nonoperated controls; however, in two comparable experiments mean femur weights of the operated and nonoperated groups were nearly identical.

B6AF$_1$ female mice were thymectomized (13) or sham-operated (12) at 10 weeks of age and the survivors, seven thymectomized and five sham-operated, were killed when they were 29.5 months old. The femur ash weights of the survivors were not significantly different (sham, 21.4 ± 1.3 mg; thymectomy, 21.9 ± 1.4 mg).

Promethazine HCl. In five experiments a total of 101 mice, 26 to 30 months old, were

TABLE I. EFFECT ON FEMUR ASH WEIGHT OF THE TRANSFER OF 21 TO 35 × 10^6 SPLEEN CELLS FROM YOUNG OR OLD DONORS TO LETHALLY IRRADIATED YOUNG SYNGENEIC HOSTS

Strain sex	Age of host (months)	Age of donor (months)	No. of femurs	Weight of ashed femur (mg ± SD)	SEM	*P*
B6D2F$_1$F	4	4	54	24.2 ± 1.7	0.3	<0.001
	4	30	43	22.7 ± 1.2		

Note. The mice were killed 4 to 5 months later.

TABLE II. EFFECT ON FEMUR ASH WEIGHT OF THE TRANSFER OF BONE MARROW AND SPLEEN CELLS FROM YOUNG OR OLD SYNGENEIC DONORS TO LETHALLY IRRADIATED MICE OF VARIOUS AGES

Strain sex	Age of host (months)	Age of donor (months)	No. of femurs	Weight of ashed femur (mg ± SD)	SEM	*P*
B6D2F$_1$F	3–5	3–5	89	24.9 ± 2.1	0.30	<0.001
	3–5	23–36	55	21.7 ± 1.5		
	12	3–5	27	25.4 ± 2.2	0.59	<0.001
	12	36	17	21.3 ± 1.7		

Note. The mice were killed 4 to 5 months after cell transfer.

given promethazine HCl in the drinking water (0.1, 1.0, 2.0, or 4.0 mg/dl) and 101 control mice of the same age, sex, and weight were given acidified tap water. Four to 5 months later the survivors were killed and the femur ash weights and in three experiments the ash weights of the ilium/sacrum were determined on mice free of disease at autopsy. With the exception of the lowest dose level (0.1 mg/dl), it was found that bone ash weights were greater in the promethazine-treated groups and that the differences were statistically significant in all cases except for the weights of the femurs of B6D2F$_1$ males killed at 30 months of age (Table VII). The mice tolerated the promethazine well and manifested no evidence of sedation, increased mortality, nor increased frequency of tumors (Table VIII).

The pH of the acidified tap water and the water to which promethazine had been added were 2.0 and 5.5, respectively. Repeated urine pHs, however, were 5.5 ± 0.35 (acidified water, n = 20) and 5.5 ± 0.5 (promethazine water, n = 24). Arterial blood gas values determined on pooled blood from mice given acidified tap water were within normal limits (pH, 7.39; PaO_2, 110; $PaCO_2$, 23). Thus, there was no evidence that the acidified tap water produced a metabolic acidosis.

Histological sections were prepared from the distal ends of femurs taken from mice given acidified tap water or promethazine for 1 year. There was no evidence of abnormal bone formation in the group given promethazine and osteoclastic activity appeared decreased on the surfaces of trabecular bone when compared with that noted in the group given acidified tap water. The tissue sections were not satisfactory for the quantitative assessment of trabecular or cortical bone volumes.

Discussion. A universal loss of bone normally begins in the fourth or fifth decade of life affecting the strength of cancellous trabecular bone to a greater degree than cortical bone. The age-related loss of skeletal mass does not accelerate with age, begins earlier in women, is accelerated by menopause, is more rapid in short individuals, occurs at

TABLE III. EFFECT ON FEMUR ASH WEIGHT OF THE TRANSFER OF MARROW OR SPLEEN CELLS FROM MATURE OR OLD DONORS TO LETHALLY IRRADIATED MATURE MICE

Strain (sex)	Age of host (months)	Age of donor (months)	Donor cells	No. of donor cells ($\times 10^6$)	Radiation to sacrifice (months)	Femurs (*n*)	Weight of femurs (mg ± SD)	*P*[a]
B6AF$_1$ (F)	12	12	B.M.[b]	22	5	18	23.9 ± 1.8	<0.01
	12	29	B.M.	25		18	21.8 ± 2.0	
	12	12	Spleen	61	5	19	24.0 ± 1.8	<0.01
	12	29	Spleen	80		12	21.9 ± 1.9	

[a] Student's *t* test.
[b] B.M., bone marrow.

TABLE IV. FEMUR ASH WEIGHTS 9.5 MONTHS AFTER THE TRANSFER OF MARROW OR SPLEEN CELLS FROM 8- OR 20-MONTH-OLD DONORS TO LETHALLY IRRADIATED 20-MONTH-OLD MICE

Strain (sex)	Age of host (months)	Age of donor (months)	Donor cells	No. of donor cells ($\times 10^6$)	Radiation to sacrifice (months)	Femurs (n)	Weight of femurs (mg ± SD)	P^a
$B6AF_1$ (F)	20	8	B.M.	21	9.5	11	23.4 ± 1.1[b]	
	20	20	B.M.	22	9.5	19	23.8 ± 2.0	
	20	8	Spleen	60	9.5	20	24.4 ± 1.4[b]	<0.01
	20	20	Spleen	73	9.5	12	23.0 ± 1.2	

[a] Student's t test.
[b] Young B.M. (bone marrow) vs young spleen, $P < 0.05$.

the same rate in whites and blacks although bone mass at maturity is greater in blacks, and in well-nourished individuals is unrelated to calcium intake (4).

Osteoporosis of the senile or postmenopausal type, perhaps a specific form of this generalized osteopenia of the aged is a skeletal disorder in which there is a reduction in bone mass below that which normally characterizes the skeleton for the age, sex, and race of the individual, and in which the remaining bone is normal in chemical composition (4–6). As such, it is a description of a state, not a disease. Osteoporosis may be caused by increased bone resorption without a concomitant increase in bone formation, by impaired formation or by a combination of the two. It presently is thought that in the majority of cases of menopausal and senile osteoporosis bone formation is near normal but bone resorption is increased (7).

Rodents also develop age-related osteo-

TABLE V. EFFECT ON FEMUR ASH WEIGHT OF THE TRANSFER OF MARROW AND/OR SPLEEN CELLS FROM YOUNG OR OLD DONORS TO SUBLETHALLY IRRADIATED YOUNG, MATURE, OR LATE MIDDLE-AGED MICE

Strain (sex)	Age of host (months)	Age of donor (months)	Donor cells	No. of donor cells ($\times 10^6$)	Radiation (r)	Radiation to sacrifice (months)	Femurs (n)	Weight of femurs (mg ± SD)	P^a
$B6D2F_1$ (F)	5	6	B.M.[b]	28	600	5	17	24.5 ± 1.7	<0.01
	5	34	B.M.	30			6	22.7 ± 1.4	
$B6D2F_1$ (F)	4	4	B.M.	26	600	5	16	24.3 ± 1.6	<0.01
	4	34	B.M.	26			16	22.2 ± 1.3	
	4	4	Spleen	67	600	5	16	24.1 ± 1.2	<0.01
	4	34	Spleen	88			14	22.4 ± 1.4	
	4	4	B.M. and Spleen	13 & 33	600	5	19	24.5 ± 0.9	<0.01
	4	34	B.M. and Spleen	13 & 44			12	22.8 ± 1.4	
$B6D2F_1$ (M)	23	8	B.M.	19	500	6	10	24.5 ± 3.0	>0.1
	23	23	B.M.	22			11	22.9 ± 1.7	
	23	8	Spleen	74	500	6	12	25.8 ± 2.0	<0.02
	23	23	Spleen	62			14	23.4 ± 1.7	
$B6AF_1$ (F)	14	5	B.M.	21	500	15	10	26.2 ± 1.7	<0.05
	14	14	B.M.	31			4	21.5 ± 2.8	

[a] Student's t test.
[b] B.M., bone marrow.

TABLE VI. EFFECT OF HOST THYMECTOMY AND/OR TREATMENT OF DONOR CELLS WITH ANTI-θ SERUM AND COMPLEMENT ON FEMUR ASH WEIGHT OF LETHALLY IRRADIATED YOUNG MICE INJECTED WITH YOUNG OR OLD SYNGENEIC MARROW OR SPLEEN CELLS

Strain and sex	Age of host (months)	Age of donor (months)	No. of B.M. or spleen cells ($\times 10^6$)	Anti-θ treatment of B.M.	Host Tx	No. of femurs	Weight of ashed femur (mg ± SD)
B6D2F$_1$, F	3–5	3–5	5, B.M.	No	No	50	23.9 ± 1.4[a]
				Yes	No	38	23.7 ± 1.9
				No	Yes	28	21.5 ± 2.3[a]
				Yes	Yes	25	21.6 ± 1.7
	3–5	30	5, B.M.	No	No	55	23.6 ± 2.6
				Yes	No	30	23.4 ± 2.1
				No	Yes	21	23.1 ± 1.2
				Yes	Yes	10	23.4 ± 1.5
B6D2F$_1$, F	4–5	4–5	>10, B.M.	No	No	75	23.9 ± 2.1[b]
				No	Yes	70	23.5 ± 2.0[c]
	4–5	30–32	>10, B.M.	No	No	16	21.8 ± 2.2[b]
				No	Yes	7	20.3 ± 1.2[c]
B6D2F$_1$, F	4–5	4–5	>35, spleen	No	No	20	23.0 ± 1.7[d]
				No	Yes	19	22.6 ± 1.6
	4–5	30–32	>35, spleen	No	No	8	19.3 ± 1.3[d]
				No	Yes	6	18.9 ± 1.8

Note. The mice were killed 4 to 5 months after cell transfer. B.M., bone marrow.
[a–d] $P < 0.001$.

TABLE VII. EFFECT OF PROMETHAZINE ON BONE MASS WHEN GIVEN ORALLY IN THE DRINKING WATER FOR 4 TO 5 MONTHS TO 26 TO 30 MONTH-OLD MICE

Strain	Sex	Age at sacrifice (months)	Promethazine (mg/dl)	Bone assayed	Weight of ashed bone (mg ± SD)	*n*	*P*<
B6D2F$_1$	F	31.5	None	Femur	21.3 ± 2.5	20	
			0.1	Femur	21.0 ± 2.0	22	NS
			None	Ilium	15.4 ± 1.2	14	
			0.1	Ilium	15.2 ± 0.8	7	NS
			None	Sacrum	8.5 ± 0.6	6	
			0.1	Sacrum	8.7 ± 1.0	7	NS
B6AF$_1$	F	34	None	Femur	22.1 ± 1.3	18	
			1.0	Femur	23.5 ± 1.6	22	0.01
B6D2F$_1$	M	32.5	None	Femur	21.6 ± 1.9	20	
			2.0	Femur	23.0 ± 2.7	24	0.05
			None	Sacrum	8.2 ± 1.4	10	
			2.0	Sacrum	9.9 ± 1.3	12	0.05
B6D2F$_1$	F	31.5	None	Femur	20.9 ± 1.7	15	
			2.0	Femur	22.8 ± 1.1	18	0.01
B6D2F$_1$	M	30.0	None	Femur	21.9 ± 2.0	28	
			4.0	Femur	22.6 ± 2.3	21	NS
			None	Ilium	15.8 ± 1.2	25	
			4.0	Ilium	16.7 ± 1.8	22	0.05
			None	Sacrum	8.4 ± 0.6	11	
			4.0	Sacrum	10.4 ± 1.0	8	0.001

TABLE VIII. MORTALITY RATE AND FREQUENCY OF TUMORS AMONG MICE GIVEN PROMETHAZINE IN THE DRINKING WATER FOR 4 TO 5 MONTHS

Treatment	Number dead/total	Number with tumors/ total alive
None	39/81[a]	5/42[b]
Promethazine	30/81[a]	12/51[b]

Note. Data from experimental groups receiving 1.0 to 4.0 mg/dl as shown in Table VII.
[a,b] Differences not significantly different; χ^2.

penia, and work in mice has shown that the impaired bone resorption seen in osteopetrosis and the excessive bone resorption associated with age-related osteopenia may be reversed or produced by cells which normally reside in the marrow or spleen. Walker and others have demonstrated that normal bone resorption can be restored to osteopetrotic mice by the transfer of marrow or spleen cells from phenotypically normal littermates and that decreased bone resorption can be transmitted to normal mice with hematopoietic cells from osteopetrotic sibs (3, 7, 8). The macrophage has been shown to be involved in bone resorption as a precursor of the osteoclast and/or as an auxiliary or helper cell (10–12), and this mononuclear phagocyte has been found to have a defect in chemotaxis in osteopetrotic mice (13).

The studies presented here and in a previous report (1) have shown that spleen and marrow cells from old mice effectively reduce bone growth in immature hosts and cause loss of bone mass when transferred to mature mice. Further, spleen cells alone or spleen cells mixed with marrow from young donors were found to prevent the loss of bone mass normally seen in aging mice. No consistent evidence was found to suggest that the thymus or T cells play a significant role in bone growth or resorption. These observations taken together with those derived from the studies on osteopetrosis suggest that in aging mice the loss of bone mass is caused by an intrinsic defect in a hematopoietic cell population, most likely the macrophage/osteoclast or its precursor, which results directly or indirectly in increased bone resorption, or less likely, in decreased bone formation. The defect may be an increased sensitivity of the cells to hormonal regulation by parathyroid hormone, vitamin D, osteoclast activating factor or other agents, or it may be independent of external stimuli.

It has been shown that promethazine HCl inhibits the metabolism and phagocytic activities of polymorphonuclear leukocytes and macrophages (14, 15) and that it abrogates parathyroid extract-induced bone resorption *in vitro* but has no evident effect on the *in vitro* formation of new osteoid (16). On this basis promethazine was added to the drinking water of aging mice (0.1–4.0 mg/dl) in five separate experiments. It was found that 4 to 5 months later bone ash weights (femur, sacrum, ilium) in the promethazine-treated groups (1.0–4.0 mg/dl) were greater than those of the controls and near the weights seen in mature healthy mice. These results suggest that the proximate cause of the loss of bone mass in aging mice may be an absolute or relative increase in macrophage, osteoclast, or other phagocytic cell activity which can be inhibited by relatively low and well-tolerated doses of promethazine. Promethazine is a phenothiazine with H1 blocker activity; the agent has many other pharmacological actions, such as anticholinergic activity, increasing prolactin, and decreasing growth hormone and adrenal corticosteroid secretion, and promoting mild diuresis (17). It is not clear at this point how the agent reduces bone resorption or if the effect is expressed exclusively through macrophages or osteoclasts.

Although both mouse and man experience an age-related loss of bone mass, it has not been demonstrated that the mechanisms are similar in the two species.

1. Tyan ML. Femur mass: Modulation by marrow cells from young and old donors. Proc Soc Exp Biol Med **164**:89–92, 1980.
2. Tyan ML. Marrow colony-forming units: Age-related changes in responses to anti-θ sensitive helper/suppressor stimuli. Proc Soc Exp Biol Med **165**:354–360, 1980.
3. Loutit JF, Sansom JM. Osteopetrosis of microphthalmic mice. A defect of the hematopoietic stem cell? Calcif Tissue Res **20**:251–255, 1976.

4. Avioli LV. Senile and postmenopausal osteoporosis. Adv Intern Med **21**:391–404, 1976.
5. Wheeler M. Osteoporosis. Medical Clinics of N America **60**:1213–1232, 1976.
6. Avioli LV. The osteoporosis problem. In: Winick M, ed. Nutritional Disorders of American Women. New York, Wiley, pp99–110, 1977.
7. Heaney RP, Recker RR, Saville PD. Menopausal changes in bone remodeling. J Lab Clin Med **92**: 964–971, 1978.
8. Walker DG. Bone resorption restored in osteopetrotic mice by transplants of normal bone marrow and spleen cells. Science (Washington, DC) **190**:784–785, 1975.
9. Walker DG. Spleen cells transmit osteopetrosis in mice. Science (Washington, DC) **190**:785–786, 1975.
10. Kahn AJ, Stewart CC, Teitlebaum SL. Contact-mediated bone resorption by human monocytes *in vitro*. Science (Washington, DC) **199**:988–989, 1978.
11. Horton JE, Openheim JJ, Mergenhagen SF, Raisz LG. Macrophage-lymphocyte synergy in the production of osteoclast activating factor. J Immunol **113**: 1278–1284, 1974.
12. Ash P, Loutit JF, Townsend KMS. Osteoclasts are derived from hematopoietic stem cells. Nature (London) **283**:669–670, 1980.
13. Minkin C. Defective macrophage chemotaxis in osteopetrotic mice. Calcif Tissue Int **33**:677–679, 1981.
14. De Chatelet LR, *et al.* Effects of promethazine-hydrochloride on human polymorphonuclear leukocytes. Infect Immun **7**:403–410, 1973.
15. De Chatelet LR, *et al.* Effects of promethazine-hydrochloride on the metabolism of rabbit alveolar macrophages. Proc Soc Exp Biol Med **153**:392–396, 1976.
16. Goldhaber P, Rabadjija L. Effect of promethazine-hydrochloride on bone resorption in tissue culture. Proc Soc Exp Biol Med **169**:105–109, 1982.
17. Douglas WW. Histamine and 5-hydroxytryptamine and their antagonists. In: Gilman AG, Goodman LS, Gilman A, eds. The Pharmacological Basis of Therapeutics. New York, MacMillan, pp609–646, 1980.

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