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NATURAL KILLER CELLS, BONE, AND THE BONE MARROW: STUDIES IN ESTROGEN-TREATED MICE AND IN CONGENITALLY OSTEOPETROTIC (*mi/mi*) MICE[1]

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Mice lose natural killer cells after 6 weeks of treatment with 17β-estradiol. We here demonstrate that the same protocol leads to loss of genetic resistance to bone marrow transplantation and to significant osteoproliferation with loss of bone marrow. We also show that mice with reduced marrow because of congenital osteopetrosis are deficient in natural killing. These findings are consistent with previous evidence that natural killing and genetic resistance to bone marrow transplantation are dependent upon the marrow.

Temporal studies of bone histology and radiology during and after treatment with estrogen reveal that alterations in natural killing proceed more rapidly than changes in bone marrow volume. These studies also demonstrate that estrogens induce osteoproliferation only at endosteal surfaces that are adjacent to hematopoietic marrow. From these observations, we conclude that estrogens do not reduce natural killer cells simply by reducing the volume of bone marrow. Estrogens may instead have an effect on bone marrow cells that leads both to osteoproliferation and to a deficiency of marrow-dependent cells.

The bone marrow is the preferred environment for hematopoiesis and myelopoiesis in adults. Both functions can, however, be assumed by other organs, particularly the spleen and liver (1, 2). This occurs, for example, when mice are given radioactive strontium (^{89}Sr), a powerful β-emitter that localizes to bone and provides intense irradiation of the marrow (3, 4). Such treatment leads to fibrosis of the bone marrow, but the formed blood elements can be sustained by extra-medullary production, particularly in the spleen (3, 5). Mice treated with ^{89}Sr under defined protocols also show little or no loss of T or B lymphocyte function (5).

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Treatment of mice with ^{89}Sr does, however, reduce certain cells. These cells have been termed "marrow-dependent" cells or "M" cells because ^{89}Sr appears to ablate cells in the marrow, with relatively less effect on other organs (5, 6). Marrow-dependent cells include cells that mediate or participate in i) natural cell-mediated cytotoxicity, also called natural killing (7); ii) genetic resistance to bone marrow transplantation (6); iii) genetic resistance to certain viral infections (8, 9); and iv) resistance to intracellular infections with *Listeria monocytogenes* (5). The evidence that these cell functions are uniquely dependent on the bone marrow rests largely on their sensitivity to ^{89}Sr, and little is known of the properties of the marrow that might provide a specialized environment for these cells.

We recently demonstrated that the administration of estrogen (17β-estradiol) to mice for a period of 4 to 6 weeks led to a marked reduction in one of the ^{89}Sr-sensitive functions, natural killing (10). Androgen (5α-methyltestosterone) had little or no effect. The loss of natural killing in estrogen-treated mice was not due to a humoral or a cellular suppressor of natural killer cells, and it appeared to be independent of the known thymolytic effect of estrogen (10, 11). Because natural killer cells are sensitive to ^{89}Sr, we considered that the loss of natural killing might be due to an effect of estrogen on the bone marrow. It has been known for over 40 years that pharmacologic doses of estrogen lead to osteoproliferation in young mice, with replacement of the marrow by bone (12–14). This osteosclerotic effect of estrogens was noted in our estrogen-treated mice. Thus, the loss of natural killing could be due to a loss of bone marrow secondary to estrogen-induced osteoproliferation. This hypothesis leads to three predictions, each of which is examined in this paper.

First, if estrogens reduce natural killing because natural killer cells are marrow-dependent, then estrogens should reduce other marrow-dependent cell functions. We have examined genetic resistance to bone marrow transplantation.

Second, if estrogens reduce natural killer cells because of the replacement of marrow by bone, then mice with congenital osteopetrosis, in which the marrow is also replaced by bone, should similarly have low levels of natural killer cells.

Third, if the effects of estrogens on natural killer cells are due to a loss of marrow, then the loss of marrow should precede, or at least parallel, the loss of natural killer cells.

The results presented here are consistent with the hypothesis that estrogens reduce natural killer cells by altering the bone marrow, but it appears that this effect involves more than just a reduction in the amount of marrow due to replacement by bone.

MATERIALS AND METHODS

Mice. C57BL/10Sn, BALB/c, B10.D2ns, DBA/2, and NZB/J mice were purchased from Jackson Laboratories.[3] NZB/NZW mice were bred in our vivarium, using NZB mice from Jackson Laboratories and NZW mice from our colony. Congenitally osteopetrotic (*mi/mi*) mice were bred from Grüneberg (*mi/+*) heterozygotes and CBA (*mi/+*) heterozygotes kindly supplied by J. F. Loutit, Harwell, England.

Assay for natural killing. Natural killing by spleen cells was carried out in microtiter plates as previously described (10), using as a target YAC-1, a Moloney virus-induced lymphoma from A/J mice (kindly supplied by Dr. George Klein, Stockholm) that we carry *in vitro*. Target cells were labeled by incubation in ^{51}Cr. Percent cytotoxicity was determined by:

$$\left[\frac{CPM_{effectors} - CPM_{spontaneous\ release}}{CPM_{maximum\ release} - CPM_{spontaneous\ release}} \times 100\right]$$

Maximum release was determined by incubation in 15% saponin, which releases 95 to 100% of the ^{51}Cr. We find that cytotoxicity in this assay is increased or unchanged if effectors are a) pretreated with anti-Thy 1.2 plus complement (C); b) depleted of cells adherent to plastic; or c) depleted of B lymphocytes on nylon wool columns.

Administration of estrogen. Mice were subjected to sustained administration of 17β-estradiol as previously described (10). Briefly, at 3 to 4 weeks a 2-cm Silastic tube containing approximately 15 mg of 17β-estradiol was implanted in the dorsal subcutaneous tissue, using pentobarbital anesthesia. Such implants rapidly provide a constant level of serum estrogen that is sustained for at least several months (15). The mean level of 17β-estradiol in NZB/NZW female mice 6 weeks after implantation was at least 9 times the level in sham-treated (empty implant) female mice (assay kindly performed by Dr. William Crowley, Massachusetts General Hospital, Boston, Massachusetts).

Assay for genetic resistance to bone marrow transplantation. Genetic resistance to bone marrow transplantation was assayed by the method of Cudkowicz *et al.* (16, 17). Donor bone marrow cells were collected into Eagle's minimal essential medium (with Hanks' and HEPES buffers) by cutting the ends of both femurs and flushing the marrow with a 25-gauge needle. Recipient (NZB or NZB/NZW) mice were exposed to 800 R of x-irradiation at 122 R/min. Within 15 min before irradiation, each mouse was given 25 mg of cytosine arabinoside i.p. to further suppress endogenous splenic colonies (18). Four to 6 hr after irradiation, mice were injected i.v. with 10^7 bone marrow cells in 0.2 ml via the lateral tail vein. Four days later, the mice were injected i.p. with 1.0 mCi ^{125}IUdR (5-iodo[^{125}I]-2-deoxyuridine, Amersham, Chicago, Ill.), a thymidine analogue, as an assay for DNA formation. To suppress endogenous thymine formation, the mice received 10^{-7} moles of unlabeled 5-fluoro-2-deoxyuridine 1 hr before the ^{125}IUdR.

Sixteen to 18 hr after injection of ^{125}IUdR, the spleen was removed from each animal and DNA synthesis was determined by measurement of ^{125}I in the intact spleen, using a Packard gamma scintillation spectrometer. Results are expressed as a percent of the injected dose.

Bone histology. Bones were dissected free of soft tissue and fixed in phosphate-buffered formalin. They were then decalcified in a solution containing 15% sodium citrate and 45% formic acid. After embedding in paraffin, 5 μ longitudinal sections of the entire femur were cut and stained with hematoxylin and eosin. The slides were coded and scored in a blind manner by two histopathologists (T.G. and J.G.). The system for scoring was based on an estimate of the amount of cellular marrow that had been replaced by new bone (see Table I). There was agreement in scoring of virtually all slides.

Radiographic evaluation. Mice were killed by cervical dislocation, and whole-body radiographs were taken with a Hewlett-Packard Faxitron x-ray system (43807N, Hewlett-Packard, Santa Clara, Calif.) set at 40 Kvp on the automatic exposure mode. Exposures were made on Kodak RP/M X-omat mammography film (Catalog No. 157-1389, Eastman Kodak, Rochester, N. Y.)

RESULTS

Effects of estrogen on genetic resistance to bone marrow transplantation. NZB/NZW mice possess unusually strong genetic resistance to bone marrow transplantation in that they can reject more than 10^7 parental cells, a dose that overcomes the resistance of most other strains (19). Resistance to bone marrow transplantation, like natural killing, is a marrow-dependent function (8). Because estrogens reduce natural killing in mice, we tested the effects of 6 weeks of estrogen therapy on the resistance of NZB/NZW mice to NZB marrow cells. As shown in Figure 1, the splenic activity in estrogen-treated NZB/NZW recipients was nearly 10-fold greater than the activity in sham-treated recipients. The activity in estrogen-treated recipients was equal to the levels in syngeneic NZB transfers. This result was not due to an effect of estrogens on donor cells, since NZB marrow cells did not show a substantial difference in activity when transferred into estrogen-treated or sham-treated syngeneic NZB recipients. The effect of estrogens was also not due to an enhancement of the ability of the NZB/NZW recipients to sustain cell growth, since there was no difference in splenic activity between estrogen-treated and sham-treated NZB/NZW mice that received syngeneic NZB/NZW marrow cells. Estrogen-treated recipients had larger spleens than sham-

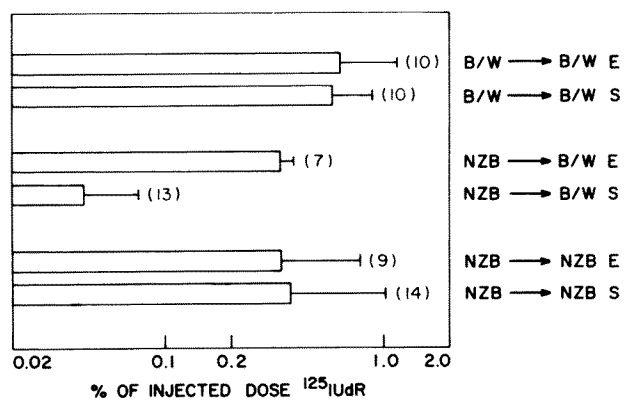

Figure 1. Genetic resistance to parental (NZB) bone marrow transplantation by NZB/NZW F_1 mice pretreated for 6 weeks with estrogen (E) or sham (S) implant. Bars show per cent of ^{125}I retained in the spleen 16 to 18 hr after injection of ^{125}IUdR (plus standard deviation). The number of mice assayed for each transfer is shown in parentheses. Controls include the transfer of marrow cells from NZB/NZW mice (*two upper bars*) and from NZB mice (*two lower bars*) into syngeneic recipients that received either estrogen or sham implants. Mice that received no cells had less than 0.02% uptake (NZB or NZB/NZW, estrogen or sham).

[3] Abbreviations used in this paper: NZB, New Zealand Black; NZW, New Zealand White; *mi*, microphthalmia (recessive gene for osteopetrosis); +, normal gene at relevant locus; HEPES, *N*-2-hydroxypiperazine-*N'*-2-ethanesulfonic acid.

treated recipients, but the syngeneic transfers showed that spleen size did not account for the increased activity in estrogen-treated recipients.

Natural killing in congenitally osteopetrotic mice. For studies of natural killing in congenitally osteopetrotic mice we chose microphthalmic (*mi/mi*) mice, which develop osteopetrosis *in utero* (20, 21). These mice lack eyes and teeth, and many do not survive weaning, even when provided with liquid food (20). Because survival beyond weaning may reflect reduced expression of the disease, we chose to examine natural killing in unweaned mice, 4 to 5 weeks old. We determined first that if normal mice were not weaned they nonetheless developed levels of natural killer cells that were equal, at 5 weeks, to levels in mice that had been weaned at 3 weeks. We examined natural killing in CBA (*mi/mi*) and Grüneberg (*mi/mi*) mice, because both of these strains show high levels of natural killing in normal (+/+) or heterozygous (*mi/+*) mice. As shown in Figure 2, CBA (*mi/mi*) mice had markedly reduced levels of natural killing at 4 weeks when compared to their unaffected littermates. The percent killing by *mi/mi* cells from all seven animals tested was less than three S.E.M. below the normal levels at all effector/target ratios. Results with 5-week Grüneberg (*mi/mi*) mice were more variable; seven of the nine (*mi/mi*) mice had levels of killing that were less than 5 S.E.M. below the normal mean, but one (*mi/mi*) mouse had normal levels of natural killing and one had high levels (Fig. 2). Nonetheless, killing by (*mi/mi*) mice was significantly lower than killing by normals ($p < 0.02$ by the Wilcoxen rank order test for two samples).

Bone histology from (*mi/mi*) mice showed typical changes of osteopetrosis: short, wide bones and at least 50% replacement of the marrow (Fig. 3*e*).

Histology and radiology of estrogen-induced osteosclerosis: Relation to natural killing. The time course of estrogen-induced new bone formation was examined both by bone histology and by whole-body radiographs. In serial studies of the femurs from estrogen-treated NZB/NZW mice, we observed a temporal correlation between the appearance of new bone and the loss of natural killer cells from the spleen. No change was found in the femurs from estrogen-treated mice after 1 week, but new bone formation was prominent after 2 weeks. New bone was formed principally in the femoral metaphyses and, to some extent, at the endosteal surface of the cortex of the shaft (see Fig. 3*a–c* and Table I). After 4 weeks of estrogen administration, there was extensive growth of new bone, which penetrated and replaced the marrow. The remaining marrow, although normal in appearance, was largely confined to the center of the shaft and was subdivided by new trabeculae. After 6 weeks of estrogens, less than 10% of the femoral marrow in NZB/NZW mice remained. These changes in the femoral histology of NZB/NZW mice correlated well with the levels of natural killer cells in the spleen, which, as we previously demonstrated (10), are normal or slightly reduced after 2 weeks of estrogens, but which fall dramatically over the next two to 4 weeks (Table I).

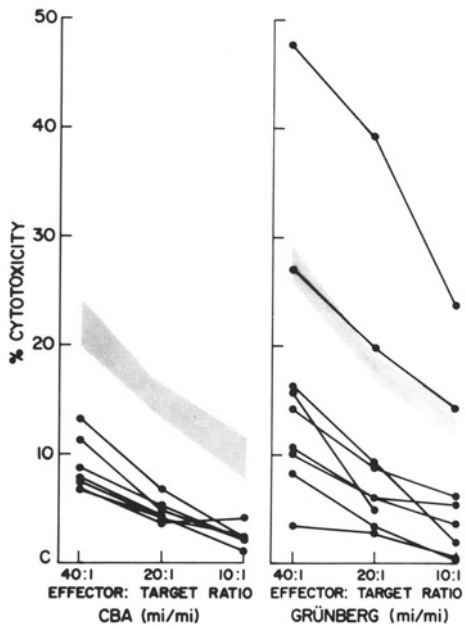

Figure 2. Natural killing by spleen cells from 4-week CBA (*mi/mi*) mice (Fig. 2, *left*) and from 5-week Grüneberg (*mi/mi*) mice (Fig. 2, *right*). Solid lines show results for individual (*mi/mi*) mice. The shaded area is the mean ± S.E.M. for their normal littermates. Normals include both (*mi/+*) and (+/+) mice, as these did not differ in natural killing (total 9 Grüneberg and 12 CBA normals).

Despite this correlation, however, natural killing activity did not correlate simply with the quantity of remaining marrow in estrogen-treated mice. There were three lines of evidence against this.

First, whole-body radiographs confirmed a previous observation that the femur is an early site of new-bone formation in estrogen-treated mice (13). In our studies, there was considerable marrow remaining in other bones after 6 weeks of estrogens, particularly in the vertebral column, which normally contains about 40% of the total marrow in mice (22). This was true even in NZB/NZW mice which (as is shown in Table I) were particularly responsive to the effects of estrogen on bone. Figure 4 shows representative radiographs from NZB/NZW mice after 2, 4, and 6 weeks of estrogens. After 4 to 6 weeks of estrogens, there was extensive hyperostosis of the long bones (except the distal tibiae), the pelvis, the ribs, the sternum, and the clavicles. Males and females were equally affected. Partial hyperostosis of the vertebrae was also evident, but there was not complete obliteration of the vertebral marrow space even in mice that had been treated for 18 weeks. Histologic sections confirmed the presence of marrow in the vertebral columns of these mice. Even in mice treated with estrogen for 14 to 18 weeks, at least one-half of the vertebral marrow remained (at least 20% of the total-body marrow), yet the spleens of these mice had very low levels of natural killing, despite the persistence of marrow (see Table I and Reference 10).

Second, cessation of estrogen administration after 6 weeks in NZB/NZW mice was followed by a recovery of natural killing that was more rapid than the recovery of marrow. For these studies, the histology of the femur was again followed. Natural killing did not begin to recover before 2 weeks after cessation of estrogen therapy. At that time, some revascularization of metaphyseal bone was first apparent in the femur, with recovery of small islands of marrow surrounded by osteoclasts. However, 8 weeks after cessation of estrogens, natural killing was fully restored in the spleen, yet only about one-quarter of the femoral marrow had returned (Fig. 3*d*). The relative lack of recovery of the bone marrow volume in the femur may be partially compensated by more rapid recovery of marrow in other bones (23). Nonetheless, it is clear that splenic natural killing is restored before full restoration of the normal bone marrow volume.

Third, examination of other strains of mice revealed strain differences in the effects of estrogen on new bone formation and these did not correlate with the effects of estrogen on natural

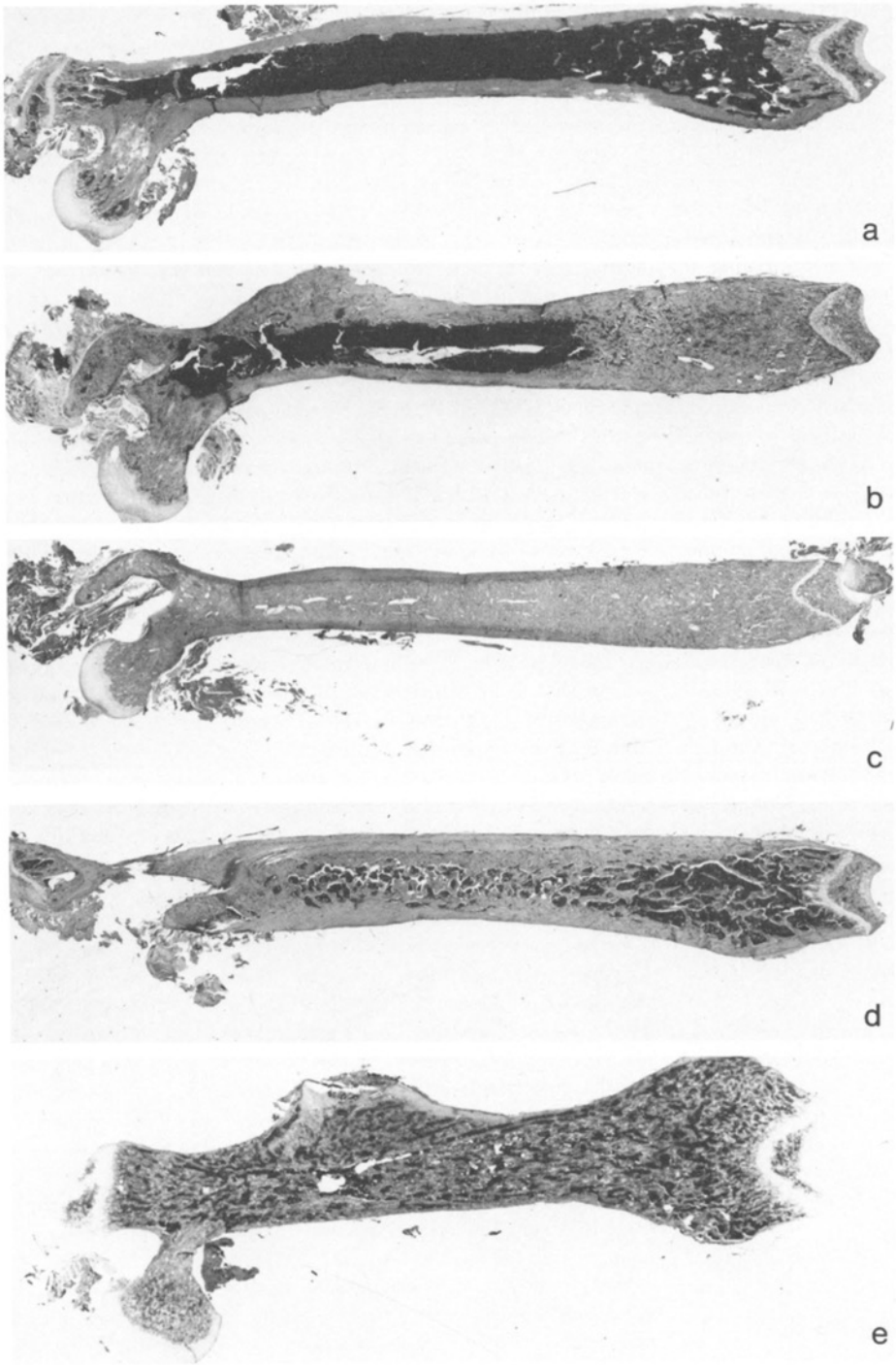

Figure 3. Histology of the femur in estrogen-treated and in congenitally osteopetrotic mice. Figures 3*a* to 3*d* show femurs (5.3X) from NZB/NZW mice that received a Silastic implant at 4 weeks; *a*, sham implant for 6 weeks; *b*, estrogen implant for 2 weeks showing "moderate" replacement of marrow by bone (see Table I); *c*, estrogen implant for 8 weeks, showing complete replacement of marrow; *d*, estrogen implant for 6 weeks, then removed, mouse killed 8 weeks after removal, showing partial marrow regeneration; *e*, 5-week-old Grüneberg osteopetrotic (*mi/mi*) mouse, showing increased skeletal mass and reduced marrow (× 13).

killing. In Table I, note that 6 weeks of estrogen treatment in most strains other than NZB/NZW resulted in a loss of only about 50% of the femoral marrow. Nonetheless, such treatment produced a marked loss of natural killing in C57BL/10 and BALB/c mice.

These three observations do not support the idea that marrow-dependent cells are diminished in estrogen-treated mice simply as a result of the replacement of marrow by new bone. Notably, natural killing can be virtually eliminated by estrogen, despite the persistence of marrow, and natural killing recovers more rapidly than the bone marrow volume when estrogens are stopped.

One alternative explanation for the effects of estrogen on natural killing and on genetic resistance to bone marrow transplantation is that estrogens act on the bone marrow, rather than on bone, with consequent effects both on marrow-dependent cells and on osteoproliferation. With regard to osteoproliferation, there is evidence, detailed in *Discussion*, that estrogen directly affects the marrow, and that osteoproliferation is secondary to cellular changes in the marrow. This sequence was supported in our studies by examination of the sites of estrogen-induced bone formation, noting their relation to the marrow. First, new bone formation in the femurs from estrogen-treated mice occurred only on endosteal surfaces of the cortex; there was no increase in bone formation at the periosteum or the epiphyseal plate. Thus, new bone was formed only at sites in apposition to the marrow. This is in accord with previous studies (13, 14). Second, whole body radiographs of estrogen-

TABLE I

Extent of marrow replacement by new bone in the femurs from estrogen-treated mice: comparison with splenic natural killing

Strain	Duration of Estrogen Implant	Total Mice for Histology	No. of Mice with Bone Changes Indicated (% of Total)[a]				Splenic Natural Killing (% of Sham)[b]
			Minimum	Moderate	Severe	Complete	
	weeks						
NZB/NZW	1[c]	4	4 (100)	0	0	0	ND
	2	25	3 (12)	22 (88)	0	0	96
	4	22	0	22 (100)	0	0	50
	6	30	0	6 (20)	22 (73)	2 (7)	27
	8	10	0	0	5 (50)	5 (50)	25
	10	7	0	0	2 (29)	5 (71)	21
	14	4	0	0	0	4 (100)	8
	In 6, out 2[e]	8	0	0	8 (100)	0	30
	In 6, out 4	9	0	0	9 (100)	0	70
	In 6, out 8	5	0	2 (40)	3 (60)	0	96
BALB/c	6	6	0	6 (100)	0	0	11
C57BL/10	6	8	0	6 (75)	2 (25)	0	18
B10.D2ns	6	6	0	6 (100)	0	0	48
DBA/2	6	8	0	5 (62)	3 (38)	0	38

[a] Marrow replacement was scored as follows: *minimal*, normal or only slight deviation from normal (see Fig. 3*a*); *moderate*, replacement of one-third to two-thirds of the normal volume (see Fig. 3*b*); *severe*, incomplete, but more than two-thirds marrow replacement; *complete*, no marrow (Fig. 3*c*). Loss of marrow did not vary between sexes, and values for both sexes are combined. All sham-treated mice (more than 150) were classed as minimal.

[b] Natural killing by spleen cells from estrogen-treated mice was assessed at an effector to target ratio of 40:1 and is expressed as a per cent of killing by cells from sham-treated mice at the same ratio. Values did not vary consistently between the sexes and are therefore combined.

[c] All mice were given an estrogen implant at 4 weeks of age.

[d] Not done.

[e] Estrogen implant removed after 6 weeks, mice tested 2 weeks later.

treated NZB/NZW mice revealed a lack of new bone formation in the tail bodies and in the distal tibiae (see Fig. 4). Histologic examination of weanling mice revealed that these areas contained marrow that was predominantly fatty. Figure 5 shows a section of the distal tibia from a 5-week NZB/NZW mouse; the marrow is virtually devoid of hematopoietic marrow. At 10 weeks some hematopoietic marrow was present in distal tibiae from both estrogen-treated and sham-treated mice, but fatty marrow still predominated, and estrogens produced relatively little excess bone. Thus, bone formation occurred only in apposition to marrow and was decreased when hematopoietic marrow was replaced by fat cells, even at different sites within the same bone.

DISCUSSION

Both natural killing and genetic resistance to bone marrow transplantation appear to be dependent on the unique environment of the bone marrow because both are reduced by ^{89}Sr *in vivo* (6, 7). We find that both functions are markedly reduced in mice after 6 weeks of sustained estrogen administration. Such treatment also leads to osteoproliferation, with boney overgrowth of the marrow. We also demonstrate here that natural killing is diminished in congenitally osteopetrotic (*mi/mi*) mice, which have reduced marrow because of a failure of bone resorption (21).

These findings support the hypothesis that certain cell functions are uniquely dependent on the marrow and suggest that estrogens reduce such cells by reducing or altering the marrow. We initially thought that estrogens act simply by stimulating new bone growth with consequent reduction in the marrow volume. However, despite a correlation between the temporal loss of natural killing and the induction of osteoproliferation in the femur, we demonstrate here that significant marrow remains in mice in whom natural killing is virtually ablated. Moreover, cessation of estrogens leads to full recovery of natural killing before the normal marrow volume is restored. Thus, alterations in natural killing do not temporally follow alterations in bone marrow volume.

Our findings could be explained by an effect of estrogen on bone marrow, rather than on bone, with consequent effects both on marrow-dependent cells and on osteoproliferation. We do not have direct evidence that estrogens affect marrow-dependent cells in this manner, but the pattern of new bone formation in estrogen-treated mice indicates that estrogens induce new bone because of their effect on the marrow: new bone forms in response to estrogens only at sites in apposition to hematopoietic marrow.

Previous observations have also supported the concept that estrogens have a primary effect on the bone marrow, with secondary effects on bone. First, in estrogen-treated mice there is a loss of erythropoietic colony-forming units from the marrow before the intrusion of bone (24). Second, the bone matrix itself does not appear to contain cells with estrogen receptors (25); estrogens must therefore exert their primary effect elsewhere, with secondary stimulation of bone growth. Third, autoradiographic studies of whole bones reveal that labeled estrogen given *in vivo* concentrates in marrow near the endosteal surface before it can be detected in bone (26). This suggests that cells near the endosteum may be subject to the influence of estrogens, and this possibility is further supported by studies in which marrow cells are pulse-labeled with thymidine after estrogen administration. Estrogen has an early effect on the metaphysis, where undifferentiated marrow cells are stimulated to form osteoblasts (27).

This evidence favors a primary effect of estrogen on the bone marrow. It is possible that estrogens alter the marrow so that

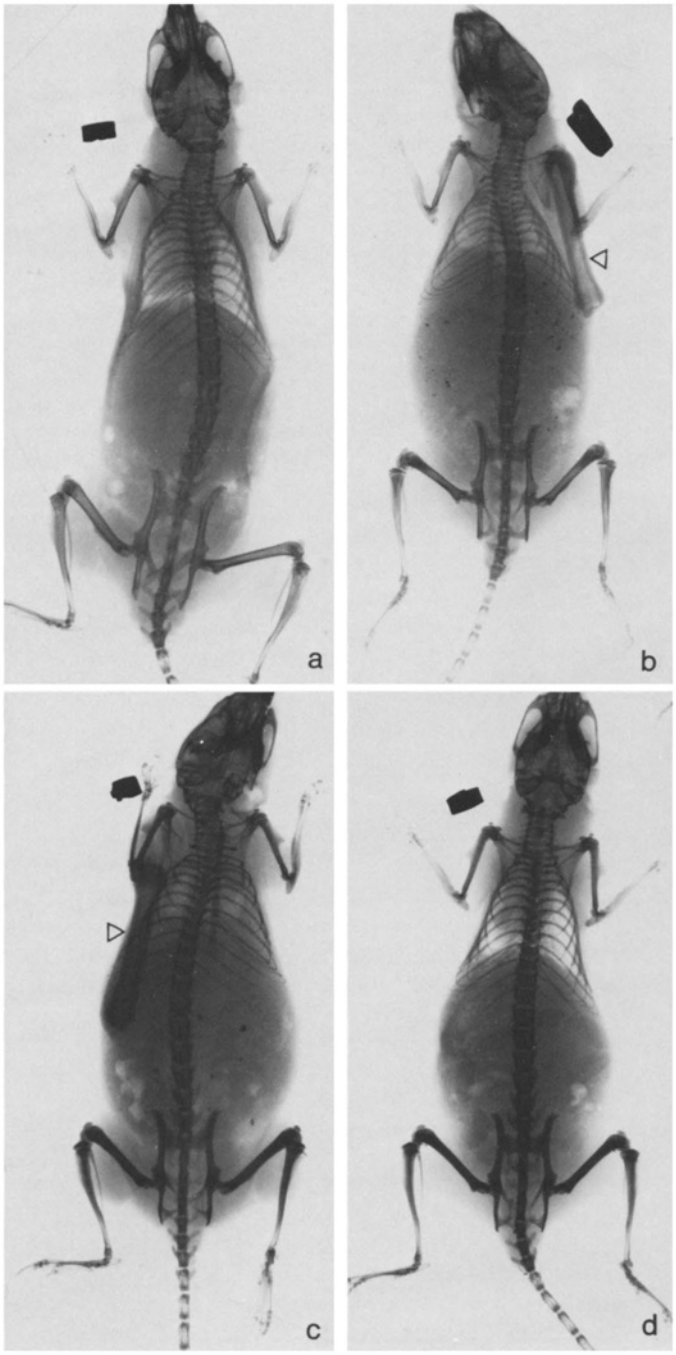

Figure 4. Radiology of estrogen-treated mice. All mice received implants at 4 weeks of age: *a*, sham implant for 6 weeks; *b*, estrogen implant for 2 weeks; *c*, estrogen implant for 4 weeks; *d*, estrogen implant for 6 weeks. In Figures 4*b* and 4*c*, the implant was not removed before the radiograph (*arrow*). By 6 weeks, the pelvis, femurs, humeri, ribs, and proximal tibiae appear solid, but note sparing of the distal tibiae and the tail bones.

the production of marrow-dependent cells is reduced. Similarly, in the osteopetrotic mouse reduced natural killing may reflect the relative inability of an altered marrow to generate natural killer cells, rather than simply reflect a loss of marrow volume.

Recently, cells from osteopetrotic mice have been shown to be deficient in their response to both T cell and B cell mitogens (28). Similar defects are found in osteopetrotic (*op*) rats (29). The relationship of these defects to the deficiency in natural killer cells and the defect in bone resorption is as yet uncertain.

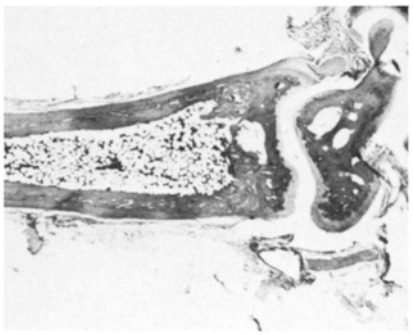

Figure 5. Distal tibia from a normal NZB/NZW mouse at 6 weeks (× 16). The marrow cavity contains largely fat cells with few hematopoietic cells present.

Osteopetrosis in (*mi/mi*) mice can be reversed by the transfusion of spleen or marrow cells from normal littermates (30, 31) even though relatively few donor cells survive in the host (32). It will be of interest to examine the effects of such treatment on the defects in mitogen responsiveness and on the reduced levels of natural killing in (*mi/mi*) mice.

Although reduced levels of natural killing are found in association with excess bone in both estrogen-treated mice and in microphthalmic mice, it is unlikely that the loss of natural killing is causally related to the defects in bone growth. First, a reduction in natural killer cells by ^{89}Sr does not substantially alter bone morphology (3). Second, the two animal models reflect different defects; estrogen-treated mice appear to have an excess of bone formation (27), while the primary defect in microphthalmic mice is a lack of bone resorption (21). Further examination of these two animal models, however, should lead to a better understanding of the relation between marrow-dependent cells, bone, and the bone marrow.

REFERENCES

1. Patt, H. M., and M. A. Maloney. 1976. Regulation of stem cells after local marrow injury: the role of an osseous environment. *In* Stem Cells and Renewing Cell Populations. Edited by A. B. Cairnie, P. K. Lala, and D. G. Osmond. Academic Press, New York. P. 239.
2. Trentin, J. J. 1976. Hemopoietic inductive microenvironments. *In* Stem Cells and Renewing Cell Populations. Edited by A. B. Cairnie, P. K. Lala, and D. G. Osmond. Academic Press, New York. P. 255.
3. Jacobson, L. O., E. L. Simmons, and M. H. Block. 1949. The effect of splenectomy on the toxicity of Sr^{89} to the hematopoietic system of mice. J. Lab. Clin. Med. 34:1640.
4. Fried, W., C. W. Gurney, and M. Swatek. 1966. The effect of strontium-89 on the stem cell compartment of the spleen. 1966. Radiat. Res. 29:50.
5. Bennett, M., E. E. Baker, J. W. Eastcott, V. Kumar, and D. Yonkosky. 1976. Selective elimination of bone marrow precursors with the bone-seeking isotope ^{89}Sr: implications for hemopoiesis, lymphopoiesis, viral leukemogenesis and infection. J. Reticuloendothel. Soc. 20:71.
6. Bennett, M. 1973. Prevention of marrow allograft rejection with radioactive strontium: evidence for marrow-dependent effector cells. J. Immunol. 110:510.
7. Haller, O., H. Wigzell. 1977. Suppression of natural killer cell activity with radioactive strontium: effector cells are marrow dependent. J. Immunol. 118:1503.
8. Kumar, V., M. Bennett, and R. J. Eckner. 1974. Mechanisms of genetic resistance to Friend virus leukemia in mice. I. Role of ^{89}Sr-sensitive effector cells responsible for rejection of bone marrow allografts. J. Exp. Med. 139:1093.
9. Lopez, C. 1978. Genetic resistance to HSV-1 in the mouse is mediated by the "M" cell of allogeneic resistance. Fed. Proc. 37:

1560 (Abstr.)
10. Seaman, W. E., M. A. Blackman, T. Gindhart, J. R. Roubinian, J. M. Loeb, and N. Talal. 1978. β-Estradiol reduces natural killer cells in mice. J. Immunol. 121:2193.
11. Dougherty, T. F. 1952. Effect of hormones on lymphatic tissue. Physiol. Rev. 32:379.
12. Gardner, W. U., and C. A. Pfeiffer. 1938. Skeletal changes in mice receiving estrogens. Proc. Soc. Exp. Biol. Med. 37:678.
13. Urist, M. R., A. M. Budy, and F. C. McLean. 1950. Endosteal-bone formation in estrogen treated mice. J. Bone Joint Surg. 32-A:143.
14. Silberg, M., and R. Silberg. 1971. Steroid hormones and bone. *In* The Biochemistry and Physiology of Bone. 2nd ed. Vol. 3. Edited by G. Bourne. Academic Press, New York. P. 401.
15. Roubinian, J. R., N. Talal, J. S. Greenspan, J. R. Goodman, and P. K. Siiteri. 1978. Effect of castration and sex hormone treatment on survival, anti-nucleic acid antibodies, and glomerulonephritis in NZB/NZW F_1 mice. J. Exp. Med. 147:1568.
16. Cudkowicz, G., A. C. Upton, L. H. Smith, D. G. Gosslee, and W. L. Hughes. 1964. An approach to the characterization of stem cells in mouse bone marrow. Ann. N. Y. Acad. Sci. 114:571.
17. Bennett, M., G. Cudkowicz, R. S. Foster, Jr., and D. Metcalf. 1974. Hemopoietic progenitor cells of *W* anemic mice studied *in vivo* and *in vitro*. J. Cell Physiol. 71:211.
18. Boggs, S. S., D. R. Boggs, G. L. Neil, and G. Sartiano. 1973. Cycling characteristics of endogenous spleen colony-forming cells as measured with cytosine arabinoside and methotrexate. J. Lab. Clin. Med. 82:727.
19. Lotzová, E., and G. Cudkowicz. 1973. Resistance of irradiated F_1 hybrid and allogeneic mice to bone marrow grafts of NZB donors. J. Immunol. 110:791.
20. Murphy, H. M. 1973. The osteopetrotic syndrome in the microphthalmic mutant mouse. Calcif. Tissue Res. 13:19.
21. Marks, S. C., Jr., and D. G. Walker. 1976. Mammalian osteopetrosis—a model for studying cellular and humoral factors in bone resorption. *In* The Physiology and Biochemistry of Bone. 2nd ed., Vol. 4. Edited by G. Bourne. Academic Press, New York. P. 227.
22. Briganti, G., V. Corelli, G. Silini, and P. N. Srivastava. 1970. The distribution of erythropoietic bone marrow in the mouse. Acta Haematol. 44:355.
23. Morse, B. S., D. Giuliani, M. Soremekun, S. Defino, and E. R. Giuliani. 1974. Adaptation of hemopoietic tissue resulting from estrone-induced osteosclerosis in mice. Cell Tissue Kinet. 7:113.
24. Fried, W., T. Tichler, I. Dennenberg, J. Barone, and F. Wang. 1974. Effects of estrogens on hematopoietic stem cells and on hematopoiesis of mice. J. Lab. Clin. Med. 83:807.
25. Nutik, G., and R. L. Cruess. 1974. Estrogen receptors in bone. An evaluation of the uptake of estrogen into bone cells. Proc. Soc. Exp. Biol. Med. 146:265.
26. Budy, A. M. 1960. Skeletal distribution of estrone-16-C^{14}. Clin. Orthop. 17:176.
27. Simmons, D. J. 1963. Cellular changes in the bones of mice as studied with tritiated thymidine and the effects of estrogen. Clin. Orthop. 26:176.
28. Olsen, C. E., S. M. Wahl, A. L. Sandberg, and S. E. Mergenhagen. 1978. Immunologic defects in osteopetrotic mice. *In* Mechanisms of Localized Bone Loss. Edited by J. E. Horton, T. M. Tarpley, and W. F. Davis. Special Supplement to Calcified Tissue Abstracts. Information Retrieval, Inc., Washington, D. C. P. 389.
29. Milhaud, G., M.-L. Labat, M. Parant, C. Damais, and L. Chedid. 1977. Immunological defect and its correction in the osteopetrotic mutant rat. Proc. Natl. Acad. Sci., 74:339.
30. Walker, D. G. 1975. Control of bone resorption by hematopoietic tissue. The induction and reversal of congenital osteopetrosis in mice through use of bone marrow and splenic transplants. J. Exp. Med. 142:651.
31. Loutit, J. F., and J. M. Sanson. 1976. Osteopetrosis of microphthalmic mice—a defect of the hematopoietic stem cell? Calcif. Tissue Res. 20:251.
32. Nisbet, N. W., J. Menage, and J. F. Loutit. 1978. Host-donor cellular interactions in the treatment of experimental osteopetrosis. Nature 271:464.