Effect of Estrogen on Bone Mineral Turnover in Mature Female Rats as Measured by Strontium-85[1] (35164)

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(Introduced by H. H. Draper)

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The action of estrogens on bone metabolism throughout gestation is of interest because of their involvement in the regulation of blood calcium concentration. It has been suggested, for example, that the skeletal end organ response to parathyroid hormone may be depressed in parturient cows, especially those that develop hypocalcemia and parturient paresis or milk fever (1, 2). Two reports (3, 4) presented evidence that pointed to an antagonism between the actions of parathyroid hormone and estrogen on skeletal tissue. Stimulation of bone resorption by parathyroid hormone is well known, but the effect of estrogen on bone is not so clearly established. Lindquist *et al.* (5) concluded that resorption of bone in estrogen-treated immature rats was inhibited only in regions of bones not undergoing remodeling.

Investigations in man have also supported the idea that estrogen inhibits bone resorption (6–8). Recently, Tapp (9) has provided evidence that estrogen inhibits bone resorption; and Woessner (10) has generalized from studies of rat uterine involution that estrogen blocks the metabolic degradation of collagen in various tissues, including bone.

The present experiments were undertaken to test the hypothesis that daily injections of physiological doses of estrogen cause an enhanced retention of bone mineral, as measured by whole-body retention of strontium-85. ^{85}Sr was chosen as the radionuclide because it is an excellent tracer of calcium and it is a photon-emitter, which facilitates estimation of whole-body radioactivity for a prolonged period of time.

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Materials and Methods. Mature female and mature male rats only were used in these studies. In Expt. 1 one half (12 rats) of a group of female Sprague-Dawley rats (Madison, Wisc.) were castrated prior to administration of ^{85}Sr, whereas the other half (12 rats) of the females and 9 male rats (also Sprague-Dawley) remained intact. In Expt. 2, bilateral ovariectomy was performed on 20 female Holtzman rats (Madison, Wisc.), less than 2 weeks prior to ^{85}Sr injection, whereas 20 other female Holtzman rats served as unoperated controls. The group of castrate rats was further subdivided, so that 10 rats were placed on a restricted dietary intake of 15 g of rat chow/day (about 80% of the *ad libitum* feed intake of controls). Otherwise all rats in Expts. 1 and 2 were offered food and water *ad libitum*. Purina rat chow was used exclusively (Ralston-Purina, St. Louis). The animals were housed individually in wire cages and were weighed daily.

Subcutaneous injections of 5 μg of 17-β-estradiol benzoate (Mann Laboratories, New York) in 0.5 ml of vegetable oil were administered to half of the castrate and normal rats in Exp. 2 (see Table II for precise schedule of subgroups) on each day of the 45-day experimental period. Injections were delivered on alternate sides of abdomen.

Experiments 1 and 2 began on the day of administration of ^{85}Sr, which was designated as day 0. The ^{85}Sr dose (Nuclear Science and Engineering, Pittsburgh), 25 μCi in Expt. 1 and 5 μCi in Expt. 2, was administered intraperitoneally as the chloride (pH 6).

In both experiments on days 0 through 5, and then weekly to day 45, rats were placed in a small plastic cup with perforated lid

TABLE I. Mean Fractional Whole-Body Retention (± SEM) of Strontium-85 and Weight Changes of Castrate and Normal Mature Female Rats and of Normal Mature Male Rats (Sprague-Dawley) Over the 45-Day Experimental Period.

	Group		
	1 ($N = 12$) Normal females	2 ($N = 12$) Castrate females	3 ($N = 9$) Normal males
Day PI			
1	0.44 ± 0.02	0.46 ± 0.01	0.56 ± 0.02
45	0.17 ± 0.01[b]	0.19 ± 0.01[a]	0.27 ± 0.01[ab]
Net change in wt (g)	254–268 +14	283–320 +37	363–412 +49
Mean R_t/mean wt on day 45 ($\times 10^{-4}$)	6.3	5.9	6.5

[ab] Significantly different ($p < .01$).

(cottage cheese type) and whole-body counted at a distance of 8 in. from a lead-shielded solid 1.5 × 1.5-in. sodium iodide (thallianated) scintillation counting system (Baird Atomic, Cambridge, Mass.). An ^{85}Sr dose similar to that administered to the animals was distributed in a volume approximating that of a rat and thus served as a machine standard. Radioactivity in the standard was measured as counts per minute (cpm) each day the rats were counted and whole-body fractional retention (R_t) at any time, t, was calculated as follows:

$$R_t = \frac{\text{animal cpm at time } t}{\text{animal cpm at time 0}} \times \frac{\text{standard cpm at time 0}}{\text{standard cpm at time } t}.$$

At the conclusion of Expt. 2, both femurs were removed from all rats, carefully cleaned of soft tissue, and placed in vials for counting in a well-type NaI (Tl) crystal scintillation detector (Nuclear-Chicago, Chicago). Tests of significance were made by Duncan's new multiple-range test.

Results. Expt. 1. Table I presents the mean fractional ^{85}Sr retention and the standard error of the mean of mature castrate and normal female and normal male Sprague-Dawley rats. Retention by the males on day 45 postinjection was significantly ($p < .01$) greater than that of the females, whether castrate or not. Castration appeared to have no effect on retention, but the castrate rats were hyperphagic compared to the normal unoperated females, as evidenced by their net gain in weight, by a factor of nearly 3 greater than the normals (Table I). Thus, calculation of mean retention divided by mean weight on day 45 revealed a lower value for the castrates, 5.9×10^{-4}, compared to normal females, 6.3×10^{-4}, and normal males, 6.5×10^{-4}.

Expt. 2. Mean fractional ^{85}Sr retention (± SEM) of mature castrate and normal female Holtzman rats is given in Table II. At day 45, retention in the untreated castrates was the same as in the untreated normal rats, whereas retention was significantly greater by 12 to 15% in rats treated with a physiological dose of estrogen whether castrate or not. Restriction of dietary intake caused an even greater retention (24%) in estrogen-treated rats compared to untreated rats allowed to feed *ad libitum*.

Table II also shows the net changes in body weight during the course of the experiment. The two groups of estrogen-treated castrate rats demonstrated declines in weight, whereas the untreated castrate rats feeding *ad libitum* were hyperphagic and increased in weight. In the normal rats weight gain was depressed by half (about 1 g/day) in those administered estrogen compared to those not. When mean retention divided by mean weight on day 45 (Table II) was calculated for each group, the greatest values were found for the estrogen-treated groups and the least value for the untreated castrate group

TABLE II. Mean Fractional Whole-Body Retention (± SEM) of Strontium-85 and Weight Changes of Castrate and Normal Mature Female Rats (Holtzman) Over the 45-Day Experimental Period.

	Group					
	1 ($N = 5$)	2 ($N = 4$)	3 ($N = 5$)	4 ($N = 4$)	5 ($N = 10$)	6 ($N = 7$)
Diet:	*Ad libitum*		Restricted		*Ad libitum*	
Treatment:	None Castrate	Estrogen Castrate	Estrogen Castrate	None Castrate	None Normal	Estrogen Normal
Day PI						
1	0.55 ± 0.03	0.56 ± 0.04	0.61 ± 0.01	0.55 ± 0.1	0.52 ± 0.02	0.56 ± 0.02
45	0.29 ± 0.01[a]	0.33 ± 0.02[bc]	0.38 ± 0.01	0.30 ± 0.01[ab]	0.29 ± 0.01[a]	0.34 ± 0.01[c]
Net change in wt (g)	285–351 +66	288–279 −9	276–264 −12	266–290 +24	264–305 +41	263–283 +20
Mean R_t/mean wt on day 45 ($\times 10^{-4}$)	8.3	11.8	14.4	10.3	9.5	12.0

[abc] Day 45 means bearing a common superscript letter are not significantly different ($p < .05$).

on an *ad libitum* dietary intake.

It should be noted that ^{85}Sr retention by female Sprague-Dawley rats in *Expt. 1* was considerably less than that of female Holtzman rats of approximately the same weight and age whether castrated or not (Tables I and II). Additionally, Sprague-Dawley males, although approximately 100 g heavier, retained significantly more ^{85}Sr in their skeletons than Sprague-Dawley females (Table I). No satisfactory reasons can be offered to explain these discrepancies.

Table III lists the mean radioactivity of ^{85}Sr (cpm) in the femurs removed from the rats of all groups at the conclusion of the 45-day experimental period. With the exception of group 2, femurs of rats treated with estrogen had significantly more radioactivity than did similar rats not receiving estrogen. The small sampling of only 4 rats may have contributed to this unexpected finding. Femur radioactivity was in general agreement with whole-body radioactivity on a group basis.

Discussion. The major findings of these experiments are that daily estrogen treatment of mature female rats caused an increase in skeletal retention of ^{85}Sr at 45 days PI compared to nontreated females whether castrate or not and that a restriction of dietary intake further enhanced the retention of ^{85}Sr in estrogen-treated castrate rats (Table II). Ovariectomy itself had no effect on retention, but castrate rats became hyperphagic which lowered the value of their mean R_t/mean wt (Table I). Thus, some castrate rats were

TABLE III. Mean Radioactivity (cpm ^{85}Sr) in Femurs Removed from Castrate and Normal Mature Female Rats at Conclusion of 45-Day Experimental Period.

	Group					
	1	2	3	4	5	6
Status:	Castrate				Normal	
Feed:	*Ad libitum*		Restricted		*Ad libitum*	
Treatment:	None	Estrogen	Estrogen	None	None	Estrogen
N	5	4	5	4	10	7
Net cpm	17,732[ab]	16,624[ab]	19,744[b]	14,661[a]	16,123[a]	19,654[b]

[ab] Net cpms bearing a common superscript letter are not significantly different ($p < .05$).

subsequently restricted in their food intake, which only enhanced ^{85}Sr retention at 45 days.

These observations that estrogen enhanced the whole-body ^{85}Sr retention in mature female rats over a 45-day period are in accord with the findings of other investigators using different methods of ^{45}Ca bone and blood measurements (3–5) and of tetracycline measurements (9). Our data suggest that estrogen acted by inhibiting bone resorption but, since our rats were mature females with a low rate of bone turnover, unlike the immature rats of Lindquist *et al.* (5), stimulation of bone accretion cannot be ruled out as an alternative effect of estrogen. However, resorptive mechanisms should not be excluded from consideration of the action of estrogen as they appear to have been by Ranney (4) in a much shorter time study than ours. A more likely explanation of bone turnover, as suggested by Woessner (11), is that inhibition of bone resorptive processes would be accompanied concurrently by a reduction in bone accretion resulting in a net decrease in bone turnover. If the less likely opposite case had occurred, namely, increased resorption accompanied by increased accretion, a greater loss of bone-incorporated ^{85}Sr in the treated rats might be anticipated over the 45-day experimental period than was observed.

The parathyroids were intact in all rats and there was no indication of other than normal stimulation of them. Dietary Ca and phosphorus were adequate and estrogen-enhanced mineral retention, though significant, would not appear to have been a major triggering event sufficient to stimulate homeostatic readjustment via the parathyroids. However, such might be the case in parturient hypocalcemic cows when estrogen levels are known to be high. Future experiments will be undertaken to investigate the interaction of estrogen and parathyroid hormone.

Summary. On a per weight basis, normal mature male and female rats retained the same amount of ^{85}Sr at 45-days postinjection, whereas hyperphagic castrate females retained less. However, no difference in ^{85}Sr retention was observed between castrate rats on an *ad libitum* diet and those on a restricted diet. When daily injections of a physiological dose (5μg) of estradiol were administered, retention was (i) significantly greater (12–15%) in estrogen-treated castrate and normal rats on an *ad libitum* intake compared to untreated rats; (ii) significantly greater (24%) in estrogen-treated castrate rats on a restricted intake compared to untreated castrate rats on an *ad libitum* intake; and (iii) generally significantly greater in the femurs of estrogen-treated rats compared to untreated rats. Possible mechanisms by which retention was enhanced by the action of estrogen are discussed.

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