Relationship Between Estradiol and Parathyroid on Retention of Ca^{45} in Bone and Blood Serum of Rats.* (23087)

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In several species the parathyroid glands of female animals have been reported to be heavier than the male of equal body weight. These reports include data on rats(1-4), man (5,6), and Brown Leghorn fowls(7). In rabbits, goats(4) and bantams(8) no sex difference was observed. The reported influence of ovarian hormones on calcium metabolism has been variable. In the rat, estrogen has been reported to increase blood calcium (9,10), or have little or no effect(11-14). In the guinea pig no effect was observed(4). In rabbits, the reports varied from no effect(9, 12,13,15) to decrease in blood calcium(16-18). In dogs, the reports vary from an increase(9,19,20) to no effect(12,21). In cattle a slight decrease in blood calcium was noted(22,23). These variable results may be due to the use of non-physiological doses of estrogen or to other unknown factors. The osteogenetic properties of estrogenic hormones in birds, mice and rats have been reported. A recent review of this literature has appeared(24). Recently, it was demonstrated in rats that after parathyroidectomy females lived longer than males(25). If, in addition to parathyroidectomy, the females were previously ovariectomized, their life span was as short as the males(26,25), and if the males were treated with a small quantity of estradiol, they lived as long as non-castrated females(27).

In the light of variable observations concerning the role of estrogenic hormone in calcium metabolism, it seemed desirable to extend the previous observations using Ca^{45} as a measure of effect of estrogen.

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Procedure. Thirty-nine adult male albino rats were divided into 6 groups and treated as indicated (Table I). Prior to and during the experimental period the animals were fed a stock diet. All animals were housed in wide-mouthed glass jars which contained wire screens approximately 2 inches from the bottom and prevented the rats from eating their feces. Estradiol benzoate was injected subcutaneously daily, at a level of 82.5 μg in 0.25 ml of sesame oil. Parathyroid extract was administered subcutaneously daily at a level of 50 U.S.P. parathyroid hormone units. Parathyroidectomized rats were treated for 2 days post-operatively with Penicillin G. Ca^{45}, in the form of $CaCl_2$, (specific activity >0.2 mc/g of Ca) was injected intraperitoneally at total dosage of 2.65 μc/100 g body weight during 4 days. Considering radioactive decay each rat received a total of approximately 20 to 28 mg of calcium/100 g body weight. Throughout all operational procedures of parathyroidectomy or of blood sampling the anesthetic was chloroform. The animals were killed 2 days after last injection by exsanguination from the left carotid artery. After centrifugation of the clotted blood 2 aliquots of 0.5 ml of serum were dried in metal counting cups and radioactivity measured in duplicate. To measure radioactivity in bone, the right femur was carefully cleaned of muscular and tendinous tissue, weighed and completely digested (approximately 24 hours) in 2 ml of hydrochloric acid (1.18 sp. gr., 37% HCl). The digest was filtered through glass wool and the filtrates diluted to 10 ml with distilled water which had been used to wash the digestion flask and digest retained on glass wool. From this solution 3-1 ml aliquots were dried in glass counting cups and the radioactivity of the femur measured in triplicate. Corrections were not made for sample self-absorption.

Results. Mean values in counts per min-

TABLE I. Radioactivity of Serum and Bones of Male Rats Injected with Ca^{45} for 4 Days.

Group	Treatment	Bone, mean count/min./g (± S.D.)	Serum, mean count/min./ml (± S.D.)
1	Control (8)‡	21168 ± 4143.9	199 ± 42.1
2	Estradiol (8)	20233 ± 3454.8	†344 ± 61.5
3	Parathyroidectomy (6)	*16254 ± 2082.0	174 ± 52.4
4	" + estradiol (7)	17309 ± 3691.6	†300 ± 113.4
5	Parathyroid extract (4)	†12602 ± 1003.6	171 ± 30.1
6	" " + estradiol (6)	*16076 ± 2607.2	257 ± 74.4

* $P < 0.05$. † $P < 0.01$. ‡ No. of animals in group.

ute and their standard deviations (σ) for bone and serum are presented (Table I). The difference in the amount of Ca^{45} found in bone of estrogen-treated and control groups was not significant.

After parathyroidectomy the amount of Ca^{45} present in bone was decreased ($P<0.05$) confirming the results of Talmage *et al.*(28) who reported a significant decrease in rate of calcium turnover in the absence of parathyroids. Although not significant, injection of estrogen tended to increase the uptake of Ca^{45} in parathyroidectomized animals.

Administration of parathyroid extract caused a significant ($P<0.01$) decrease in the amount of Ca^{45} present in bone. Employing autoradiographic technic, Talmage *et al.*(28) similarly found that 24 hours after treatment with parathormone there was a detectable amount of Ca^{45} removed from the bone.

Comparison of Groups 1, 5 and 6 demonstrates that when estrogen is administered to rats receiving parathyroid extract, there was a tendency to inhibit removal, or induce deposition of Ca^{45} in bone. Inhibition of parathormone action on bone demineralization was not complete as seen by the fact that the difference between the controls and group(6) treated with both hormones was still significant ($P<0.05$).

Following treatment with estradiol the amount of Ca^{45} still present in serum at the time the rats were killed was significantly higher than in the control group ($P<0.01$). Although this confirms the findings of Manunta(29) who reported that in rats treated with synthetic estrogens there was a significant decrease in urinary calcium, it is contrary to the results of other workers who found no difference in serum calcium levels of rats similarly treated. Re-examination of the controversial data showed results that were variable but still within the physiological range and non-significant. It is believed that the different results are due to the different procedures used and sensitivity of methods. To test the sensitivity of the method an *in vitro* technic was employed. To normal blood serum sufficient Ca^{40} and Ca^{45} was added to raise the calcium level 1 mg. The radioactivity of serum was 1.216 $\mu\mu$c/ml. With the apparatus used this gave 180 counts/minute ml. It is evident that this method allows measurements of slight differences in serum Ca^{45} levels.

In the parathyroidectomized - estrogen-treated group Ca^{45} was higher in blood and the differences statistically different from control and parathyroidectomized groups. The latter group had a lower amount of serum Ca^{45}, but it was not statistically different from the controls, probably because of a continuous removal from the bone.

It has been known and recently reaffirmed (28,30) that parathyroid extract causes a rise in serum calcium. Under the same treatment (Group 5) a non-significant decrease was found. This is not interpreted as a lack of hypercalcemia, but as a prompt excretion of Ca^{45}. The simultaneous treatment with estrogen tended to increase the serum level of Ca^{45} (Group 6).

The present experiment supports the belief that estrogen acts independent of the parathyroids to influence calcium metabolism in bone and serum. From these observations it appears that the 2 hormones may be antagonistic in some aspects of calcium metabolism, and a proper balance is necessary for normal calcium equilibrium. If the estrogenic action

is pronounced, calcium deposition takes place in bones in greater amount and the excretion of this element is decreased. When the parathyroidal secretion predominates, calcium excretion is increased but may be retarded by estrogen. Estrogen also increases the absorption of calcium from the gut (Dallemagne *et al.*)(30), and parathyroidectomy or estrogenic treatment influences the proteins binding capacity of calcium in blood serum(31).

Summary. Administration of estrogen to normal male rats increased the radioactivity of blood serum following Ca^{45} injection, whereas bone remained unchanged. Parathyroid extract depressed radioactivity of both bone and blood serum but when estrogen was given in addition radioactivity was increased in both. Parathyroidectomy depressed the radioactivity of bones and serum but estrogen again increased the radioactivity of bones slightly and serum significantly. These data indicate that estrogen plays a role in calcium metabolism independent of the parathyroid hormone in aiding retention of calcium.

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