

1974

Effects of Estrogen and Progesterone on Serum Ionized and Total Calcium in Ovariectomized Rats

Edward L. Hatcher
University of Northern Iowa

Robert J. Simpson
University of Northern Iowa

Copyright ©1974 Iowa Academy of Science, Inc.

Follow this and additional works at: <https://scholarworks.uni.edu/pias>

Recommended Citation

Hatcher, Edward L. and Simpson, Robert J. (1974) "Effects of Estrogen and Progesterone on Serum Ionized and Total Calcium in Ovariectomized Rats," *Proceedings of the Iowa Academy of Science*, 81(2), 68-71.

Available at: <https://scholarworks.uni.edu/pias/vol81/iss2/8>

This Research is brought to you for free and open access by the Iowa Academy of Science at UNI ScholarWorks. It has been accepted for inclusion in Proceedings of the Iowa Academy of Science by an authorized editor of UNI ScholarWorks. For more information, please contact scholarworks@uni.edu.

Effects of Estrogen and Progesterone on Serum Ionized and Total Calcium in Ovariectomized Rats

EDWARD L. HATCHER¹ and ROBERT J. SIMPSON²

HATCHER, EDWARD L., and ROBERT J. SIMPSON (Department of Biology, University of Northern Iowa, Cedar Falls, Iowa 50613). Effects of Estrogen and Progesterone on Serum Ionized and Total Calcium in Ovariectomized Rats. *Proc. Iowa Acad. Sci.* 81(2): 68-71, 1974.

The effects of estrogen and estrogen plus progesterone on the serum ionized calcium levels were studied in ovariectomized rats. Concentrations of total and ionized calcium were measured in the serum of rats following three- and six-day treatments with estrogen (0.1 μ g daily). Concentrations of ionized calcium in estrogen-treated ovariectomized rats were significantly higher than in

controls and significantly higher values were noted in the ionized: total serum calcium ratios in estrogen treated rats as compared with controls. The addition of progesterone to the estrogen treatment procedure resulted in increased total and ionized serum calcium with the ionized values remaining significantly higher than in the ovariectomized controls. The results clearly indicate an estrogenic effect on calcium metabolism resulting in a significant increase in the ionized serum calcium. Suggested possible estrogen and progesterone mechanisms of action are given.

INDEX DESCRIPTORS: Total Serum Calcium, Ionized Serum Calcium, Estrogen, Progesterone, Ovariectomy, Rats.

The total serum calcium in the body can be broken down into three distinct calcium fractions: nondiffusible calcium, composed primarily of protein-bound calcium; diffusible non-ionized calcium, primarily in complexes, chelates, etc.; and ionized calcium. Of these three fractions, the nondiffusible form makes up approximately 30-35 percent of the total, and the diffusible non-ionized portion represents about 5-15 percent of the total (Lloyd and Rose, 1958; Moore, 1970).

Of the three major serum calcium fractions, the physiologically active component is the ionized fraction, and it has been clearly demonstrated to play the central role in overall calcium metabolism and to be under the influence of the known calcium regulatory factors (Hirsch and Munson, 1969).

There is also evidence in the literature indicating that estrogens are a possible factor in calcium homeostasis. Estrogen effects on serum calcium and bone metabolism have been described in birds (Riddle and McDonald, 1945; Riddle, Rauch and Smith, 1945). Estrogens have also been shown to participate in the serum calcium regulation in fish (Hess, 1928; Bailey, 1957) and in amphibia (Zwarenstein and Shapiro, 1933; Clark, 1967).

The role of estrogens in mammalian calcium metabolism is less certain, and workers have disagreed regarding an estrogenic influence (Riddle and Dotti, 1936; Gardner and Pfeiffer, 1938; Urist, Budy and McLean, 1948). Work in mice and rats indicates that estrogens influence calcium metabolism (Manunta, Saroff and Turner, 1957; Ranney, 1959) and positive calcium balance has been reported in some osteoporotic individuals (Geschwind, 1961; Young *et al.*, 1968).

The experiments reported here were undertaken in an attempt to describe the possible effects of estrogen treatment

on total and ionized serum calcium. In addition, the effects of combining progesterone treatment with estrogen were investigated.

MATERIALS AND METHODS

Female Holtzman rats (Madison, Wisconsin) were ovariectomized using pentobarbital sodium anesthesia. After a minimum of seven days, when the animals weighed 200-220 gm, the rats were grouped for treatment as follows: three- and six-day estrogen treatment and controls and six-day estrogen and progesterone treatment plus controls. Throughout the experimental period the animals were maintained on Purina Laboratory Chow (Ralston-Purina, St. Louis) and tap water *ad libitum*.

The first day of estrogen administration was designated day 0. Hormones were injected subcutaneously in the right shoulder. Daily injections of estradiol 17- β -cyclopentylpropionate (Depo®-Estradiol Cypionate, Upjohn Company, Kalamazoo, Michigan) amounted to 0.1 μ g dissolved in 0.1 ml of sesame oil. A dose of 0.05 ml was given twice daily. Control rats were given sesame oil injections in the same volumes.

The six-day estrogen and progesterone treatment was carried out fundamentally the same way as indicated above for estrogen treatment alone. Progesterone treatment (Progesterone, Eli Lilly and Company, Indianapolis) consisted of a daily injection of 0.25 mg (0.25 mg = 0.1 ml) given in the same manner as for estrogen. The injection was given between the two half dosages of estrogen, and control rats were given the sesame oil vehicles.

Blood samples were collected at the end of the three- and six-day periods by cardiac puncture and were centrifuged for approximately five minutes at 1300g. A portion of the plasma obtained was immediately analyzed for ionized calcium (recorded in mg/100 ml) by a potentiometric method using an Orion Model 99-20 Calcium Activity Flo-Thru System. The remaining portion of the plasma was stored at 0° C for total serum calcium determination, which was made by atomic absorption spectroscopy procedures outlined by Sunderman and Carroll (1965). The serum samples used in this procedure were diluted 1:50 with distilled water (0.2

This work was supported in part by NSF Grant GY-4704.

¹ This paper presents work required for partial fulfillment of the requirements for the degree Master of Arts in Biology. Address: Department of Biology, University of Northern Iowa, Cedar Falls, Iowa 50613.

² Address reprint request to Robert J. Simpson, Department of Biology, University of Northern Iowa, Cedar Falls, Iowa 50613.

ml serum in 10 ml volume) with 1.0 ml of lanthanum chloride (5 percent) added to each sample as a phosphate binder. The total calcium in each sample was read from a standard curve and calculated in mg/100 ml.

The data obtained were subjected to analysis and the differences in means were compared using the Student's t-test.

RESULTS

Daily injections of estrogen resulted in a significant rise in the serum ionized calcium of the ovariectomized rats (Table 1). After three days the ionized calcium values had increased from a mean value of 5.70 mg/100 ml to a mean value of 6.36 mg/100 ml. This represented a significant increase at the $p < 0.001$ level. Continued injections of estrogen up to six days further increased the level of ionized calcium to 6.51 mg/100 ml, but the rate of increase had declined with no significant difference in rats treated for three or six days.

At the same time that the ionized calcium values showed increases, the total calcium values were decreasing. After the six-day treatment period, total calcium had declined from a normal value of 9.50 mg/100 ml to 8.82 mg/100 ml, a significant decrease at the $p < 0.05$ level (Table 1). Significantly higher values were noted in the ionized:total serum calcium ratios in the estrogen-treated rats as compared to the controls at both three- and six-day treatment periods ($p < 0.001$). The increase was rapid between the initial injection of estrogen and day three of the treatment period (60.2 percent to 70.2 percent) with further increase at a reduced rate (70.2 percent to 74.8 percent) with no significant difference between day three and day six (Table 1).

Progesterone treatment produced further increases in the ionized calcium values in animals treated for six days (Table 1). The ionized calcium values in animals given both hormones were greater than the ionized levels detected with just estrogen treatment alone. There was a significant difference ($p < 0.001$) between those animals having six days of estrogen treatment and those having six days of estrogen plus progesterone treatment, with a serum ionized calcium increase of approximately 1 mg/100 ml.

The most interesting aspect of the addition of progesterone

to estrogen treatment was that the total calcium did not decrease as it did in estrogen treatment alone. Total calcium actually increased slightly over the control value (9.50 mg/100 ml to 10.10 mg/100 ml) but the increase is not significant at the 95 percent confidence level ($p < 0.1$). Since the ionized calcium remained high, the ionized:total calcium ratio remained at approximately the same level as that found in the serum of the rats treated with estrogen alone (Table 1).

DISCUSSION AND CONCLUSIONS

Estrogen influence on serum calcium levels has been clearly demonstrated in egg-laying vertebrates, and effects have also been shown in male birds where the need for calcium for eggshell formation or for yolk is nonexistent (Riddle, 1942). These studies indicate a temporary increase in total calcium. It is also quite evident that estrogen, either directly or indirectly, affects mammalian bone (Urist, Budy and McLean, 1948; Simmons, 1966).

The data presented in Table 1 clearly demonstrate that in estrogen-treated rats the serum ionized calcium values, in general, were increasing. This indicates that the mechanism of action involves a change in the serum ionized-total calcium ratio.

Interpretations of our data resolve themselves into two possible categories of estrogenic effects on calcium metabolism: direct effects on calcium partition in serum and/or in mobilization from bone, and indirect effects mediated via hormones which control calcium metabolism.

With respect to the second type of effects, either parathyroid hormone (PTH) and/or thyrocalcitonin (TCT) could be involved. Since Bernstein *et al.* (1969) noted that TCT decreased serum ionized calcium activity, it could be suggested that our observations mean that estrogen interferes with this action of TCT. Raman (1971) observed that TCT at sufficient dose levels decreased both total and ionized calcium, and Sorensen and Hindberg (1971) reported that short- and long-term estrogen treatment in rats resulted in decreased responsiveness to TCT, as measured by changes in total serum calcium. Since our data indicated that total and

TABLE 1. MEAN VALUES OF SERUM TOTAL AND IONIZED CALCIUM IN OVARIECTOMIZED RATS

Treatment	Number of Rats	Total Calcium mg/100 ml \pm SD	Ionized Calcium mg/100 ml \pm SD	Bound Calcium mg/100 ml	Ionized Calcium/Total Calcium (Percent) \pm SD
Control	32	9.50 \pm 0.69	5.70 \pm 0.44	3.80	60.19 \pm 5.66
Three Day Estrogen	17	9.10 \pm 0.80	6.36 \pm 0.40**	2.74	70.27 \pm 6.34**
Six Day Estrogen	16	8.82 \pm 0.77***	6.51 \pm 0.40**	2.31	74.28 \pm 7.31**
Six Day Estrogen Plus Progesterone	14	10.10 \pm 0.72*	7.51 \pm 0.57**	2.59	74.79 \pm 8.24**

* Significant increase over control, $p < 0.1$.
 ** Significant increase over control, $p < 0.001$.
 *** Significant decrease over control, $p < 0.05$.

ionized calcium moved in opposite directions, it is difficult to conclude satisfactorily that the estrogen influence on calcium is by means of an interaction with TCT.

It was also difficult to explain our observations by singling out a potential estrogen-parathyroid interaction. Orimo *et al.* (1972) suggest that estrogen absence (*i.e.*, ovariectomy) enhanced PTH-induced resorption of bone. If one assumes the converse regarding estrogen presence, our data on total calcium can be explained. However, it is still not clear why the serum ionized calcium values increase during bone deposition unless this effect is coupled with a simultaneous enhancement of intestinal calcium absorption. It would be further assumed that the additional calcium absorbed remained largely in ionized form. An enhancement by estrogen-gestogen treatment has been observed (Caniggia *et al.*, 1970) but there is no clear evidence to suggest that only ionized calcium increases or that estrogens alone cause enhancement.

Direct effects of the estrogen on partition of calcium in the serum offer a possible explanation for our results. Very little data are reported in this area. Polin and Sturkie (1957) reported significant fluctuations in diffusible calcium levels in estrogen-treated chickens, but they did not feel that these fluctuations were large enough to affect the total calcium levels.

Manunta *et al.* (1957) have reported that estrogen affected the serum protein binding of calcium and specifically that in estrogen treatment there is a shift of calcium binding from albumin and α -globulin to binding by β - and λ -globulins. Thus far, protein binding of calcium has not been studied in our experiments, so that the possibility remains open that the estrogen affects the ionized to total calcium ratios through a possible alteration in the ability of serum proteins to bind calcium. If the initial effect of estrogen is on calcium binding capacity, then one may observe increased excretion of calcium (and thus lower total calcium) and a concomitant PTH stimulation resulting in mobilization of bone calcium with an increase in the percentage of ionized calcium. While this seems to be a possible explanation, the observations by Simmons (1966) and others indicating that estrogens stimulate endosteal bone formation is in apparent contradiction to a suggested calcium mobilization from bone.

At the present time, a completely satisfactory theoretical resolution of the problem presented by the observed estrogen-induced changes in the serum ionized: total calcium ratio is not feasible. It is evident that further analysis must consider changes occurring in potential calcium binding elements of the serum (*i.e.*, anions and proteins) and that one must attempt to define the initial change which estrogen induces. It is particularly interesting to note whether the changes in serum calcium are due to changes in partitioning or to effects on the homeostatic controls. The addition of progesterone to the experiment prevents the diminution in the total calcium but seems to raise the ionized values further. Thus, progesterone synergizes with the estrogen mechanism affecting ionized calcium but antagonizes estrogen's effects on total calcium. This probably means that the change in one calcium parameter is secondary to the other. Studies which define the effects of various progesterone/estrogen ratios on calcium partition in the serum are needed for clarification. The observation by Caniggia *et al.* (1970) is helpful in interpreting the increased total serum calcium ob-

served since these authors concluded that intestinal absorption is increased in combined estrogen-gestogen treatment. It will be necessary to determine if estrogen alone has any effect on intestinal calcium absorption under our experimental conditions to assess what effect this aspect of calcium metabolism is having on our observed data.

Our data clearly support the view that estrogen has an effect on calcium metabolism and specifically, in ovariectomized rats, that there is an increase in the ionized calcium fraction. The experiments indicate that attempts to define the mechanism of estrogen effects must consider partitioning between ionized and bound calcium, the possible action of estrogen on intestinal absorption of calcium and the possibility of effects on calcium regulating hormones. In addition, the progesterone modification of the estrogen effects must be considered.

LITERATURE CITED

- BAILEY, R. E. 1957. The effect of estradiol on serum calcium, phosphorus, and protein of goldfish. *J. Exp. Zool.* 136(3):455-470.
- BERNSTEIN, D. S., M. A. ALIAPOLIOS, R. S. HATTNER, A. WACHMAN and B. ROSE. 1969. Serum calcium ion activity: Effects of thyrocalcitonin and parathyroid extract in the rat. *Endocrinology* 85:589-592.
- CANIGGIA, A., C. GENNARI, G. BORRELLO, M. BENCINI, L. CESARI, C. POGGI and S. ESCOBAR. 1970. Intestinal absorption of calcium-47 after treatment with oral oestrogen-gestogens in senile osteoporosis. *Brit. Med. J.* 4(5726):30-32.
- CLARK, N. B. 1967. Influence of estrogens upon serum calcium, phosphate and protein concentrations of freshwater turtles. *Comp. Biochem. Physiol.* 20(3):823-834.
- GARDNER, W. U., and G. A. PFEIFFER. 1938. Skeletal changes in mice receiving estrogens. *Proc. Soc. Exp. Biol. Med.* 37:678-683.
- GESCHWIND, I. I. 1961. Hormonal control of calcium, phosphorus, iodine, iron, sulfur, and magnesium metabolism. Vol. I(B), p. 387-472. In C. L. Comar and F. Bronner, eds. *Mineral Metabolism: An Advanced Treatise*. Academic Press, New York.
- HESS, A. F. 1928. Differences in calcium level of the blood between the male and female cod. *Proc. Soc. Exp. Biol. Med.* 25:349-359.
- HIRSCH, P. F., and P. L. MUNSON. 1969. Thyrocalcitonin. *Physiol. Revs.* 49:548-622.
- LLOYD, H. M., and G. A. ROSE. 1958. Ionized, protein-bound, and complexed calcium in the plasma in primary hyperparathyroidism. *Lancet* 2:1258-1266.
- MANUNTA, G., J. SAROFF and C. W. TURNER. 1957. Metabolism of Ca⁴⁵ in blood, bones, and young of lactating rats treated with estradiol. *Proc. Soc. Exp. Biol. Med.* 94:788-789.
- MOORE, E. W. 1970. Ionized calcium in normal serum, ultrafiltrates and whole blood determined by ion-exchange electrodes. *J. Clin. Investigations* 49(2):318-334.
- ORIMO, H., T. FUJITA, M. YOSHIKAWA and K. HAYANO. 1972. Increased sensitivity of bone to parathyroid hormone in ovariectomized rats. *Endocrinology* 90:760-763.
- POLIN, D., and P. D. STURKIE. 1957. The influence of parathyroids on blood calcium levels and shell deposition in laying hens. *Endocrinology* 60:778-784.
- RAMAN, A. 1971. The effects of thyrocalcitonin on ionic and total plasma calcium in monkeys. *Horm. Metab. Res.* 3:48-51.
- RANNEY, R. E. 1959. Antagonism between estrone and parathyroid extract in their effects upon bone accretion. *Endocrinology* 65:594-601.

- RIDDLE, O. 1942. Cyclic changes in blood calcium, phosphorus and fat in relation to egg laying and estrogen production. *Endocrinology* 31:498-506.
- , and L. B. DOTTI. 1936. Blood calcium in relation to anterior pituitary and sex hormones. *Science* 84:557-559.
- , and M. R. McDONALD. 1945. The partition of plasma calcium and inorganic phosphorus in estrogen-treated normal and parathyroidectomized pigeons. *Endocrinology* 36:48-52.
- , V. M. RAUCH and G. C. SMITH. 1945. Action of estrogen on plasma calcium and endosteal bone formation in parathyroidectomized pigeons. *Endocrinology* 36:41-47.
- SIMMONS, D. J. 1966. Collagen formation and endochondral ossification in estrogen-treated mice. *Proc. Soc. Exp. Biol. Med.* 121(4):1165-1168.
- SORENSEN, O. H., and I. HINDBERG. 1971. Influence of gonadal hormones on the action of calcitonin in the rat. *Acta Endocrinologica* 68:585-596.
- SUNDERMAN, F. W., JR., and J. E. CARROLL. 1965. Measurements of serum calcium and magnesium by atomic absorption spectrometry. *Am. J. Clin. Path.* 43(4):302-310.
- URIST, M. R., A. M. BUDY, and F. C. MCLEAN. 1948. Species differences in the reaction of the mammalian skeleton to estrogens. *Proc. Soc. Exp. Biol. Med.* 68:324-327.
- YOUNG, M. M., C. JASANI, D. A. SMITH and B. E. C. NORDIN. 1968. Some effects of ethinyl oestradiol on calcium and phosphorus metabolism in osteoporosis. *Clin. Sci. (London)* 34(3):411-417.
- ZWARENSTEIN, H., and H. A. SHAPIRO. 1933. Metabolic changes associated with endocrine activity and the reproductive cycle in *Xenopus laevis*. *J. Exp. Biol.* 10:372-378.