Effect of Zinc on Rat Uterine Metabolism

by

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Honors

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ACKNOWLEDGMENT

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INTRODUCTION

The discovery by Zipper (1) that a copper or zinc wire on a polyethylene intrauterine devide (IUD) enhances the contraceptive effect has stimulated interest in these divalent cations as contraceptive materials. The bulk of subsequent research has centered upon the use of copper rather than zinc. These studies have shown that enhancement of the contraceptive effect apparently requires the release of copper ions and is not due to the physical presence of the IUD (2). A brief review of the biological roles of zinc follows.

More than 25 zinc containing proteins, most of which are enzymes, have been identified (3). The integrity and stability of the DNA double helix, ribosomes and biomembranes depend upon zinc (4,5). Plasma zinc concentration decreases to a deficiency range if zinc is removed from the diet. Nonfunctional zinc is tightly bound and cannot be shifted within the body to meet crucial needs (6).

At a low concentration (2μM), zinc inhibits the electron transport chain in mitochondria. The low respiratory activity of spermatozoa may be due to the high cellular content of zinc. This suggests a specific and effective role of zinc in the control of respiration (4).

Many studies have shown that the contraceptive effect is manifested through an action on the endometrium by rendering it hostile to implantation (7). Hagenfeldt (8) has shown that a

continuous decrease of the zinc concentration in the secretory endometrium occurs when the copper IUD is *in situ*. This suggests a decrese in the synthesis of zinc containing enzymes, which may contribute to the contraceptive effect. Oster has reported the inhibition of the activities of the zinc enzymes alkaline phosphatase and carbonic anhydrase(9). Other possibilities explaining this contraceptive effect, such as the role of estradiol action and possibly its uptake, have been suggested.

Some investigations using rats have indicated a possible alteration of estradiol uptake by uteri exposed to copper IUD's. The results of these experiments are, however, contradictory. Chang and Tatum reported no alteration of uptake (10). Aedo and Zipper found an increse in estradiol uptake by the uterus (11), whereas Ghosh found a decrease in uptake (12).

It has been found that *in vitro* binding of estradiol to uterine receptors is affected by divalent cations. Emanuel and Oakey found an increase of estradiol binding to uterine tissue, zinc proving to be the most effective of those tested (13). Most researchers have found an increase of binding by divalent cations, though Brecher found zinc to decrease estradiol binding (14).

Therefore, there is good evidence that zinc and copper may affect estradiol binding in the uterus. One of the questions these findings pose is whether this copper and zinc induced binding is of physiological importance. That is, does the increased estradiol binding significantly alter the basal physiological state of the

uterus? If estradiol binding is altered and if that alteration is of physiological importance, then a corresponding effect on uterine metabolism is expected. Nicolette and Gorski have shown that estrogen causes a two fold increase in the incorporation of ^{14}C from labeled glucose into uterine carbon dioxide, lipid, protein and RNA (15). Maxwell (16) has reported that copper elicits a similar stimulation of uterine glucose metabolism. The primary objective of this project was to monitor the incorporation of glucose derived carbon into these metabolic end points.

MATERIALS AND METHODS

Immature (21-26 days of age) Holtzman rats were used in all experiments. The animals were housed in the same room and fed Rat Chow from Carolina Biological Supply ad libitum.

Control animals received a 0.5 ml intraperitoneal injection of 0.9% NaCl two hours before sacrifice. Estradiol treated rats received an 0.5 ml intraperitoneal inhection of estradiol-17β (10 μg/ml). This was prepared by dissolving 10 mg of estradiol in 10 ml of absolute alcohol (1 mg/ml). One tenth of a ml of this solution was added to 10 ml of 0.9% NaCl, resulting in the desired dose of 5 μg estradiol per 0.5 ml of solution. Length of treatment was two hours. Zinc treated rats received a 0.5 ml intraperioneal injection of 4.8 mM of zinc chloride (.156 mg of zinc per dose) six hours before sacrifice. A fourth group of animals was given both zinc chloride and estradiol treatments.

After treatment, the rats were sacrificed by decapitation.

Other methods of sacrifice were considered unsuitable because of undo stress on the animal. This stress would cause the release of a number of hormones which would disrupt the normal state of the animal which in turn could markedly affect the results of the experiment.

After sacrifice, the uteri were excised from the animals and all adhering fat and connective tissue was removed. The tissue was placed in a 25 ml flask containing 2 ml of Robinson's (17) medium which contained 0.125 μci/ml glucose-U-^{14}C (268 mci/mM; New England Nuclear Corporation). Each flask was aerated on ice for one minute with oxygen and sealed with a rubber septum stopper fitted with a plastic well containing a 2" x 3/4" piece of folded filter paper (Whatman #1). The flasks were then placed in a shaking water bath at 37^{o}C for one hour. At the end of this incubation period, 1 ml of 3N H_2SO_4 was injected into the flask through the stopper to kill the tissue and stop uterine metabolism. Then, 0.2 ml of hydroxide of hyamine (a carbon dioxide absorbant) was injected through the stopper onto the filter paper. The flasks were then incubated for 30 minutes, during which time the CO_2 was absorbed on the paper. At the end of this second incubation, the filter paper was removed and placed in a vial containing 10 ml of scintillation fluid (5.0 g PPO and .1 g M_2POPOP per liter). The radioactivity (the amount of ^{14}C trapped on the filter paper in the form of $^{14}CO_2$) was determined by a Packard Tri-Carb liquid scintillation spectrometer.

After the second incubation period, the uteri were blotted

dry, washed twice in a cold isotonic saline solution and stored in 2 ml of 5% trichloroacetic acid (TCA). In order to extract the lipid portion of the tissue, the uteri were macerated in TCA, the resulting homogenate centrifuged and the supernatant discarded. The pellet was again treated with 2 ml of TCA, recentrifuged and the supernatant again discarded. The TCA dissolves any free amino acids and glycogen and in effect removes these compounds from the tissue. Sequential suspension and centrifugation of the pellet in the following organic solvents extracted the lipid portion of the tissue. The pellet was treated with 5 ml of: 100% ethanol, a mixture of two parts 95% ethanol and one part chloroform and two washings of ethyl alcohol. The combined supernatants were collected in a 100 ml beaker and evaporated to dryness under a hood. The resulting dried lipid fraction was redissolved in 1 ml of ethyl ether and transferred to a scintillation vial and evaporated. To each vial 10 ml of scintillation fluid was added and the radioactivity (the amount of ^{14}C incorporated into lipid) was determined.

The remaining pellet after the lipid extraction represents the protein-nucleic acid fracion of the uterine tissue. This was dried at room temperature for 24 hours, then placed in a scintillation vial to which .2 ml of hydroxide of hyamine (also a digester) was added. The vial was capped and placed in a $45^{\circ}C$ oven for 48 hours. Subsequently, 10 mls of scintillation fluid was added and the radioactivity was determined.

In analysis of data, $p<.05$ confidence level was used unless otherwise stated.

RESULTS AND DISCUSSION

Figure 1 illustrates the effect of these treatments on the rate of $^{14}CO_2$ production in the rat uterus. These results indicate that the rate of CO_2 formation in zinc treated rats was significantly greater than in control rats (student's t-test). Figure 1 also shows the expected stimulatory effect of estradiol on the rate of CO_2 formation (15). Treatment with both zinc and estradiol also significantly increased the production of CO_2 as compared to control. Importantly, this increase was not significantly different when compared to those treated with estradiol alone.

The effect of estradiol, zinc chloride, and zinc chloride: estradiol treated rats on lipid production is illustrated in Figure 2. Again, as expected, the estradiol treated animals demonstrated a significant increase of ^{14}C incorporation into lipid (15). The zinc chloride and zinc chloride:estradiol treated animals were not significantly different from control at the $p<.05$ level but were different at the $p<.10$ level. Again, there proved to be no significant difference between the estradiol treated rats and those treated with zinc and estradiol in combination.

Figure 3 illustrates the effect of these treatments on the rate of protein-nucleic acid production. All three treatments demonstrated a significant difference from control, including the expected (15) effect of estradiol. Again, no differenct between zinc

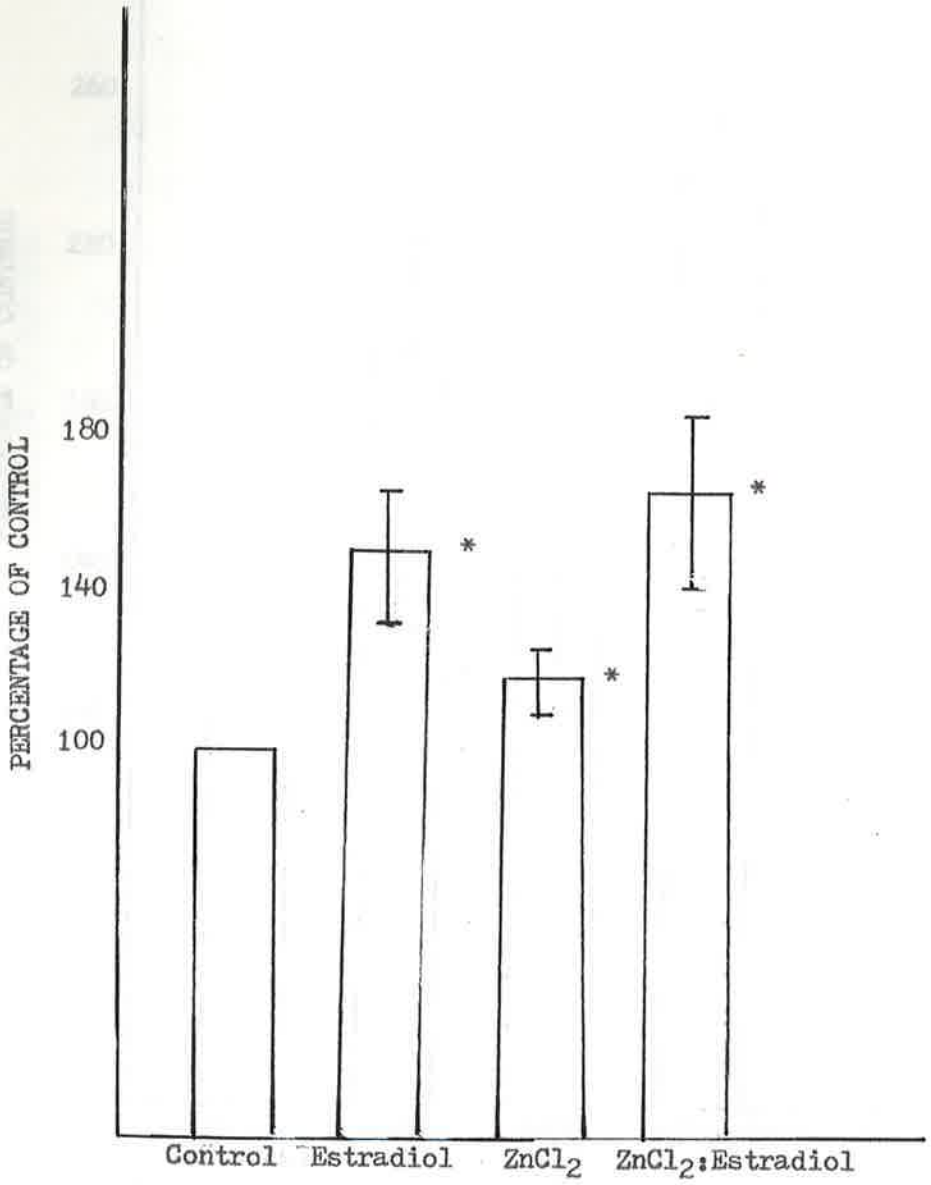

Figure 1. Effect of estradiol, zinc chloride and zinc chloride: estradiol on the rate of $^{14}CO_2$ production in the rat uterus. Each value is the mean (± SE) based on 6 experiments. Values are fiven as percentage of control values which averaged 4747 counts per minute.
* = significantly different from controls ar $p<.05$.

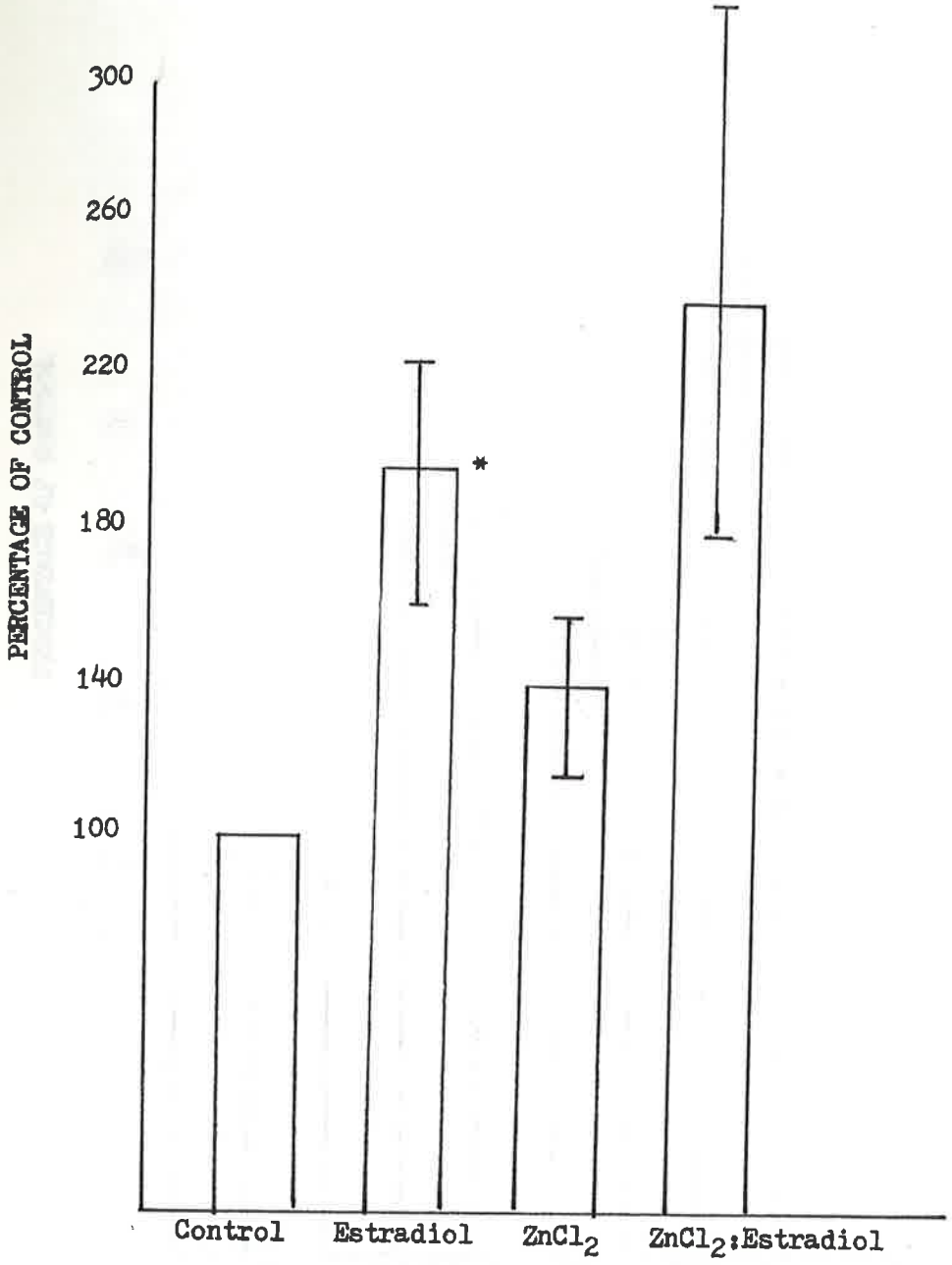

Figure 2. Effect of estradiol, zinc chloride and zinc chloride: estradiol on the rate of lipid production in the rat uterus. Each value is the mean (± SE) based on 7 experiments. Values are given as percentage of control values which averaged 387 counts per minute.
* = significantly different from control at $p<.05$.

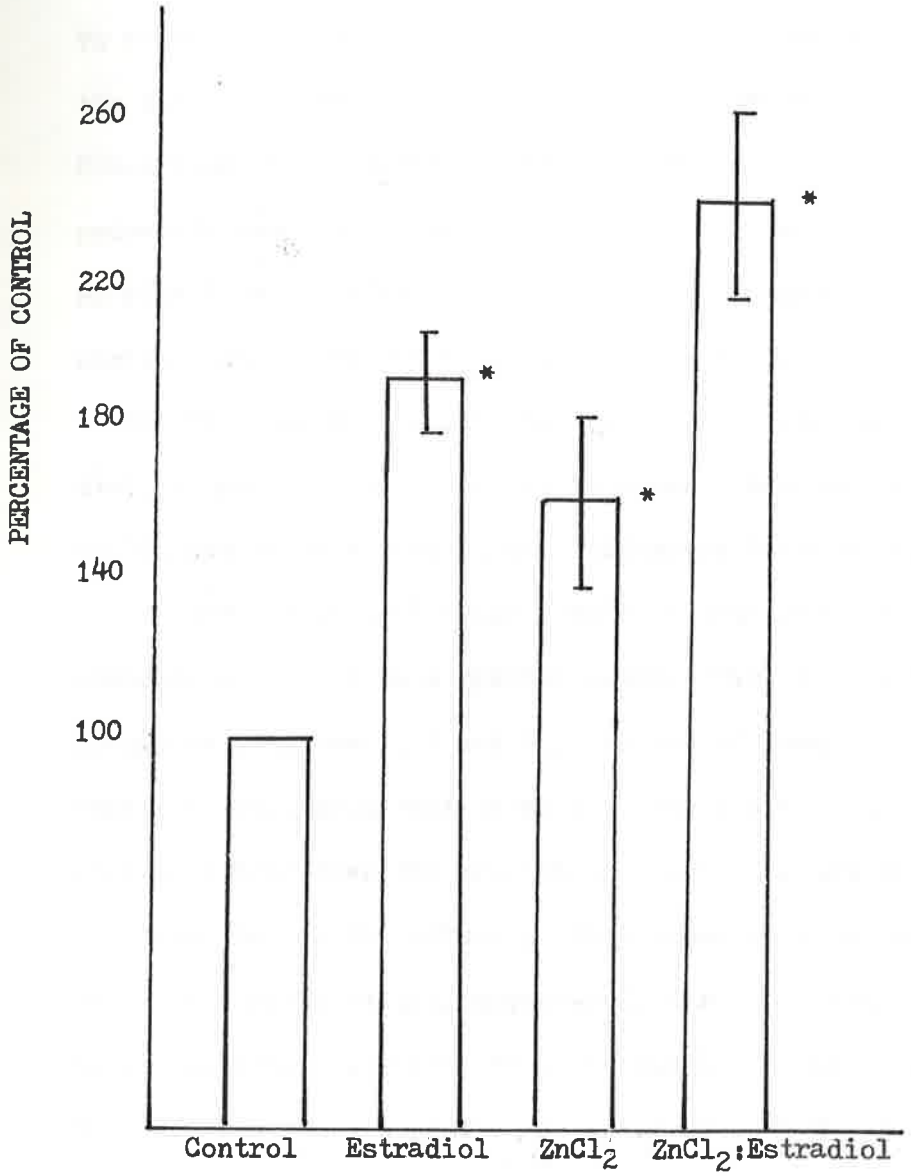

Figure 3. Effect of estradiol, zinc chloride and zinc chloride: estradiol on the rate of protein and nucleic acid production in the rat uterus. Each value is the mean (± SE) based on 7-8 experiments. Values are given as percentage of control values which averaged 815 counts per minute.
* = significantly different from control at $p<.05$.

chloride:estradiol and estradiol treated rats was established.

At this point, one may speculate if zinc follows a similar or different pathway to estradiol when eliciting the above effects. To answer this question, estradiol was considered the control and the zinc chloride:estradiol data calculated as percent of estradiol. Comparison of the means of these groups, for each of the metabolic endpoints (CO_2, lipid and the protein-nucleic acid fraction) showed no significant difference. This indicates that zinc follows a similar pathway as estradiol when stimulating glucose utilization by the rat uterus. If zinc followed a different pathway from estradiol, an additive effect would be evident and the resultant t-test would have shown a significant difference between the two groups.

These experiments also indicate that zinc chloride stimulates anabolic processes to a greater extent than it stimulates catabolic processes (Figures 1, 2 and 3). Smith and Stultz have shown that that estradiol treatment stimulates the anabolic process to a greater extent than the process of glucose oxidation (which is indicated by the CO_2 values). They found that in control uteri, 26% of the radioactivity appeared in the lipid and protein-nucleic acid fraction, while estradiol increased this percent to 36%. Maxwell (16) found a similar shift of glucose metabolism along the anabolic pathway elicited by estradiol and copper. His results showed a 21% incorporation of ^{14}C into lipid and protein-nucleic acid by control uteri. Treatment with copper and estradiol caused an increase in this percentage to 25% and 26% respectively.

As illustrated in Figure 4, in control uteri, 20% of the total radioactivity of the sample is incorporated into the lipid and protein-nucleic acid fractions (anabolic products). In zinc treated rats this percentage was significantly increased to 24%. Surprisingly, a similar shift to anabolic products following estradiol treatment was not evident. The zinc chloride:estradiol uteri showed a 28% incorporation of ^{14}C along this pathway, a value that is significantly different from the control at the $p<.10$ confidence level. It may be noted that the protein-nucleic acid fraction constituted the major portion of the anabolic percentages.

CONCLUSIONS

1. Zinc significantly stimulates the incorporation of ^{14}C derived from glucose-U-^{14}C into carbon dioxide, the protein-nucleic acid fraction and into the lipid fraction at the $p<.10$ level.
2. Zinc apparently stimulates uterine metabolism in the incorporation of ^{14}C derived from labeled glucose along the anabolic pathway to a greater extent than the catabolic pathway.
3. The mechanism by which zinc stimulates uterine metabolism is apparently manifested through a similar pathway to that of estradiol. When zinc and estradiol were administered together, no additive effect was observed.
4. The mode of estradiol action (and possibly its uptake by the uterine tissue) is a suggested future project as a method to elucidate the mode of action of zinc.

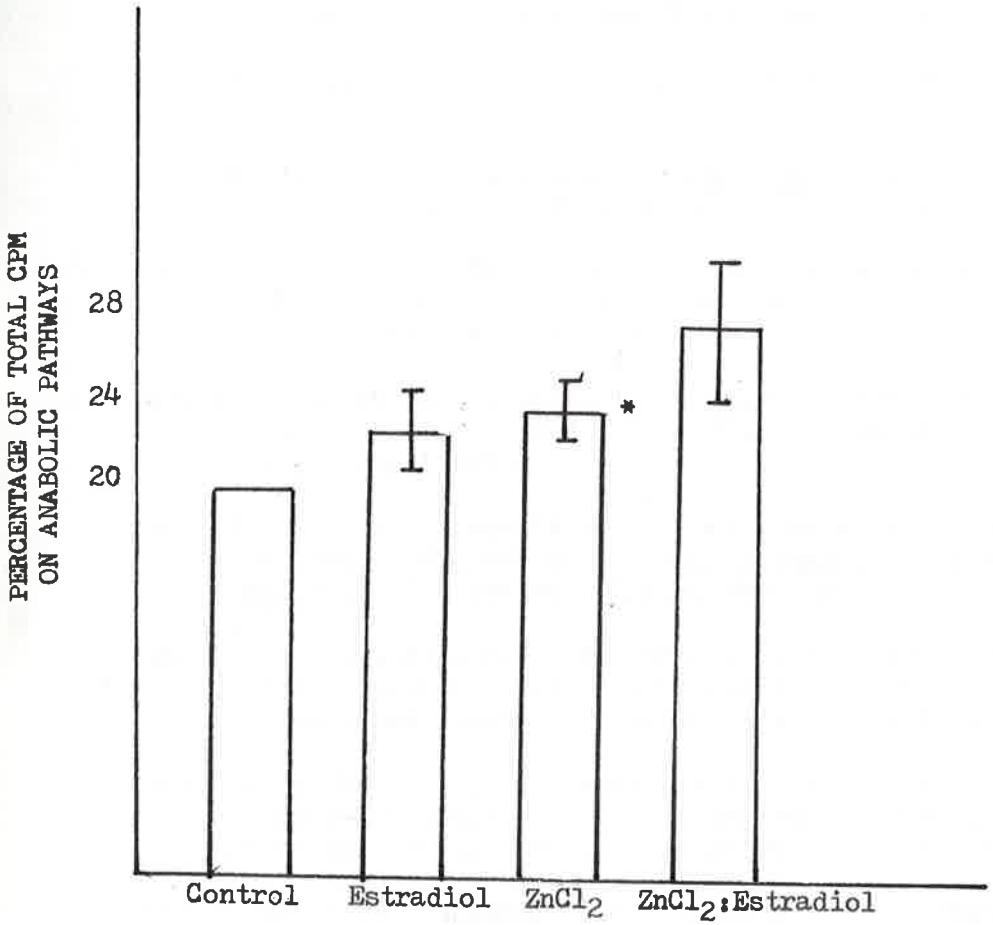

Figure 4. The effect of estradiol, zinc chloride, and zinc chloride: estradiol on the anabolic pathways in the rat uterus. Each value is the percentage (± SE) of lipid and protein-nucleic acid fractions based on 7-8 experiments. Values are based on the total counts per minute which averaged 6491, 8164, 8739 and 9571 counts per minute for control, $ZnCl_2$, estradiol and $ZnCl_2$:estradiol, respectively. * = significantly different from control at $p<.05$.

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