when exposed to #742. The increase in cell division and changes in cell morphology may be clues as to what causes the malformations.

Summary. L-fibroblast cell cultures were treated with varying concentrations of 1, 3, 5, (10), 16-estratetraen-3-ol (#742) dissolved in propylene glycol. Propylene glycol (2%) was found to stimulate the mitotic rate. With #742 added the mitotic rate and total cell number were increased markedly. The presence of propylene glycol and #742 caused a distinct change in the morphology of the cells present in the cultures.

1. Huffman, Max N., Lott, M. H., Tillotson, A., *J. Biol. Chem.*, 1955, v217, 103.

2. Paul, John, *Cell and Tissue Culture,* 2nd ed. Williams and Wilkins, Baltimore, 1960.

3. Cowdry, E. B., *Cell Physiology of Neoplasia,* N. Texas Press, Austin, 1960.

4. Smith, P. E., Copenhaver, W. M., *Bailey's Textbook of Histology,* 13th ed., Williams & Wilkins, Baltimore.

5. Earle, W. F., *J. Nat. Cancer Inst.*, 1943, v3, 555.

6. Maximow, A. A., Bloom, W., *Textbook of Histology,* 7th ed., Saunders, Philadelphia.

7. Neukomm, S. A., Boissonaz, A., Richard, M., *Acta Ant.*, 1957, v31, 289.

8. Jones, R. W., Huffman, M. N., *Trans. Am. Micro. Soc.*, 1957, v76, 177.

9. Bucher, O., Gattiker, R., *Exp. Cell Res.*, 1953, v5, 461.

10. Rothchild, R., *Endocrinology,* 1952, v50, 383.

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Calcification of the Ovarian Follicle in Rats.* (29368)

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Parathyroid extract (PTE) under certain conditions can cause deposition of calcium in soft tissues. Calcification may occur in the cortex of the kidney(1,2,3,4,5), in the gastric mucosa and in a variety of other soft tissue sites(6) after adequate dosages of PTE. There are, however, no reports of PTE-induced calcification in the reproductive organs. This communication is concerned with PTE-induced calcification of the ovarian follicular apparatus of the rat.

Materials and methods. Immature female rats of the Wistar strain weighing 30-50 g were divided into 3 groups. The experimental group was injected subcutaneously with 40 units of "Parathyroid Extract" (Lilly) twice daily for 3 days. One group of controls was given PTE, inactivated by either pepsin-digestion or formalin according to the methods of Shetlar(3). This material was given subcutaneously in amounts and for periods of time comparable to the use of the active extract in the experimental group. A third group of female rats of comparable weight and age served as uninjected controls. All animals were maintained on a diet of commercially available rat pellets and tap water administered *ad libitum.*

The experimental animals were sacrificed 24 hours after the last injection by ether asphyxiation and were autopsied immediately. Samples of ovary, uterus, cervix, vagina, and kidney were removed from these animals as well as from the controls, fixed in 10% neutral-buffered formalin, embedded in paraffin and sectioned. Alternate sections were stained with either hematoxylin and eosin, the von Kossa technique, Alizarin Red S or the PAS technique. Metachromasia was studied in the tissues by staining with Thionin.

Results. A summary of the microscopic findings is presented in Table I. Calcium

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TABLE I. Tissue Changes Induced by Parathyroid Extract.

Tissue	H and E	von Kossa	Alizarin Red S	PAS	Metachromasia
ovary	increased basophilia	calcium	calcium	increased	increased
uterus	normal	negative	negative	normal	normal
cervix	"	"	"	"	"
vagina	"	"	"	"	"
kidney	increased basophilia	calcium	calcium	increased	increased

was found only in the kidneys and ovaries of animals injected with the active PTE. Calcium deposition in the kidney was limited to the cortex and was found mainly in peritubular areas (Fig. 1, 2).

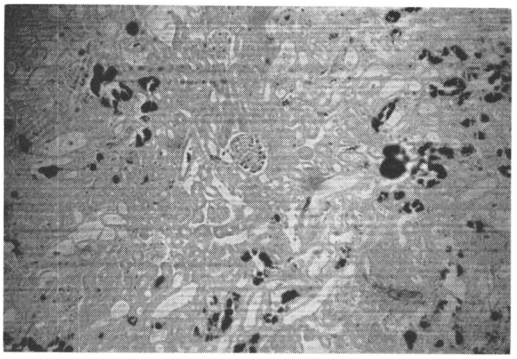

FIG. 1. Kidney of PTE-treated rat, von Kossa stain, low power, black areas indicate sites of calcium deposition.

Calcium was seen in the ovary within the cells lining intermediate-sized follicles. These follicles were in early stages of antrum formation. Maximal calcification was noted in the granulosa cell layer, with minimal calcium deposition in the theca interna (Fig. 3, 4). Calcium deposition was not detected in interfollicular areas of the ovary.

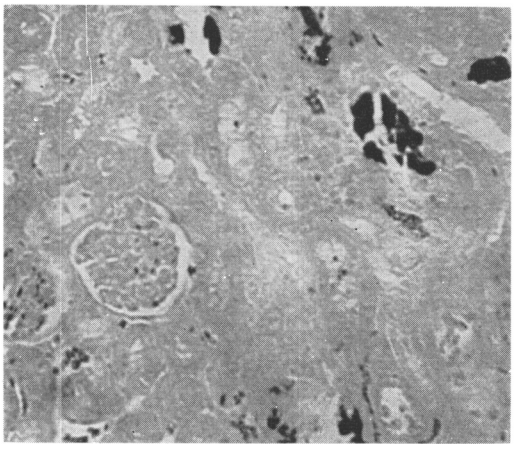

FIG. 2. High power view of PTE-treated kidney, von Kossa stain. Note that calcium deposition occurs in the kidney tubules.

Increased PAS positive material and increased metachromasia were observed only in ovaries and kidneys which contained calcium deposits when studied by the von Kossa or Alizarin Red S techniques.

The tissues taken from the control groups showed no calcium or any of the other changes which were exhibited by the experimental group.

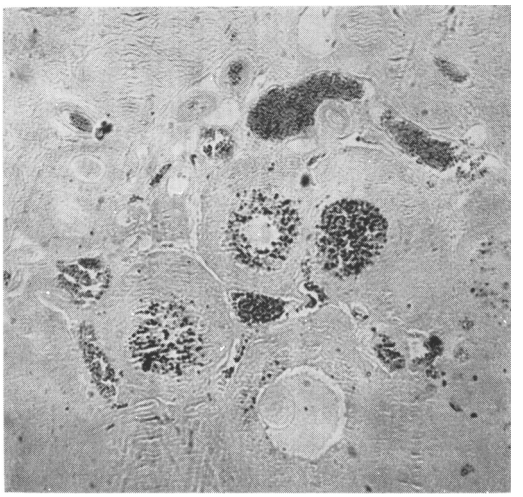

FIG. 3. Ovary of PTE-treated rat, von Kossa stain, low power, calcium deposition occurs only within follicles.

Discussion. The data reported here are the first which show experimentally induced calcification of gonadal tissue. In these studies calcium was found to be deposited only in the granulosa cells and theca interna of follicles that were undergoing antrum formation. At this time in the maturation of the follicle estrogens are being deposited in the follicular fluid. The weight of histochemical evidence indicates that the theca interna is the principal site of estrogen biosynthesis in the developing follicle(7). Corner(8), and Bullough(9), have suggested that endoge-

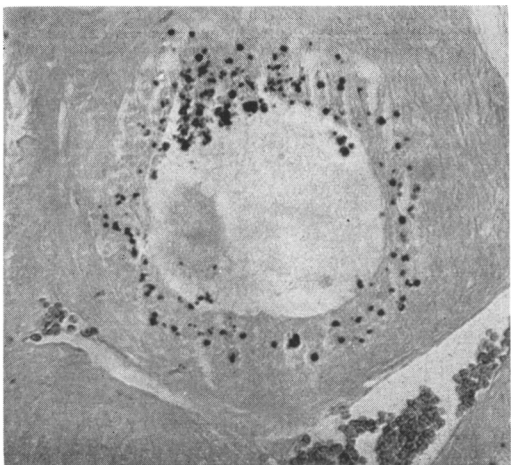

FIG. 4. High power view of PTE-treated ovary, von Kossa stain, calcium deposition is noted only in the granulosa cell layer of this follicle.

nous estrogens may exert a localized stimulatory effect upon the cells of the developing follicles. The possibility exists then that estrogens may sensitize the granulosa cells to the calcification-inducing action of PTE.

Administration of PTE results in a significant increase in serum citric acid which precedes the appearance of hypercalcemia(10, 11,12). The deposition of calcium in tissues is believed to be dependent upon the presence of an oxidizing substrate as an energy source for crystal formation(13). Estrogens are capable of increasing the utilization of citric acid(14). In addition, estrogens induce a 3- to 4-fold increase in amount of calcium deposited in PTE-induced nephrocalcinosis (15).

Villee(14,16) has postulated a mechanism of action for estrogens that depends upon the presence of a pyridine nucleotide transhydrogenase, which is estrogen-sensitive. This enzyme increases the rate of utilization of citric acid in the tricarboxylic acid cycle by making co-factors available for isocitric dehydrogenase. The estrogen-stimulable enzyme has been demonstrated in human placenta, endometrium, myometrium and mammary gland, and in rat ventral prostate, uterus, and pituitary tumors(17). To our knowledge, studies on this enzyme system have not been performed in ovarian tissue. Demonstration of such a system in the ovarian follicle would lend further support to the concept that estrogens are capable of stimulating cell layers within the follicles responsible for hormone production.

Summary. The induction of calcification in rat ovarian follicles by administration of parathyroid extract is reported. Calcium deposition is limited to the membrane granulosa and thecae interna of follicles in the early stages of antrum formation. It is suggested that the "calcifiability" of these tissues may be due in part to an action of endogenous estrogens.

1. Baker, R., Reaven, G., Sawyer, J., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 281.

2. ———, *J. Urol.,* 1954, v71, 511.

3. Shetlar, M. R., Howard, R. P., Joel, W., Courtright, C. L., Refenstein, E. C., Jr., *Endocrinol.,* 1956, v59, 532.

4. Shetlar, M. R., Howard, R. P., Joel, W., Bowling, D. C., Shetlar, C. L., *Am. J. Physiol.,* 1958, v195, 535.

5. Bradford, R. H., Howard, R. P., Joel, M., Shetlar, M. R., *Arch. Path.,* 1960, v69, 382.

6. Eisenstein, R., Trueheart, R. E., Hass, G. M., in Sognnaes, R. F., *Calcification in Biological Systems,* A.A.A.S. Washington, D.C., 1960.

7. Velardo, J. T., *The Endocrinology of Reproduction,* Oxford University Press, New York, 1958.

8. Corner, G. W., *Physiol. Revs.,* 1938, v18, 154.

9. Bullough, W. S., *J. Endocrinol.,* 1943, v3, 235.

10. Elliot, J. R., Freeman, S., *Endocrinology,* 1956, v59, 181.

11. Firschein, H. E., Neuman, W. F., Martin, G. R., Mulryan, B. J., *Rec. Progr. Horm. Res.,* 1959, v15, 427.

12. Lakan, E. C., Laskin, D. M., Engel, M. B., *Am. J. Physiol.,* 1960, v199, 856.

13. Sobel, A. E., Burger, M., Nobel, S., *Clin. Orthop.,* 1960, v17, 103.

14. Villee, C. A., *J. Biol. Chem.,* 1955, v215, 171.

15. Grob, H. S., Kupperman, H. S., Gordon, A. S., unpublished data.

16. Villee, C. A., *Ann. N. Y. Acad. Sci.,* 1959, v75, 524.

17. Hagerman, D. D., Villee, C. A., in Villee, C. A. and Engel, L. L., *Mechanism of Actions of Steroid Hormones,* Pergamon Press, New York, 1961.

Received February 24, 1964. P.S.E.B.M., 1964, v116.