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FUNCTIONAL CHANGES IN THE OVARY AND
GENITAL TRACT, AS ESTIMATED BY THE
APPEARANCE OF OVARIAN EOSINOPHILIC
GRANULATED CELLS

Thesis for the Degree of M. S.
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THESIS



ABSTRACT

FUNCTIONAL CHANGES IN THE OVARY AND GENITAL TRACT, AS ESTIMATED BY THE APPEARANCE OF OVARIAN EOSINOPHILIC GRANULATED CELLS

by Joan E. Brown

Evidence of estrogen secretion by the ovary, as estimated by changes in genital tract tissues, can be directly correlated with the appearance of eosinophilic granulated cells within the ovarian tissues. Ovarian eosinophilia and development of the genital tract of the gilt are prominent from the 11th to 15th days of the estrous cycle, between the 24th and 44th and after the 86th days of pregnancy. It is suggested that the appearance of eosinophilic granulated cells in ovarian tissues is a direct effect of ovarian estrogens and provides a means whereby the portion of the ovary secreting estrogens can be identified. Eosinophilic granulated cells were observed in thecal layers of secondary, tertiary and atretic follicles and in the connective tissue surrounding and invading recently formed corpora lutea. They were also observed between individual luteal cells from the 28th to 44th and after the 86th days of pregnancy. They were never identified in thecal layers of Graffian follicles. The eosinophilic granulated cells are not of immediate blood origin, but arose from undifferentiated mesenchymal cells.

Joan E. Brown

In the process of accumulation of eosinophilic granules and modification of nuclear chromatin to bi- or multilobulated forms, cellular death occurs and the granules and chromatin particles are scattered in the tissues.

FUNCTIONAL CHANGES IN THE OVARY AND GENITAL
TRACT, AS ESTIMATED BY THE APPEARANCE OF
OVARIAN EOSINOPHILIC GRANULATED CELLS

By

Joan E. Brown

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INTRODUCTION

Variations in the numbers and types of leucocytes have been reported in the ovaries of a variety of animals during the normal reproductive cycle. The more pronounced changes are apparent in the eosinophils. They have been reported in high concentration in the thecal layers of follicles close to the time of ovulation in cattle (McNutt, 1924; Moss, et al., 1954; Cupps, et al., 1959), or within the infiltrating layers of thecal cells in recently formed corpora lutea in swine (Corner, 1919), cattle (McNutt, 1924; Moss et al., 1954; Cupps, et al., 1959), monkey (Corner, 1945), rabbit (Moricard, 1953), and human (McKay, et al., 1961). Basophilic leucocytes were observed around recently ovulated follicles in maximal concentrations 12 hours following ovulation and degranulating during penetration into developing luteal tissues (Zacharie, et al., 1958). Cupps et al., (1959) reported an infiltration of mast cells into the ovary and their accumulation around preovulatory follicles in cattle.

It has previously been reported from this laboratory that the majority of eosinophilic polychromatin leucocyte-like cells in the genital tract of non-infected animals are from genital tract origin and originate by gonadal hormone induced metabolic and morphological modification of plasma cells (Nellor, 1963b; Nellor, 1965; Nellor and Brown,

1966). The plasma cells were described as originating from basophilic fibroblast-like cells of the loose connective tissue of the genital tract.

Preliminary studies on the ovaries of rats, cattle, sheep, and swine suggested that a similar process of tissue leucocytogenesis occurred in the ovaries. The present study was designed to determine whether the marked fluctuation in ovarian eosinophils could be correlated with gonadal hormone secretion, as estimated by genital tract changes. It was also of considerable interest to determine whether ovarian eosinophilic granulated cells were from tissue or immediate blood origin.

METHODS AND PROCEDURES

From the variety of species of animals available for this study, gilts were selected as the animal of choice. As a multiple ovulatory animal, graded follicular growth can be demonstrated at any stage of the normal estrous cycle. The length of the estrous cycle, 17 to 24 days, allows a consideration of the changes in the ovaries and genital tract tissues within the long diestrous phase, as well as clear cut separation of the early and late follicular stages of the normal cycle. The genital tissues from approximately 100 gilts were available for the selection of representative stages of the normal estrous cycle or pregnancy. They had previously served as control animals for a variety of studies on controlling ovulation (Nellor, 1960; Nellor et al., 1960), or on induced delayed parturition (Nellor, 1963a). Corner (1919, 1921) clearly defined the histological changes of the genital tract tissues of gilts during the normal estrous cycle and pregnancy, but did not include a description of the changes in genital tract leucocytes during the normal estrous cycle or pregnancy. Aside from the general classification of cycling gilts in this study into proestrous, estrous, and diestrous stages of the normal cycle, a limited consideration of the development of the epithelia of the uterus, deep uterine glands, and vagina will be included as an index of the degree of

endogenous estrogenic stimulation at the time of autopsy. Changes in genital tract leucocyte-like cells will also be discussed in order to correlate their characteristics with ovarian eosinophilia occurrence.

The description of ovarian and genital tract changes of plasma cells and eosinophilic granulated cells is included for one gilt sacrificed at 5 months of age and gilts autopsied on days 1, 3, 5, 8, 11, 12, 14, 15, 17, and 19 of the normal estrous cycle, day-1 of the estrous cycle representing the first day of physiological estrus. The ovaries and genital tract were also obtained from gilts autopsied at days 15, 24, 28, 31, 44, 53, 61, 70, 76, 86, and 97 of pregnancy. Sections of both ovaries, of the mid-portion of both uterine horns, and of the anterior portion of the vagina were removed at autopsy, placed in both 10% formalin and Carnoy's fixatives, cleared and dehydrated in routine alcohol procedures, embedded in Tissue-mat paraffin and sectioned at 5 to 7 micra. The staining procedures utilized were Harris hematoxylin and eosin, Mallory's Triple stain, Papanicolaou's stain, Giemsa stain, Toluidine Blue and periodic acid-Schiff-Alcian Blue staining of Mowery and Winkler (1956). Tissue sections were stained with periodic acid-Schiff reagent (PAS) alone or in combination with Alcian Blue stain and with Alcian Blue stain alone.

RESULTS

This study demonstrates that the ovary, as earlier reported for the genital tract, contains eosinophilic granulated polychromatin cells that originate from undifferentiated mesenchymal cells. There was also a direct relationship between increases in ovarian eosinophilic granulated cells and evidence of estrogenic stimulation of the genital tract tissues.

Regardless of the stage of the estrous cycle or pregnancy, the majority of the eosinophilic granulated cells of the genital tract originated by metabolic and morphological modification of plasma cells. This process was referred to in an earlier paper as a marked eosinophilic granulation of the cytoplasm of the plasma cells, followed by nuclear lobulation, dissolution of the nuclear and cytoplasmic membranes and scattering of nuclear and cytoplasmic particles within the tissue, (Nellor and Brown, 1966). Therefore, the majority of the eosinophilic granulated cells in the genital tract tissues are referred to as eosinophilic plasma cells or fragmenting plasma cells. In contrast, the majority of eosinophilic granulated cells in the ovary originated by the modification of connective tissue cells, either within the general ovarian stroma or in connective tissue strands within the thecal layers or corpora lutea. The most primitive cells

involved were elongate neutrophilic cells indistinguishable from undifferentiate mesenchymal cells, until acquiring eosinophilic cytoplasmic granulations, granules staining intensely with eosin or Giemsa stain. In each case it appeared that the cells rounded up during this metabolic activity, the nuclei assuming either a more spherical shape or appearing elongate with a central indentation. Nuclear lobulation was coincident with an increased accumulation of cytoplasmic granules and the number of lobules varied from 2 to 10 per cell. Death of the cells obviously accompanied this phenomenon and eosinophilic granules and chromatin particles could be observed in the tissue in areas exhibiting this activity. The cellular granules or cytoplasm of these cells did not stain with Toluidine Blue, Alcian Blue or Orange G.

The appearance of the eosinophilic granulated cells in various parts of the ovary and their relationship to the functional activity of the ovary is demonstrated by a consideration of their modification during the normal estrous cycle and pregnancy.

It has been recognized for a considerable time that although the immature animal does not ovulate, there are marked variations in the degree of estrogenic stimulation of the genital tract. The ovaries of the 5 month old gilt contained no corpora lutea but had considerable follicular growth, the maximum size of the tertiary follicles approximating 0.4 to 0.6 cm. in diameter. With the exception of

Graffian follicles, follicles were observed in all stages of development. Numerous eosinophilic granulated cells were observed in the general ovarian connective tissues but they appeared in the greatest numbers in the thecal layers surrounding healthy and atretic tertiary follicles. For the most part the eosinophilic granulated cells contained round nuclei and dispersed nuclear chromatin although occasional cells were observed with multilobulated chromatin patterns. Basophilic leucocytes or plasma cells were not observed in any of the ovarian sections. The condition of the genital tract of the immature gilt exhibited a low degree of estrogenic stimulation, although more than that observed in immature gilts of earlier ages or in ovariectomized gilts. The epithelium of the uterus and deep uterine glands was cuboidal to low columnar and the vagina stratified squamous, (4 to 6 cell layers). The connective tissues of the stroma of both the uterus and vagina contained numerous eosinophilic granulated cells, identified as eosinophilic plasma cells and fragmenting plasma cells. Basophilic preplasma cells and mature plasma cells were not observed in the genital tract tissues.

On day-1 of the normal estrous cycle, the first day of estrus, the ovaries contained regressing corpora lutea from the last cycle, numerous healthy and atretic tertiary follicles and 13 Graffian follicles. It was of considerable interest that the ovarian tissues were completely devoid of eosinophilic granulated cells. Mature plasma cells were

observed in the connective tissue of the hilus but were not present in the interstitial areas or in the thecal layers surrounding the follicles. Although in estrus, the superficial epithelium and deep uterine glands appeared progestational, that is, the epithelial cells were low pseudostratified and stained poorly with PAS. The epithelium of the vagina retained the high stratified condition typical of proestrus and estrus (6-9 cell layers). Very few preplasma cells, mature plasma cells, or eosinophilic plasma cells were observed in the genital tract tissues at this stage. Nuclear remnants of fragmented plasma cells were present in the outermost layers of the stratified epithelium of the vagina.

The gilt on day-3 of the estrous cycle still exhibited estrus at the time of autopsy. The ovaries contained regressing corpora lutea from the previous ovulation, 5 Graffian follicles (1.0 to 1.2 cm. in diameter) and 7 recently ovulated follicles. There were numerous healthy and atretic tertiary follicles in the ovaries (0.4 to 0.8 cm. in diameter). Eosinophilic granulated cells were not observed in any section of the ovaries. Mature plasma cells were only observed in small clusters in the connective tissue of the hilus. The epithelium of the uterus and deep uterine glands was pseudostratified and stained PAS negative. The vaginal epithelium was comparable to that on day-1. Eosinophilic plasma cells or fragmenting plasma cells were not observed in the genital tract tissues

at this stage of the estrous cycle. Plasma cells were observed in the uterus in greater numbers than on day-1 but there was no apparent change in the numbers of plasma cells in the vaginal tissues.

The ovaries of the gilt autopsied on day-5 of the estrous cycle contained corpora lutea in the early stages of formation, still exhibiting central cavities. Eosinophilic granular cells and plasma cells were observed in the connective tissue immediately surrounding and penetrating into the developing corpora lutea. There was an increase over the earlier stages of the estrous cycle in the numbers of eosinophilic granulated cells in the thecal layers of medium sized healthy and atretic tertiary follicles. The nuclei of the eosinophilic granulated cells could be identified in all stages, from elongate or centrally indented, to various degrees of chromatin lobulation. Large numbers of Toluidine Blue and Alcian Blue staining fibroblast-like cells, preplasma cells and plasma cells were observed in the connective tissues of the hilus and in the interstitial areas of the ovaries. They were not observed in the immediate areas of healthy or atretic follicles. There was a noticeable increase in the development of the genital tract, over early stages of the estrous cycle, changes indicative of endogenous estrogen influence. Although the height of the epithelium of the uterine lumen and deep uterine glands had not noticeably increased, the cells were larger and contained small amounts of PAS positive stained material. The

epithelium of the vagina was more stratified than on day-3 of the estrous cycle. The increased estrogen influence was also evident by the increased numbers of eosinophilic plasma cells and fragmenting plasma cells throughout the uterine tissue. There was no evidence of eosinophilic plasma cells or fragmenting plasma cells in the vaginal stroma. Alcian Blue and Toluidine Blue staining preplasma cells and mature plasma were in greater numbers than at earlier stages, in both the uterine and vaginal tissues.

The corpora lutea were fully developed in the gilt autopsied on day-8 of the estrous cycle, at which time they slightly exceeded the diameter of the mature follicles of estrus. Relatively few eosinophilic granulated cells were observed in ovarian sections at this stage of the cycle, either in the thecal layers or within the corpora lutea. Plasma cells were observed within the supporting connective tissues of the corpora lutea and there was a noticeable rounding up of the nuclei of the connective tissue elements scattered between the individual luteal cells. The uterus was considerably more convoluted than on day-5 of the estrous cycle and the epithelium of the uterus and deep uterine glands contained more PAS positive staining material. The deep uterine glands were larger than at earlier stages and the nuclei of the epithelial cells more basal. Plasma cell production had increased in the genital tract tissues over earlier stages. Large

numbers of Alcian Blue and Toluidine Blue staining pre-plasma cells and mature plasma cells were observed in the subepithelial stroma and superficial epithelium and plasma cells penetrated into the lumen of the genital tract. The numbers of eosinophilic plasma cells and fragmenting plasma cells had increased in the vaginal tissue but remained relatively the same in the uterine tissue.

There were no demonstrable changes in the functional aspects of the corpora lutea of gilts autopsied between days-11 and 15 of the normal estrous cycle. There were, however, marked changes in the numbers of eosinophilic granulated cells in the ovaries during this time. Greater numbers of eosinophilic granulated cells were noted in ovarian tissue than at any other stage of the normal cycle or during pregnancy. They were not observed to be present in the corpora lutea or in secondary and small tertiary follicles. The thecal layers of the largest tertiary and atretic follicles contained the highest concentration of eosinophilic granulated cells observed in any tissues (Plate I, Figure I). Large amounts of eosinophilic granulated material were observed in the cytoplasm of cells and scattered in the immediate vicinity of the granulated cells. The process of eosinophilic granulated cell formation appeared markedly accelerated as evidence by the large numbers of elongate mesenchymal cells displaying prominent eosinophilic granules and undergoing nuclear chromatin

lobulation. In prior stages, although elongate cells could be observed accumulating granules, a rounding up of the cell usually preceded nuclear lobulation. The genital tract reached the highest degree of development during this stage of the cycle. There were extensive foldings of the luminal surface of the uterus and the deep uterine glands were highly developed and stained strongly with PAS positive material. There was an increased edema and density in the stromal components. The numbers of genital tract eosinophilic plasma cells and fragmenting plasma cells reached a maximum on day-11 of the estrous cycle and remained fairly constant in genital tract tissues until day-15 (Plate I, Figure 2). Although plasma cell numbers remained relatively the same in the genital tract tissues from days-11 to day-15 of the estrous cycle, the marked metabolic and morphological transformation of plasma cells into eosinophilic and fragmenting plasma cells had considerably reduced their numbers as compared to days-5 and 8 of the estrous cycle.

The ovaries of gilts autopsied on days-17 and 19 of the estrous cycle contained corpora lutea in various stages of involution. Follicular growth was accelerated and follicles approached the Graffian follicle size (1.0 to 1.2 cm. in diameter) by the 19th day. The total numbers of ovarian eosinophilic granulated cells were reduced on day-17 of the estrous cycle. However, an apparent shift in sites of production had occurred. The thecal layers of the large tertiary or Graffian follicles (Plate I, Figure 3)

contained no granular cells although small or large numbers were observed in the thecal layers of small tertiary (Plate I, Figure 4) and atretic follicles. By day-19 of the estrous cycle the eosinophilic granulated cells had practically disappeared from the ovarian tissue. There were only a few eosinophilic granulated cells observed in the thecal layers of atretic follicles and none were observed in the thecal layers of Graffian follicles. The involuting corpora lutea were not observed to contain eosinophilic granulated cells. Ovarian plasma cells were in the lowest number of the estrous cycle on days-17 and 19. A precipitous decrease in genital tract eosinophilic plasma cells and fragmenting plasma cells had occurred from the 17th to 19th days of the cycle as they were only occasionally observed in the genital tissues in the gilt autopsied on day-19. Plasma cell numbers had decreased in the genital tract tissues after day-15 and were in the lowest numbers observed on day-19.

The ovaries of the gilt autopsied on the 15th day of pregnancy contained functional corpora lutea. Follicular growth in the ovaries did not exceed 0.8 cm. in diameter. Eosinophilic granulated cells were not observed in any portion of the ovaries. Plasma cells were observed in the loose connective tissue of the ovaries in numbers comparable to days-5 to 8 of the normal estrous cycle. Toluidine Blue or Alcian Blue staining cells were not present in the ovarian sections. The uterine and vaginal

tissues displayed considerably less estrogenic influence than during the mid-luteal phase of the normal estrous cycle. The epithelium of the uterus and deep uterine glands was low columnar and the vaginal epithelium was low stratified squamous. There was an abundance of mature plasma cells, of larger size than noted during the estrous cycle, in the stroma of the uterus and vagina. Eosinophilic plasma cells and fragmenting plasma cells were not observed in the genital tract tissues.

On the 24th day of pregnancy, prior to firm implantation of the fetuses, the ovaries contained prominent corpora lutea and relatively few tertiary follicles. Small numbers of eosinophilic granulated cells were observed between the luteal cells and within the strands of connective tissue dispersed throughout the corpora lutea. Small numbers of eosinophilic granulated cells were observed in the thecal layers of healthy and atretic tertiary follicles. Mature plasma cells were still abundant in genital tract tissues and eosinophilic plasma cells and fragmenting plasma cells were in very low numbers.

The ovaries of gilts autopsied on days-28, 31, and 44 of pregnancy contained increasing numbers of eosinophilic granulated cells. The numbers observed in the ovarian tissues were not as high as from the 11th to 15th days of the normal estrous cycle. Although eosinophilic granulated cells were observed in the corpora lutea in small numbers, the largest numbers were present in the

thecal layers of atretic follicles, which comprised the majority of the tertiary follicles in the ovaries at this stage of pregnancy. Preplasma cells and mature plasma cells were observed in the connective tissue of the corpora lutea in the largest numbers of any stage of the normal cycle or pregnancy. The genital tract tissues of gilts autopsied at days-28, 31 and 44 of pregnancy contained many large plasma cells and small numbers of eosinophilic plasma cells and fragmenting plasma cells.

Only small numbers of eosinophilic granulated cells and plasma cells were observed in the ovarian and genital tract tissues of gilts autopsied on the 53rd to 76th days of pregnancy. There was an increase in the numbers of eosinophilic granulated cells in the thecal layers of atretic follicles in the gilt autopsied on the 86th day of pregnancy, in numbers greater than any other stage of pregnancy but less than during the luteal phase of the normal estrous cycle. There was a definite decrease in the number of ovarian and genital tract eosinophilic granulated cells in the gilt autopsied at 97 days of pregnancy and they appeared to be comparable to the numbers observed from the 28th to 44th days of pregnancy.

DISCUSSION

This study has demonstrated that the appearance of eosinophilic granulated cells in ovarian tissues during the normal estrous cycle of the gilt can be correlated with developmental changes in the genital tract associated with estrogen secretion. This correlation is possible since progestins alone in the ovariectomized animal have little influence upon development of the superficial epithelium of the genital tract or on the secretory development of the deep uterine glands. Estrogen influence, therefore, can be estimated either in the progestin treated ovariectomized animal or normal cycling animal by the appearance of synergistic changes in the genital tract tissues. In the present study, the appearance of eosinophilic granulated cells in the ovary and genital tract of normal cycling animals was coincident with increased proliferation of the superficial epithelia and development of the deep uterine glands. This occurred from approximately the 11th to 15th days of the normal estrous cycle, from the 24th to 44th, and after the 86th day of pregnancy. There was a paucity of eosinophilic granulated cells in the ovaries and genital tract tissues close to the time of ovulation and during the early luteal phase of the estrous cycle.

Although it is generally assumed that estrogen secretion is highest at the time of physiological estrus or close to ovulation, urinary estrogen values in the gilt rise to a maximum during the follicular phase of the cycle (Velle, 1958), decrease to very low levels just prior to estrus and ovulation (Raeside, 1963), and then rise again during the luteal phase of the estrous cycle (Lunnas, 1962). This is also apparently true in the human, where urinary estrogen values show an increase during the follicular phase, sharply decrease close to the time of ovulation and then increase to a second peak on the 21st day of the menstrual cycle (Brown, 1955). Histological and histochemical studies on the genital tract tissues of the cow (Cupps, 1943; Hansel, et al., 1949; Asdell, 1960) and of the sow (Corner, 1919, 1921) have reported an increased proliferation of the genital tract epithelium from 8 to 12 days following ovulation, changes suggestive of increased estrogen influence. The type of development noted in the genital tract of the gilt from the 8th to 15th days of the normal estrous cycle can be maintained in the ovariectomized gilt by progestin-estrogen treatment, but not by either hormone alone (Nellor, 1960).

There was an increase in ovarian and genital tract eosinophilic granulated cells from the 24th to 44th days of pregnancy. This declined and very few eosinophilic granulated cells were noted until the 86th day of pregnancy. Urinary estrogens are reported to increase over the levels found

during the normal estrous cycle from the 28th day of pregnancy in the sow, reaching a peak during the 4th to 6th weeks of pregnancy (Küst and Struck, 1934; Bredeck and Mayer, 1957; Velle, 1960; Lunnas, 1962). Urinary estrogen levels remain low after the 6th week of pregnancy, start to increase from the 11th week of pregnancy, and reach a maximal level approximately 10 days from physiological term (Küst and Struck, 1934; Bredeck and Mayer, 1957). The appearance of the eosinophilic granulated cells in the ovaries and genital tract during pregnancy coincide very well with the reported increases in urinary estrogen excretion.

Previous studies from this laboratory (Nellor, 1963b; Nellor, 1965; Nellor and Brown, 1966) demonstrated that the production of eosinophilic plasma cells and fragmenting plasma cells in the genital tract tissues in normal cycling animals resulted from estrogenic modification of plasma cells. Progestational agents alone promoted the production of plasma cells but did not induce their morphological modification to polychromatin cells. Estrogen, either in the intact luteal animal or in the progestin treated ovariectomized animal, induced metabolic and morphological modification of the plasma cells to eosinophilic polychromatin cells. Chronic estrogen treatment, without progesterone, resulted in the destruction of the plasma cells and their precursors. It was evident that, although estrogen in the normal cycling animal apparently only reached levels capable of inducing fragmentation of mature plasma cells, administration of higher levels or

chronic treatment resulted in eosinophilic granulation of preplasma cells and their fibroblast-like cell precursors and eventual death of the cells. At the initiation of this study it was reasoned that if morphogenesis of the plasma cell was the result of direct action of estrogen, this would be evident within the connective tissues at the site of production of estrogens. The theca interna has been demonstrated to be one of the sites of ovarian estrogen production (Corner, 1938; Harrison, 1948).

The corpora lutea have been implicated as providing the estrogens during the luteal phase of the menstrual cycle (Furuhjelm, 1954; Diczfalusy and Laretzen, 1961; Huang and Pearlman, 1963; Rice et al., 1964), while Sturgis (1950) reported estrogen production from small follicles as well as the corpus luteum. It is of interest in this regard that although in a few instances the eosinophilic granulated cells were observed in the interstitial connective tissue of the ovaries, they were for the most part confined to the thecal layers of normal and atretic follicles. Eosinophilic granulated cells were observed within the luteal bodies during early stages of development, during the 11th to 15th days of the estrous cycle and from the 24th to 44th days of pregnancy. There were in no instances as many eosinophilic granulated cells encountered in the luteal tissues as in the thecal layers of atretic tertiary follicles on the same ovary.

It has been reported that the "second rank" follicles are associated with estrogen production at estrus, not the largest follicles on the ovary (Sturgis, 1950). Large tertiary and Graffian follicles were never observed in the current study with eosinophilic granulated cells in the thecal layers.

Estimates of estrogen concentration of ovarian vein blood can follow the total production of estrogens by the ovary but histochemical methods of identification of estrogen secreting ovarian components are not reliable. The high estrogen content of the follicular fluid is well known, yet none of the histochemical tests supposedly indicative of steroid hormones has ever been seen to give a positive reaction in the liquor folliculi (Jacoby, 1962). If the conversion of connective tissue components into eosinophilic granulated cells is a result of direct estrogen action, this might prove a valuable histological aid in assigning various structures of the ovary or placenta to estrogen secretion at various stages of the estrous cycle or during pregnancy, respectively.

There is some question as to the type of connective tissue cells in the ovary that apparently produces the eosinophilic granulated cells under estrogen stimulation. As in previous studies with tissue leucocytogenesis in genital tract tissue, a consistent finding in swine was that the cytoplasm of the precursors of the plasma cells stained intensely with Toluidine Blue, Alcian Blue, Methylene blue

or Orange G. Since under physiological conditions, the estrogen influence was confined to metabolic and morphological changes in the mature plasma cell (Plate I, Figure 5), it was relatively easy to define the precursor cells of the genital tract eosinophilic plasma cells or fragmenting cells. However, in the ovarian tissues the precursor of the eosinophilic granulated cell does not appear to be any of the basophilic blast forms, the situation depicted in Figure 5, and direct involvement of the plasma cell in eosinophil formation was only occasionally observed. The most primitive cell form participating in ovarian eosinophilic granulated cell formation resembled a completely undifferentiated mesenchymal cell, with an extremely elongate nucleus and cell body. Eosinophilic granulations could be observed accumulating in these cells. A rounding up of both the cytoplasm and nucleus accompanied the intensification of cytoplasmic eosinophilia (Plate I, Figure 6). The process of nuclear lobulation appeared to be more an attempt at aberrant cell division rather than the formation of lobules connected by fine chromatin strands. This process was not accompanied by cytoplasmic division and appeared to be a rapid process terminating in the death of the cell. These cells do not appear similar in origin or structure to the hilus cells of the menopausal ovary suggested to be involved in estrogenesis (Shaw and Dasteur, 1949) or androgenesis (Sternberg, 1949). This study does

establish, however, that the presence of the eosinophilic granulated cells in the ovaries of normal cycling and pregnant swine do not represent a simple migration and accumulation of blood eosinophilic leucocytes within the tissues.

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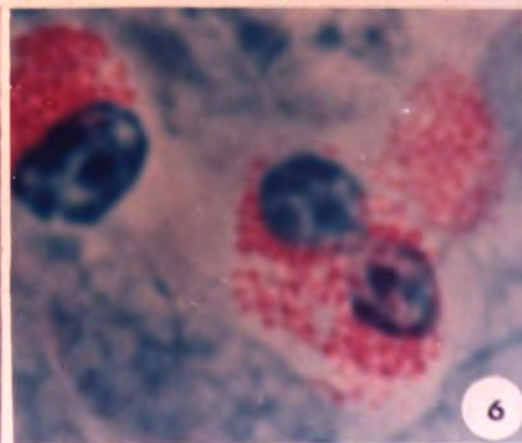
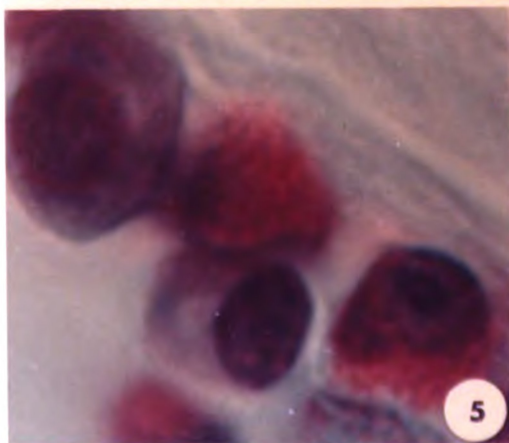
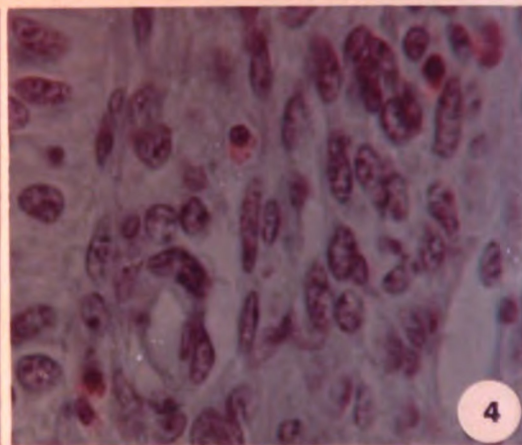
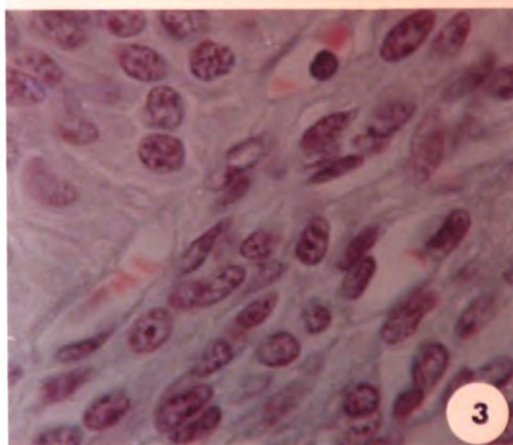
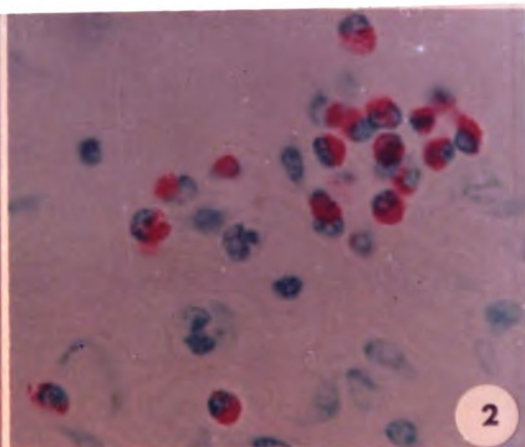
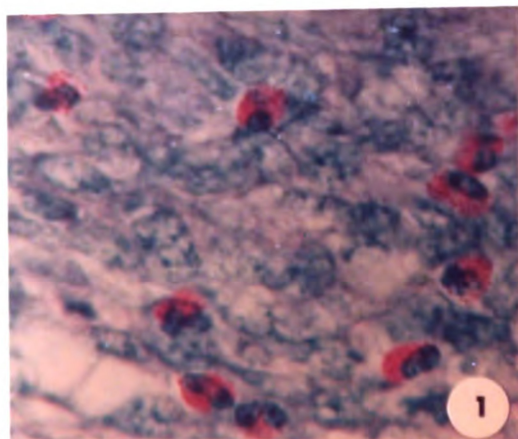
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PLATE I

Ovarian and Genital Tract Eosinophilic Granulated Cells

1. Gilt, day-11 of the normal estrous cycle. Eosinophilic granulated cells in the thecal layers of a large atretic follicle. Nuclei of cells are elongate, indented or oval. Giemsa stain. X600.
2. Gilt, day-11 of the normal estrous cycle. Eosinophilic and fragmenting plasma cells in the subepithelial stroma of the uterus. Giemsa stain. X600.
3. Gilt, day-1 of the normal estrous cycle. Thecal and granulosa layers of a Graffian follicle. Eosinophilic granulated cells were not observed in the thecal layers of large tertiary and Graffian follicles. Papanicolaou stain. X600.
4. Gilt, day-17 of the normal estrous cycle. Thecal layers of a small tertiary follicle. Minimal numbers of eosinophilic granulated cells noted in some small tertiary follicles. Papanicolaou stain. X600.
5. Gilt, day-15 of the normal estrous cycle. Subepithelial stroma of the uterus. Formation of eosinophilic plasma cells in areas of groups of basophilic plasma cells. Papanicolaou stain. X3,000.
6. Gilt, day-12 of the normal estrous cycle. Eosinophilic granulated cells in the theca interna of a small tertiary follicle. Noticeable rounding up of the nuclei in the



cells.

granule

x3,000.

cells. Nuclear division and dispersion of eosinophilic granules in the surrounding tissues. Giemsa stain.

X3,000.

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