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FINE STRUCTURE OF RAT UTERINE EOSINOPHILS AND THE POSSIBLE ROLE OF EOSINOPHILS IN THE MECHANISM OF ESTROGEN ACTION

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SUMMARY

The uterine eosinophil binding system for estrogens is considered to be responsible for some of the early estrogenic responses, such as water imbibition, histamine releasing and estrogen priming effects.

In the present report the fine structure of uterine eosinophils is described. Their pseudopodium-like elongations increase under hyperestrogenic conditions. The eosinophils are found in close proximity to the plasma membranes of other uterine cells. In hyperestrogenic animals, not only is this phenomenon more frequent, but the area of juxtaposition is increased. Pynocytotic vesicles are present in cells adjacent to the eosinophils in the areas of close cellular apposition. The disruption of uterine eosinophils with the liberation of dense granules and specific lysosomes is described. The ultrastructural findings are discussed in relation to the role of uterine eosinophils in the mechanism of estrogen action.

INTRODUCTION

TWO SEPARATE binding systems have been postulated for estrogens in the uterus [1, 2]. The first binding system, in the uterine cells, consists of a cytoplasmic receptor in the 105,000 *g* supernatant, the 8S receptor[3, 4] and the nuclear 5S receptor[5, 6], which compose the 8S–5S system[7–9]. The second system involves the uterine eosinophils.

Uterine eosinophil receptors, as shown by *in vitro* studies, have a high affinity, a great specificity and a limited capacity for compounds with estrogenic activity [1, 2, 10, 11]. The uptake of tritiated estradiol-17β by uterine eosinophils of the mature rat has also been demonstrated *in vivo* using a dry radioautographic technique for soluble compounds[12]. A differential extraction of the estrogens bound *in vivo* to their receptors in the uterus has shown different properties for the 8S–5S and eosinophil binding systems[12].

The 8S–5S binding system of the uterine cells is considered to be responsible for the genomic response, that is, the RNA synthesis, the protein synthesis and the true growth of the uterus[2]. These effects are completely blocked by Actinomycin D[13–16]. They are not counteracted by endogenous adrenocortical hypersecretion or exogenous 11-oxygenated corticosteroids[17–20]. Estradiol-17β has a much higher affinity than estriol for the 8S–5S system and therefore, is the more active estrogen for the genomic response[2].

The eosinophil binding system is apparently responsible for some of the early estrogenic responses, that is, the water imbibition, histamine releasing and estrogen priming effects[2]. These responses are not blocked by Actinomycin D [21]. However, they are diminished by conditions that produce blood or uterine eosinopenia, such as progesterone[10, 11, 22], endogenous adrenocortical hyper-

secretion or exogenous 11-oxygenated corticosteroids[17–20, 22–30], and are enhanced under conditions that produce uterine eosinophilia (intrauterine histamine or histamine liberators, intrauterine devices)[1, 31–33]. Estradiol-17β and estriol have similar affinities for the eosinophil binding system and therefore, both hormones are strong estrogens for the early estrogenic responses[2].

A morphological study of the uterine eosinophils under different hormonal conditions seems to be important in the elucidation of the role of eosinophils in the early estrogenic response. Several reports have already been published on the ultrastructure of the bone marrow and of the circulating blood eosinophils[34–47]. However, little attention has been paid to the eosinophils in the uterus. In the pioneer work of Ross and Klebanoff[48] and in our preliminary report[49] some new ultrastructural findings were described. It seemed, however, necessary to do a more extensive study under different hormonal conditions.

In the present study, we describe the fine structure of uterine eosinophils under different hormonal conditions, and discuss the ultrastructural findings in relation to the role of these cells in the mechanism of estrogen action.

EXPERIMENTAL

1. *Animals.* Sprague–Dawley and Donju mature female rats were used. The stage of the estral cycle was determined by vaginal smears. As the number of uterine eosinophils increases concomitantly with the estrogen level of the animal and decreases with progesterone[10, 11, 26, 50–53], we decided to use adult estrous rats at the beginning of these experiments. However, rats in the following hormonal conditions were also used: proestrus, diestrus and estrogen treated animals. The latter were injected subcutaneously with 5 mg of estradiol valeriate in an oil solution (Primofol* R depot) 3 days before killing the animals.

2. *Electron microscopy.* Samples of the uterus were obtained immediately after killing the animal by decapitation. The specimens were immersed in a drop of fixative and immediately trimmed into small pieces for adequate fixation.

Several fixation procedures were employed: either 4–6·5% glutaraldehyde in 0·1 M phosphate or cacodylate buffer, with a final pH of 7·2[54] or Karnowsky's formaldehyde–glutaraldehyde fixative[55] for 2 h. After washing in a buffer solution for 1 h, the specimens were postfixed in 1% osmium tetroxide in a s-collidine or in a pH 7·3 phosphate buffer[56] for 1 h at 4°C.

The tissues were dehydrated in a graded series of ethanol, absolute acetone, and then embedded in Araldite[57]. Ultrathin sections were cut on a Porter–Blum ultramicrotome, then double-stained with uranyl acetate and lead citrate [58] and studied under either a Siemens Elmiskop I or a Philips EM-300 electron microscope.

RESULTS

(a) *Localization.* The previously described localization of the uterine eosinophils under the light microscope[10] is confirmed. They are predominantly located in the deep stroma of the mucosa or in the connective tissue between the muscular layers; a few are found in the subepithelial stroma of the mucosa or between muscle cells.

(b) *Ultrastructure.* Uterine eosinophils have a characteristic multilobulated or

*Primofol R Depot (Schering A. G., Berlin).

ring-shaped nucleus with abundant aggregated clumps of chromatin in the nuclear periphery. The cytoplasm has a high electronic density, and contains only a few mitochondria, numerous specific lysosomes (for a detailed description of the lysosomes, see Ref. [42], a centrosome, a Golgi apparatus near the nucleus, and certain dense granules scattered throughout the cytoplasm (Figs. 1 and 2). These round or oval-shaped granules have not been previously described, and will be called "dense granules" in this paper. They measure 900–1200 Å in dia, are limited by a single unit membrane and its vesicular matrix embeds a spherical body of higher electronic density measuring about 600 Å in dia (Fig. 2, arrow; and Fig. 3). Some of these dense granules are always located in the vicinity of the Golgi apparatus (Fig. 1).

Uterine eosinophils have pseudopodium-like elongations of a lower electronic density than the rest of the cytoplasm. These elongations usually contain few dense granules and occasionally some specific lysosomes (Figs. 4 and 13, PS). The pseudopodium-like elongations of the uterine eosinophils are much more numerous in hyperestrogenic animals.

(c) *Proximity to other uterine elements.* A large percentage of the uterine eosinophils is in close proximity to the plasma membranes of connective tissue cells of the stroma, as previously described [48, 49]; or, to a lesser degree, to the plasma membranes of muscle cells or of other eosinophils (Figs. 5–11). The space between the membranes of the two adjacent cells is 120–200 Å, and at times even less. We have found that this close relationship between membranes is much more frequent, and the area of juxtaposition is also increased, in hyperestrogenic animals (Figs. 6, 7 and 10), particularly in those treated with high doses of estrogens. Furthermore, we have confirmed our previous finding [49] of pinocytotic vesicles, resembling coated vesicles, which may be seen in the cells adjacent to the eosinophils at the zones of close apposition (Fig. 11).

(d) *Disruption of uterine eosinophils.* The disruption and lysis of the uterine eosinophils in the rat uterus previously described [48, 49] occurs principally in estrus, in a series of well-defined stages (Fig. 12). The first step of this process is a loss of electronic density in the cytoplasm. This is followed by an increase in size of the perinuclear cystern, disruption of the cytoplasmic membranes and finally, spilling of the cytoplasmic content, viz. dense granules and specific lysosomes. The latter remain scattered in the ground substance, as it can be seen in Figs. 13 and 14. It is also possible to find free lysosomes and dense granules of eosinophilic origin inside the uterine stromal cells (Fig. 15). The last situation can only be observed in estrus or in estrogen treated animals.

In contrast with the picture of disruption and lysis of uterine eosinophils observed in estrus, large phagocytic vacuoles in the uterine stromal cells, containing whole eosinophils or large portions of them, can be seen frequently in diestrus, as previously described by Ross and Klebanoff [48]. This morphological findings in diestrus have not been observed during estrus.

DISCUSSION

The involvement of uterine eosinophils in the early estrogenic response in the uterus has previously been postulated [2, 10, 12]. The aim of the present report is to describe the ultrastructure of uterine eosinophils under different hormonal conditions, and to discuss it in relation to the role of these cells in the mechanism of estrogen action.

"Dense granules", scattered throughout the cytoplasm of the uterine eosinophil, are constantly present near the Golgi apparatus. This observation suggests that they might have originated from the Golgi vesicles and contain a secretory product synthetized by the eosinophil under the estrogenic influence in the uterus. At this point, it is interesting to compare the "dense granules" of uterine eosinophils with the "smooth vesicles" described at the periphery of the Golgi complex of immature eosinophils in the bone marrow[59]. Both are morphologically very similar. The "smooth vesicles" contain peroxidase during the myelocyte stage. Their function seems to be to transport enzymes from the Golgi apparatus to the specific lysosomes of immature eosinophils. These "smooth vesicles" do not exist in the mature eosinophil[59].

The pseudopodium-like elongations seem to be the morphological manifestation of the motility of the eosinophils in the uterus. It is known that eosinophils do not exist in the uterus of immature or ovariectomized animals and that they are attracted to this organ by estrogens[10, 11, 33, 50–53]. The higher frequence and extent of the pseudopodium-like elongations observed in uterine eosinophils under hyperestrogenic conditions may be related to an increase in the mobility of these cells in the uterus under estrogenic activity. It is still not known whether eosinophils are attracted to the uterus directly by estrogens, as suggested by Josefsson[60], or by an unknown substance produced by uterine cells under estrogenic stimulation. The mechanism of uterine eosinophilia remains to be elucidated.

The proximity of uterine eosinophils to other uterine cells, which is more marked under hyperestrogenic conditions, suggest that eosinophils might be physiologically related to adjacent cells. Pinocytotic vesicles found under hyperestrogenic conditions in the cells adjacent to the eosinophils at the zones of close cellular apposition, seem to be the anatomical support for this possibility.

The previous finding of disruption and lysis of eosinophils in the uterus [48, 49] has been confirmed in the present study. "Dense granules" and specific lysosomes from eosinophils are released to the extracellular space and even frequently found in neighbouring cells in hyperestrogenic animals. In this way it is possible that some products of the eosinophils may be released and act on the ground substance and/or neighbouring cells. Studies by other authors have demonstrated that the specific lysosomes of the eosinophils contain a hemoprotein with peroxidase activity[61–73], which has been found to be different from that of the neutrophils[74–76], beta glucuronidase, arylsulfatase, and many other enzymes[51, 59, 62, 68, 73, 77–83]. Beta glucuronidase, arylsulfatase and possibly other hydrolytic enzymes released from eosinophils during their disruption in the uterus, could be responsible for the partial depolimerization of the ground substance and imbibition of water, as well as for the increase of vascular permeability, all of which are early estrogen effects in the uterus.

In regard to the other eosinophilic enzyme, the peroxidase has been implicated in estrogen action. It has been found that hydrogen peroxide significantly increases the amount of tritiated estradiol firmly bound to uterine eosinophils, and this binding has been related to the eosinophil peroxidase[70]. Estrogenic steroids were found to be able to mediate the peroxidase-catalyzed transfer of hydrogen between $NADP^+$ and NAD^+[84, 85] or between the reduced pyridine nucleotides and either peroxide of hydrogen[86–88] or other terminal hydrogen acceptors[89, 90]. It is possible to speculate, as was previously suggested[84–90],

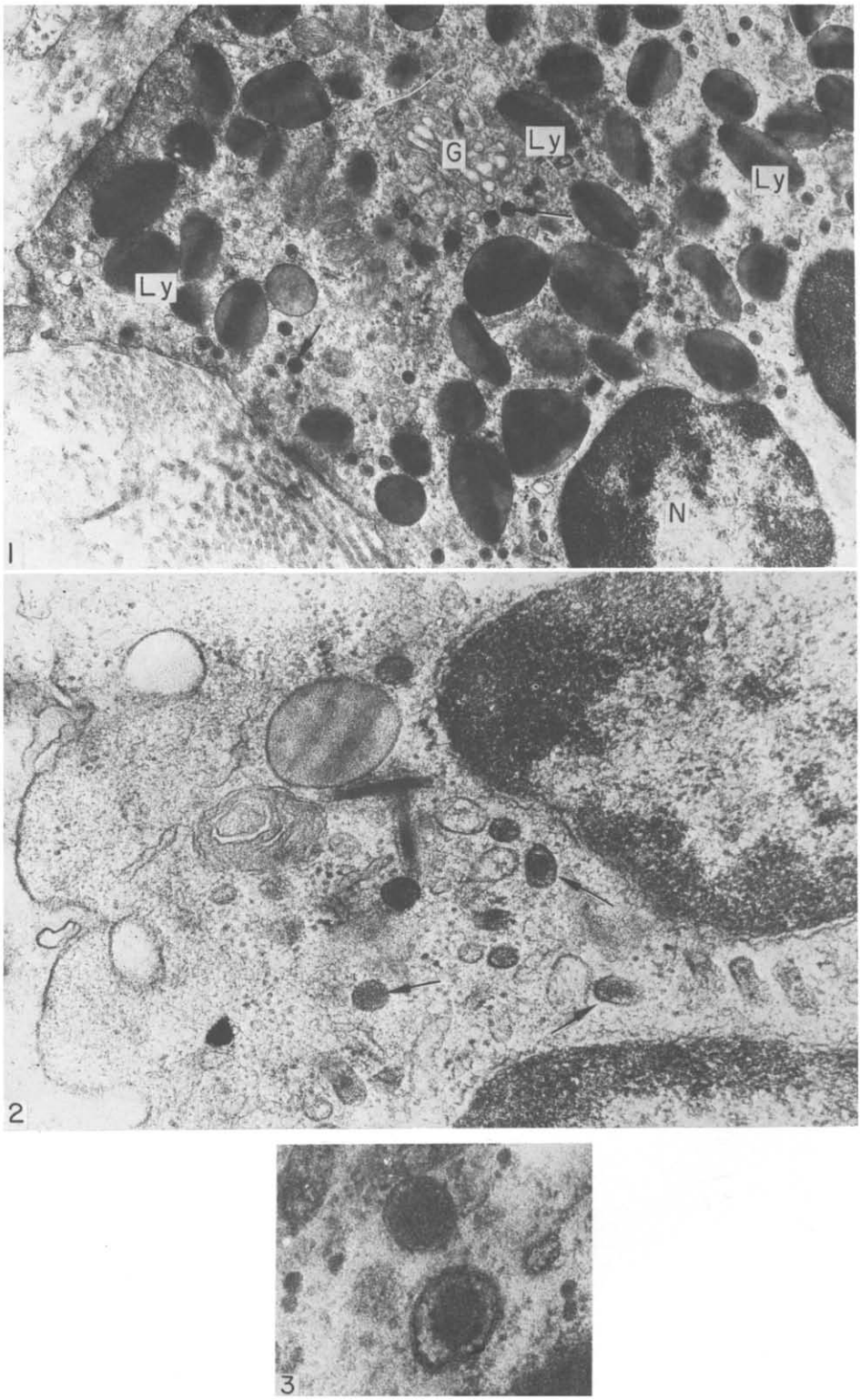


Fig. 1. Ultrastructure of uterine eosinophil. Ly specific lysosome; N nucleus; G Golgi apparatus; arrow, dense granules. (× 28,000)

Fig. 2. Uterine eosinophil. Arrow, dense granules. (× 64,000)

Fig. 3. Higher magnification of dense granules. (× 120,000) (*Facing p.* 280)

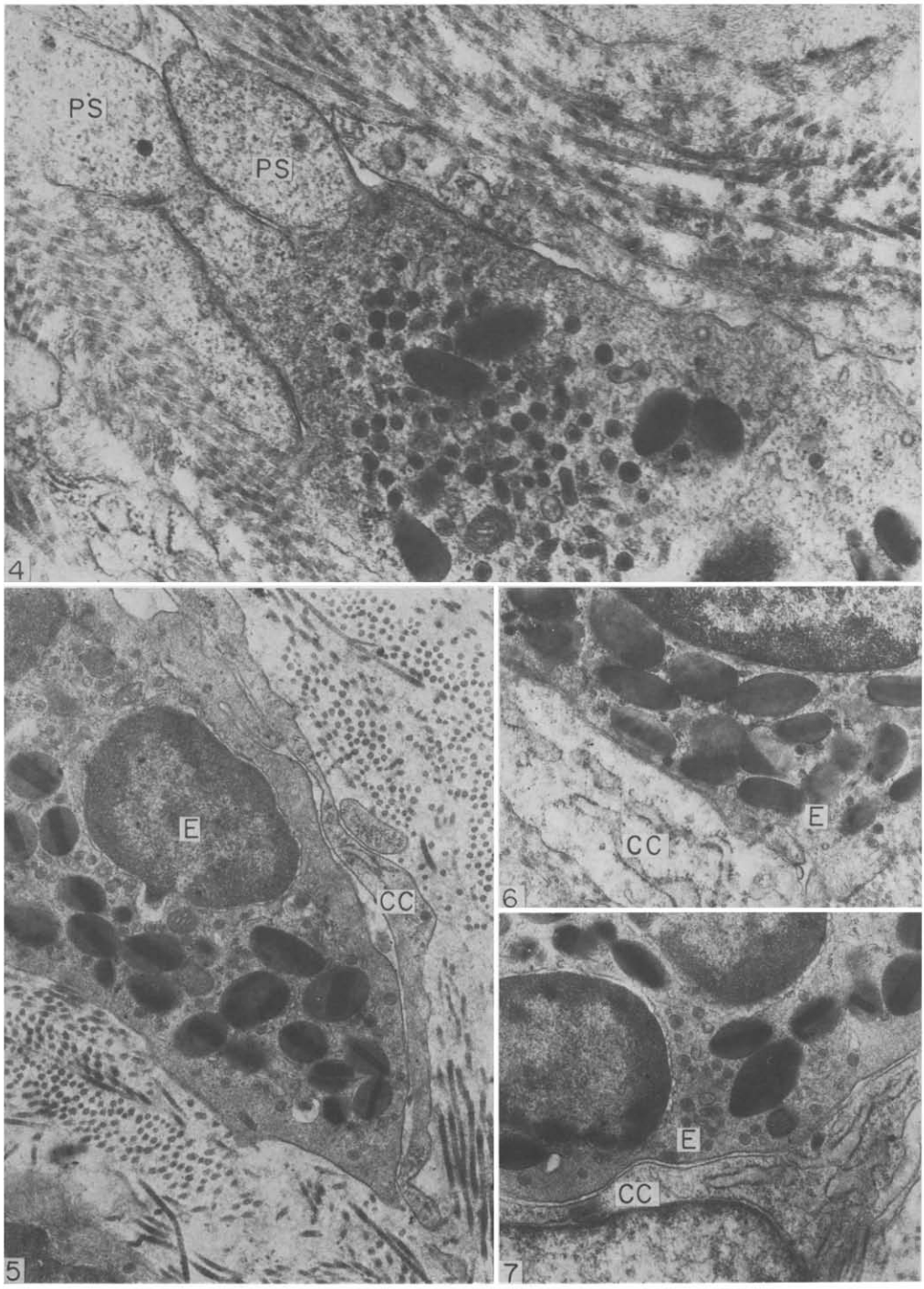
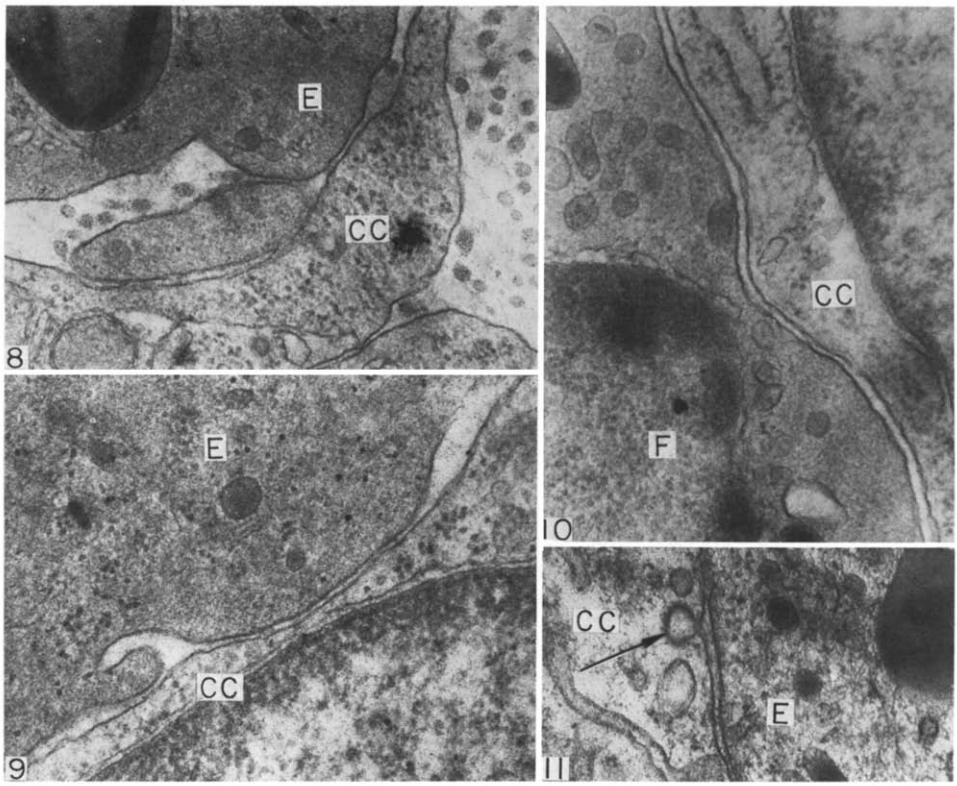


Fig. 4. Uterine eosinophil; pseudopodium-like processes (PS). (× 36,000)

Figs. 5–7. Close relationship between membranes of uterine eosinophils (E) and connective tissue cells (CC). (× 21,000)

Fig. 5. Typical aspect of this relationship in animals with a lower level of endogenous estrogens.

Figs. 6 and 7. Same situation in rats with higher level of endogenous estrogens.



Figs. 8–11. Two aspects of the cloes relationship between membranes of uterine eosinophils (E) and connective tissue cells (CC). (× 50,000)

Figs. 8 and 9 represent lower level of endogenous estrogens.

Figs. 10 and 11, same in rats with higher hormone level. Arrow in Fig. 11 shows a pynocytotic vesicle.

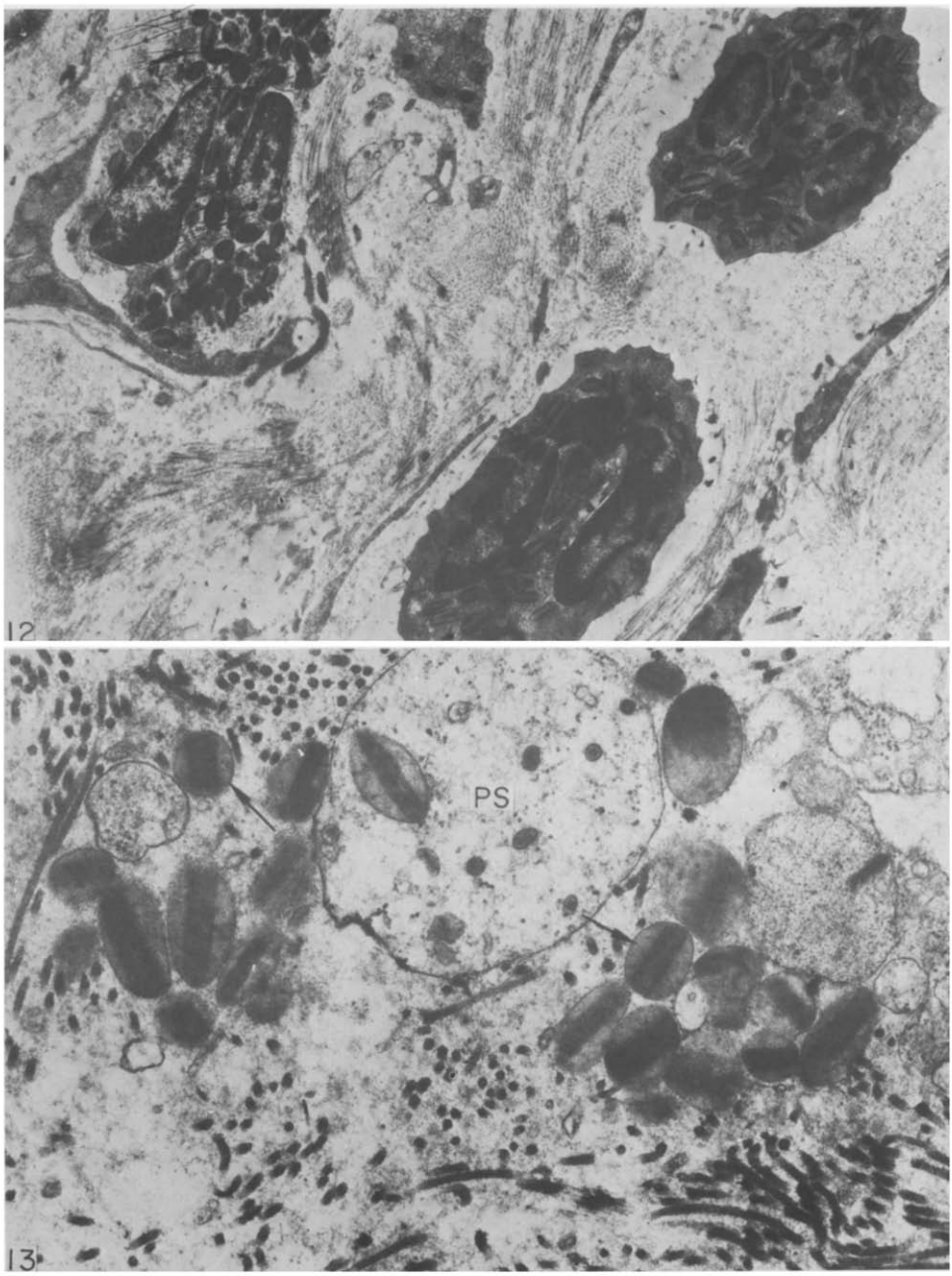
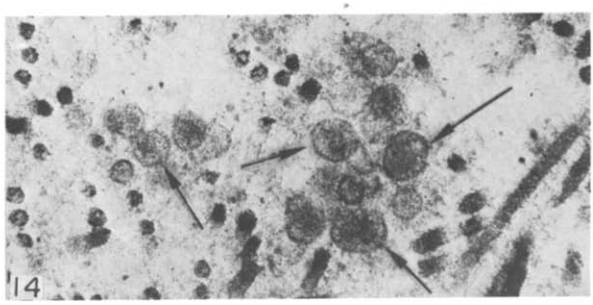


Fig. 12. Two eosinophils at right show the usual aspect of intact cells. Left side: eosinophil in advanced stage of disruption and lysis. (× 6,300)

Fig. 13. Specific lysosomes appearing free in the ground substance, between collagen fibres. Note the presence of unit membrane surrounding specific lysosomes (arrow). PS, pseudopodium-like process of an adjacent eosinophil. (× 32,000)



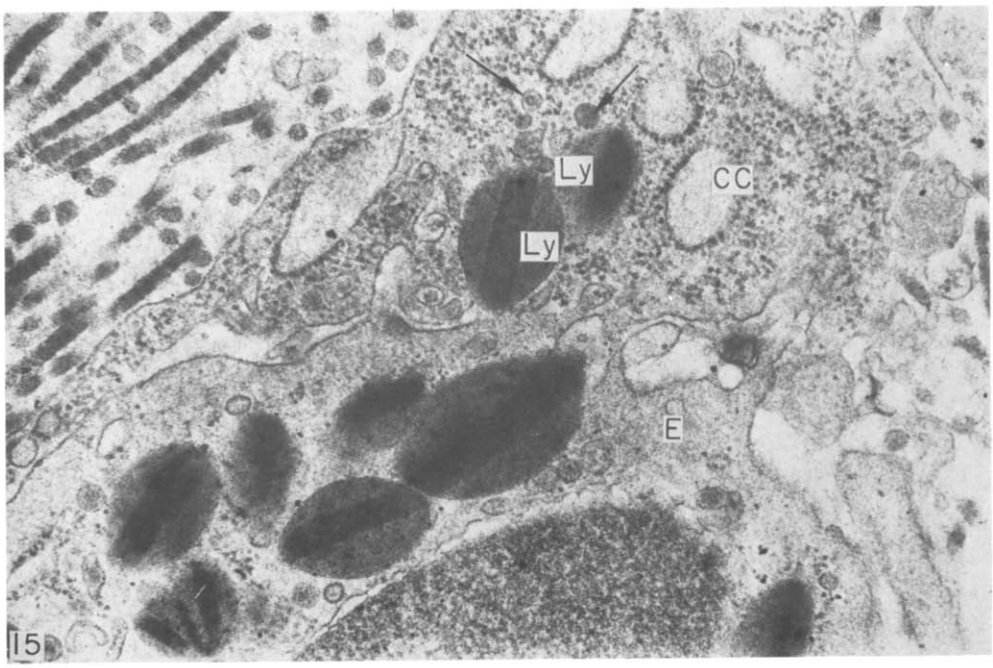


Fig. 14. Dense granules, like those found in intact uterine eosinophils, appear scattered in the ground substance. Note the presence of their unit membrane (arrow). (× 50,000)

Fig. 15. Uterine connective tissue cell (CC) closely contacting an eosinophil (E). Note the presence of specific lysosomes (Ly) and dense granules (arrow) of eosinophilic origin, inside the cytoplasm of the connective tissue cell. (× 50,000)

that estrogens bound to the eosinophil peroxidase may play a role as an intermediate hydrogen and electron carrier in a hypothetical redox cycle in essential oxydative processes.

We conclude that the ultrastructural picture described here provides further information on the involvement of uterine eosinophils in the estrogenic response in the uterus.

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