THE EOSINOPHILIC LEUKOCYTE

Fine Structure Studies of Changes in the Uterus During the Estrous Cycle

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The presence of eosinophilic leukocytes in blood has led to the consideration of the eosinophil as essentially a blood cell. However, the eosinophil passes readily into the connective tissue spaces of a number of tissues and there resides as a normal constituent of the cellular population. Rytömaa (1) has given a detailed description of the distribution of eosinophils in the organs of the rat. In this species the flow of eosinophils to the uterus under the influence of the female sex hormones is a most striking phenomenon (1–4). No eosinophils are present in the uterine connective tissues of the immature rat or of the animal late in pregnancy in contrast to the large numbers observed in the nonpregnant mature animal. In the latter, the uterine eosinophil count varies with the estrous cycle with the smallest number (approximately 150,000) present in diestrus and the largest number (approximately 8,000,000) present in estrus (1). Eosinophils are absent from the uterus of the adult ovariectomized animal but appear in large numbers after estrogen administration. Progesterone decreases the estrogen-induced accumulation of eosinophils in the uterus of the ovariectomized animal.

The cyclic nature of the uterine eosinophilia in the intact animal and the hormonal control of uterine eosinophilia in the castrate animal provides an opportunity for a morphological study of the tissue turnover of eosinophils. A number of studies of the fine structure changes in the uterus of the estrogen-primed animal have appeared; however, little attention has been paid to the uterine eosinophils in these studies. The present paper deals with the fine structure changes in the eosinophil during its lifespan in the uterus.

Materials and Methods

Thirty-eight female Simonsen albino (Sprague-Dawley) rats weighing between 190 and 250 g were used in this study. Vaginal smears were obtained daily from each animal to determine the regularity and the status of the estrous cycle. At 1 day preestrus the vaginal smear was composed almost entirely of nucleated epithelial cells. During estrus, the vaginal smear

contained squamous casts in large numbers, and 1 day postestrus the influx of large numbers of polymorphonuclear leukocytes was seen. During diestrus, the vaginal smear was composed largely of white blood cells with smaller numbers of nucleated epithelial cells and occasional squamous casts. At a predetermined time each of the animals was sacrificed by decapitation. The uterus was dissected out and cut in half. One-half was weighed and the other half was utilized for investigation by light and electron microscopy. The horn of the uterus was cut into short cylindrical segments. Some of these were placed in neutral-buffered formalin for embedding in paraffin, and others were placed in s-collidine–buffered osmium tetroxide (pH 7.3) at 4°C. These tissues were fixed in osmium for 1 hr, rinsed in buffer, then postfixed in neutral-buffered formalin for 1 hr. They were subsequently dehydrated through a graded series of alcohols and embedded in epoxy resin (Epon 812).

Paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin, the van Gieson stain, and with alkaline orcein (5). The later sections were used to count the numbers of eosinophils.

Tissues prepared for electron microscopy were double stained with lead and uranyl acetate and examined in an RCA EMU 3G electron microsope. Thin sections (1 μ) were stained with azure II methylene blue (6) for examination with the light microscope.

RESULTS

Light Microscopic Observations

The very striking cyclic changes in the numbers of tissue eosinophils in the uterus of the rat reported by Rytömma (1) have been confirmed. During diestrus, relatively few eosinophils were seen in the uterine connective tissues. However, at 1 day preestrus a number of eosinophils were present in the small vessels particularly in the endometrium adjacent to the muscular layer. Many eosinophils were also present in the perivascular spaces surrounding these vessels. Numerous eosinophils were observed in the connective tissues of the uterus just prior to estrus, during estrus, and 1 day postestrus. Although the largest number of these cells was seen in the endometrium adjacent to the muscular layer, many eosinophils were found in the connective tissue spaces between the smooth muscle cells and in the subserosal region as well. No eosinophils could be recognized in the immediate vicinity of the epithelial lining of the endometrium. The eosinophil changes just described occurred in association with marked cyclical changes in many other aspects of uterine morphology. These have been described in detail elsewhere (7).

Fine Structure Observations

Eosinophil Emigration.—At 1 day preestrus, numerous eosinophilic leukocytes could be seen within the lumen of the small blood vessels particularly in the endometrium adjacent to the muscular layer. These cells were clearly recognized as eosinophils by their characteristic large granules containing crystalloid bars. The nuclei were multilobed and, in addition to the large granules, there were large numbers of smaller granules and several mitochondria. Fig. 1 demonstrates an intravascular eosinophilic granulocyte together with several erythrocytes, a lymphocyte, a monocyte, and a platelet.

Of the blood cells, the eosinophilic granulocyte was the only one seen to emigrate between the endothelial cells of the small vessels into the perivascular connective tissue spaces. Various stages of this process can be seen in Figs. 2 to 4. Fig. 2 demonstrates the emigration of an eosinophilic leukocyte through the endothelial lining of a small vessel. A relatively granule-free portion of the cell is seen to penetrate first and is separated from the granule-rich remainder of the cell by a restriction which gives the cell an hourglass appearance. In Fig. 3 a neutrophilic leukoycte can be seen within the lumen of a vessel and an eosinophil is present between endothelial cells of the same vessel. In Fig. 4 eosinophils are seen both between endothelial cells of a small vessel and in the perivascular connective tissue spaces.

At 1 day preestrus, during estrus, and at 1 day postestrus, many eosinophils were found in the connective tissue spaces of the endometrium and the myometrium. Fig. 5 demonstrates a typical region in the endometrium just prior to estrus, in which four eosinophils can be seen in the connective tissue spaces. There was often extensive contact between the cell membrane of an eosinophilic leukocyte and fibroblasts or macrophages (Fig. 6). Contact between eosinophils and macrophages may represent the initiation of phagocytosis of an eosinophil by a macrophage (see below).

Eosinophil Lysis.—Just prior to estrus, lysed eosinophilic leukocytes were commonly observed in the connective tissue spaces of both the endometrium and the myometrium (Figs. 7 and 8). The contents of the lysed eosinophil (i.e. the large and small granules, mitochondria, nuclear debris, etc.) can be seen in the extracellular spaces. It was characteristic to see, as in Fig. 7, a lysed eosinophil adjacent to an intact eosinophil which appears normal in every way. Fig. 8 demonstrates a lysed eosinophil, represented by degenerated nuclear material and several large and small granules, in the extracellular spaces adjacent to a smooth muscle cell. The smooth muscle cells, as well as two other eosinophils in the same region, showed no signs of degeneration.

Phagocytosis of Eosinophils.—Many macrophages, containing what are apparently whole eosinophils in various stages of degeneration, were visible in the connective tissue spaces during estrus and immediately postestrus. In Fig. 9 an eosinophil can be seen in a digestive vacuole of a macrophage in which the large eosinophilic granules are readily recognized although extensive nuclear degeneration and fragmentation has occurred. In Fig. 10 the large eosinophilic granules are more densely packed within a phagocytic vacuole but remain readily recognized by their crystalloid bars. In the macrophages seen in Fig. 11 more extensive degeneration of vacuolar contents has occurred; however, several dense areas suggestive of crystalloid bars of eosinophil granules are apparent. Single eosinophil granules can also be seen in digestive vacuoles of macrophages suggesting that phagocytosis of granules released during eosinophil lysis can also occur (Fig. 9, insert). A large number of digestive vacuoles

whose contents cannot be definitely said to be of eosinophilic origin were seen in macrophages as well (Figs. 7 and 10). There were many macrophages with non-characteristic digestive vacuoles adjacent to the epithelial cells of the endometrium and, indeed, macrophages could be seen between the epithelial cells. Large digestive vacuoles also were commonly seen in epithelial cells especially during estrus.

Smooth Muscle Cells.—The smooth muscle cells also exhibited cyclical changes which are of considerable interest. Fig. 8 demonstrates a smooth muscle cell during estrus which contains an extensive enlargement of the rough endoplasmic reticulum. In view of recent studies on the effect of estrogen upon uterine protein and nucleic acid metabolism, another series of experiments were performed to determine the relationship of the ergastoplasmic changes in the smooth muscle cells to the presence of estrogen. These studies will be reported in a subsequent paper.

DISCUSSION

Although the eosinophilic leukocyte has been observed in tissues for many years, its functional role has remained obscure (see reference 8). It can act as a phagocytic cell under certain conditions (9–11) and appears to be attracted to sites of antigen-antibody reactions. The cytoplasm of the eosinophil contains primarily mitochondria, characteristic large and small granules, some smooth surface membranes and glycogen. The granules appear to be of two distinctly different morphological types. These are the large (circa 0.4 to 0.8 μ) ovoid granules with a central bar or crystalloid, and the small, round granules (circa 0.1 to 0.2 μ diameter). Archer and Hirsch (12) have isolated eosinophil granules and studied their enzyme content. The granules were found to contain cathepsin, ribonuclease, arylsulphatase, beta glucuronidase, acid phosphatase, alkaline phosphatase, and peroxidase. The first five of these enzymes are characteristically associated with lysosomes. Lutzner and Benditt (13) have separated eosinophil granules into two populations. They implicated the large granules as a repository of peroxidase and the small granules as the site of aggregates of acid hydrolytic enzymes.

The studies reported here indicate some of the fine structure changes in the eosinophil during its lifespan in the uterus. Gansler (14) has suggested that the "Spezialzellen" of the rat uterus which contain "osmiophilen Granula" similar to those of the eosinophilic leukocyte, originate from smooth muscle cells which, under the influence of follicular hormone lose their myofibrils and form osmophilic granulations from mitochondria. Our studies demonstrate eosinophils in the lumens of vessels and between endothelial cells, particularly on 1 day preestrus demonstrating that the immediate source of the uterine tissue eosinophil is, in fact, the blood eosinophil.

The eosinophils were seen in large numbers in the connective tissue spaces

and many were in intimate contact with other connective tissue cells. Intact eosinophils appeared to be destroyed in one of two ways. They were engulfed by macrophages and underwent degenerative changes within the digestive vacuoles of these cells until the vacuolar contents could no longer be clearly recognized to be of eosinophilic origin. Although intact eosinophils were not seen adjacent to the epithelial lining of the endometrium, macrophages containing digestive vacuoles were found adjacent to, and between, epithelial cells. Eosinophils also were destroyed by lysis. Lysed eosinophils were seen adjacent to intact eosinophils indicating that lysis was not an artifact of tissue preparation. The cytoplasmic contents of lysed eosinophils, including the characteristic large granules with crystalloid bars, small granules, mitochondria, etc. were present in the extracellular spaces. Macrophages with digestive vacuoles containing single eosinophil granules were seen suggesting that individual granules also have been phagocytozed.

In the intact mature animal striking cyclic variations occur in uterine structure under endogenous hormonal control. Similarly the administration of estrogen to immature or ovariectomized female animals initiates a chain of events that lead to profound chemical and morphological changes, as well as to a marked increase in the amount of uterine tissue. In the rat, eosinophils participate in large numbers in these cyclic or hormonally induced changes. The relationship of the turnover of tissue eosinophils in the estrogen primed uterus to the biochemical and morphological changes in this organ is unknown. Lucas et al. (15) have described the presence of a peroxidase in the estrogen-primed uterus. Eosinophils are exceptionally rich in peroxidase and histochemical studies suggest that the uterine peroxidase is present largely in eosinophils (1, 4). The role, if any, of the peroxidase in uterine metabolism is unknown. Certain peroxidase catalyzed reactions are stimulated by phenolic estrogens (16–18); however, it is not known whether this reaction contributes in any way to the estrogen-mediated uterine changes. The uterine (eosinophil) peroxidase can catalyze the inactivation of estradiol as well as certain other estrogens in the presence of H_2O_2 or a H_2O_2-generating system in vitro (4); however, it is not known whether this estrogen-inactivating system is operative in vivo.

Although eosinophils are phagocytic cells (9–11), there was no evidence of phagocytosis by these cells in the uterine connective tissues under the conditions of our experiments; rather the eosinophils are themselves phagocytozed by the resident macrophages of the uterine connective tissues.

Eosinophils contain large numbers of lysosomal granules. The cyclic influx and destruction of eosinophils in an organ undergoing cyclic enlargement and involution suggests the possibility that the lysosomal enzymes of the eosinophil may play a role in the tissue turnover. Finally, the uterus may be simply a repository where eosinophils end their life cycle by a process either of lysis or phagocytosis by macrophages. The question remains as to whether the emigra-

tion of eosinophils into the uterus with subsequent release of their granules into the connective tissue spaces as a result of cell lysis represents a functional role for these cells.

SUMMARY

This study has presented the fine structure changes in the eosinophilic leukocyte in the rat uterus during the estrous cycle. Eosinophils were seen to emigrate into the uterine connective tissues from the blood stream. Just prior to, during estrus, and 1 day postestrus, eosinophilic leukocytes underwent lysis releasing their contents into the extracellular spaces and both whole eosinophils and individual granules from lysed cells were ingested by resident macrophages. No phagocytic activity by eosinophils was observed.

The possible relationship of the turnover of eosinophils to the profound morphologic and chemical changes in the uterus during the estrous cycle was discussed.

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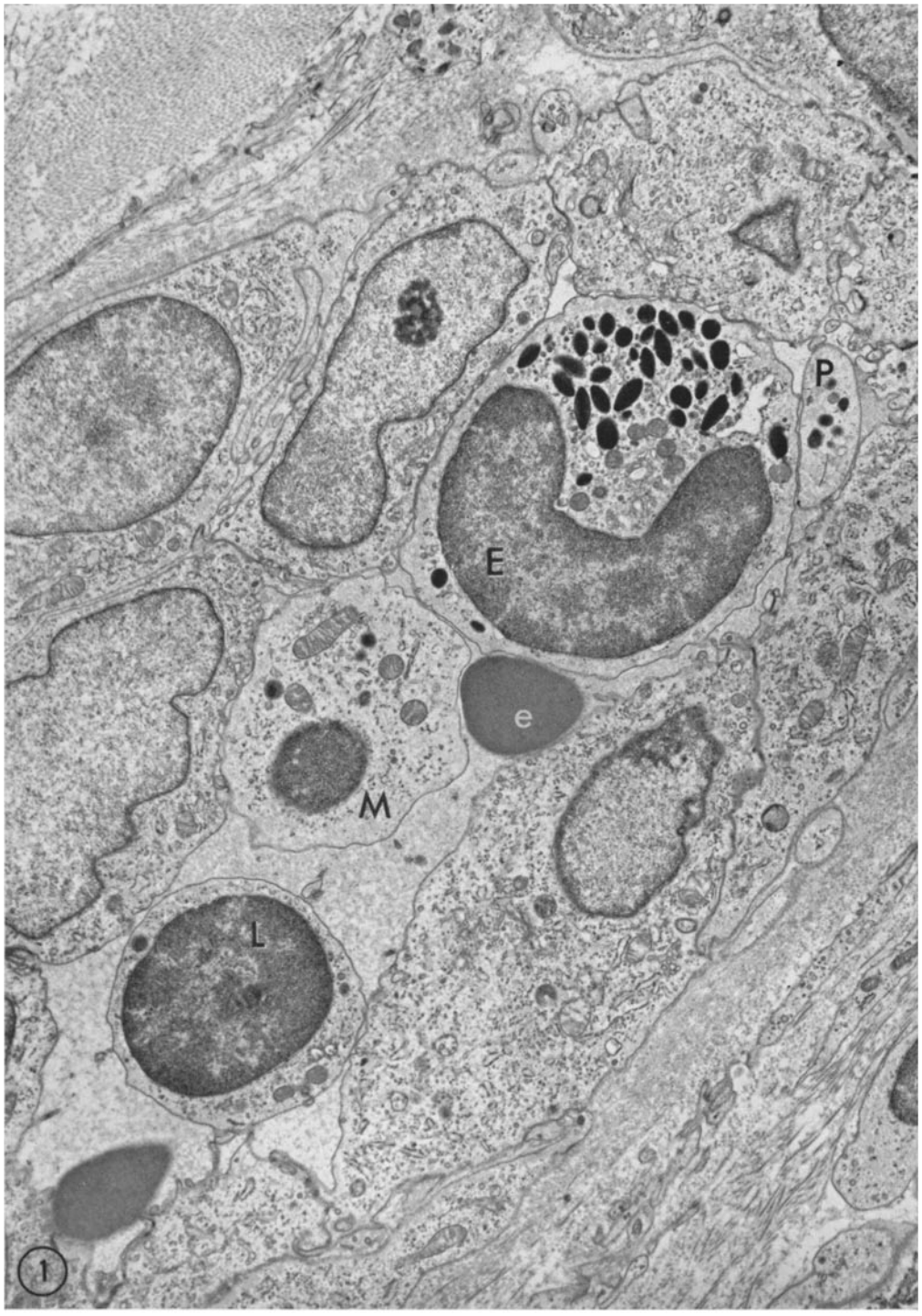
EXPLANATION OF PLATES

PLATE 64

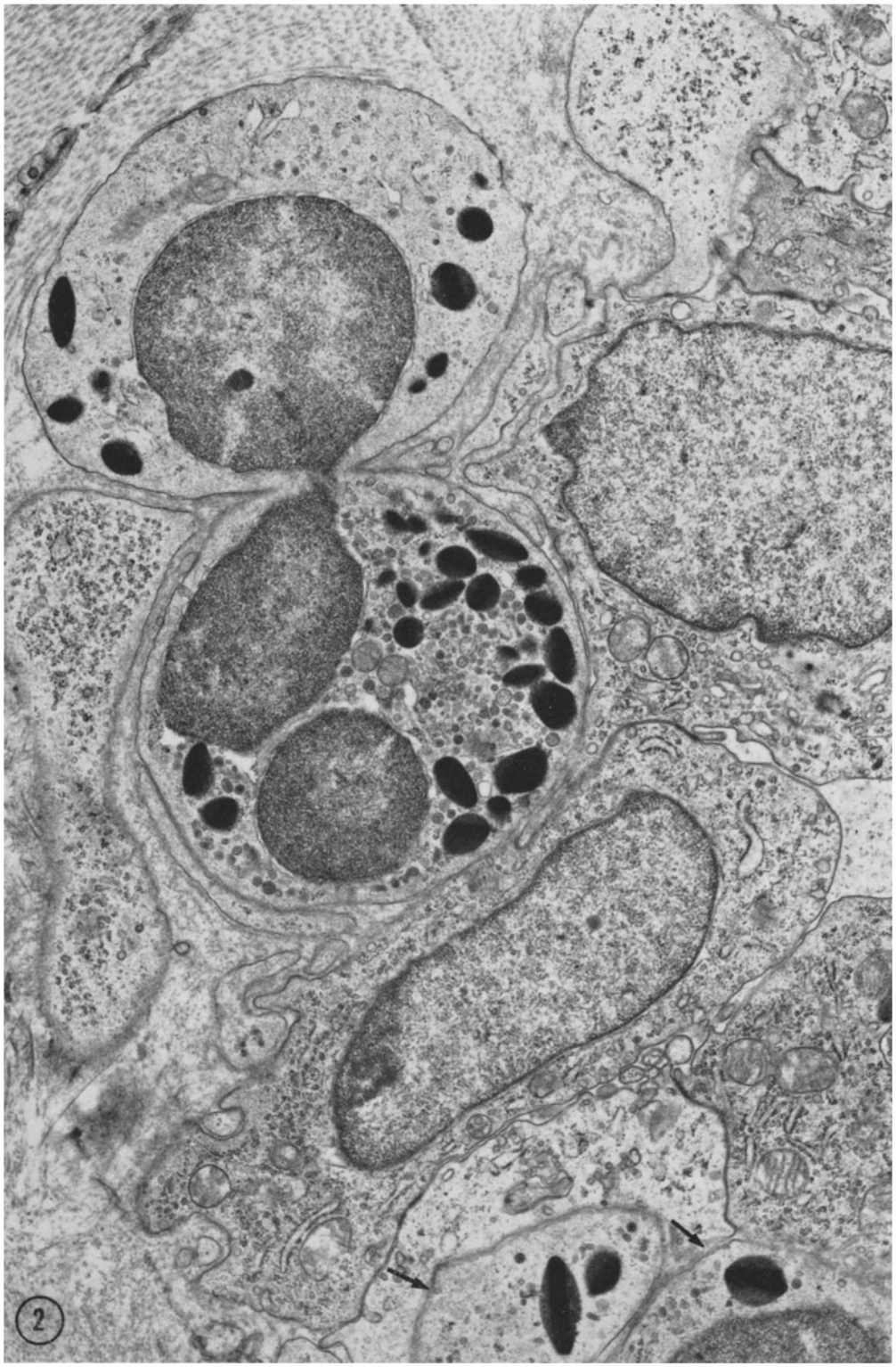
FIG. 1. This micrograph indicates a vascular area in the endometrium adjacent to the myometrium of a rat uterus 1 day prior to estrus. An eosinophil (*E*), a platelet (*P*), a monocyte (*M*), a lymphocyte (*L*), and two erythrocytes (*e*) can be seen within the lumen of a small vessel. The blood vessel is surrounded by numerous collagen fibrils and connective tissue cells and is delimited by a well defined basement membrane. × 5500.



(Ross and Klebanoff: The eosinophilic leukocyte)

PLATE 65

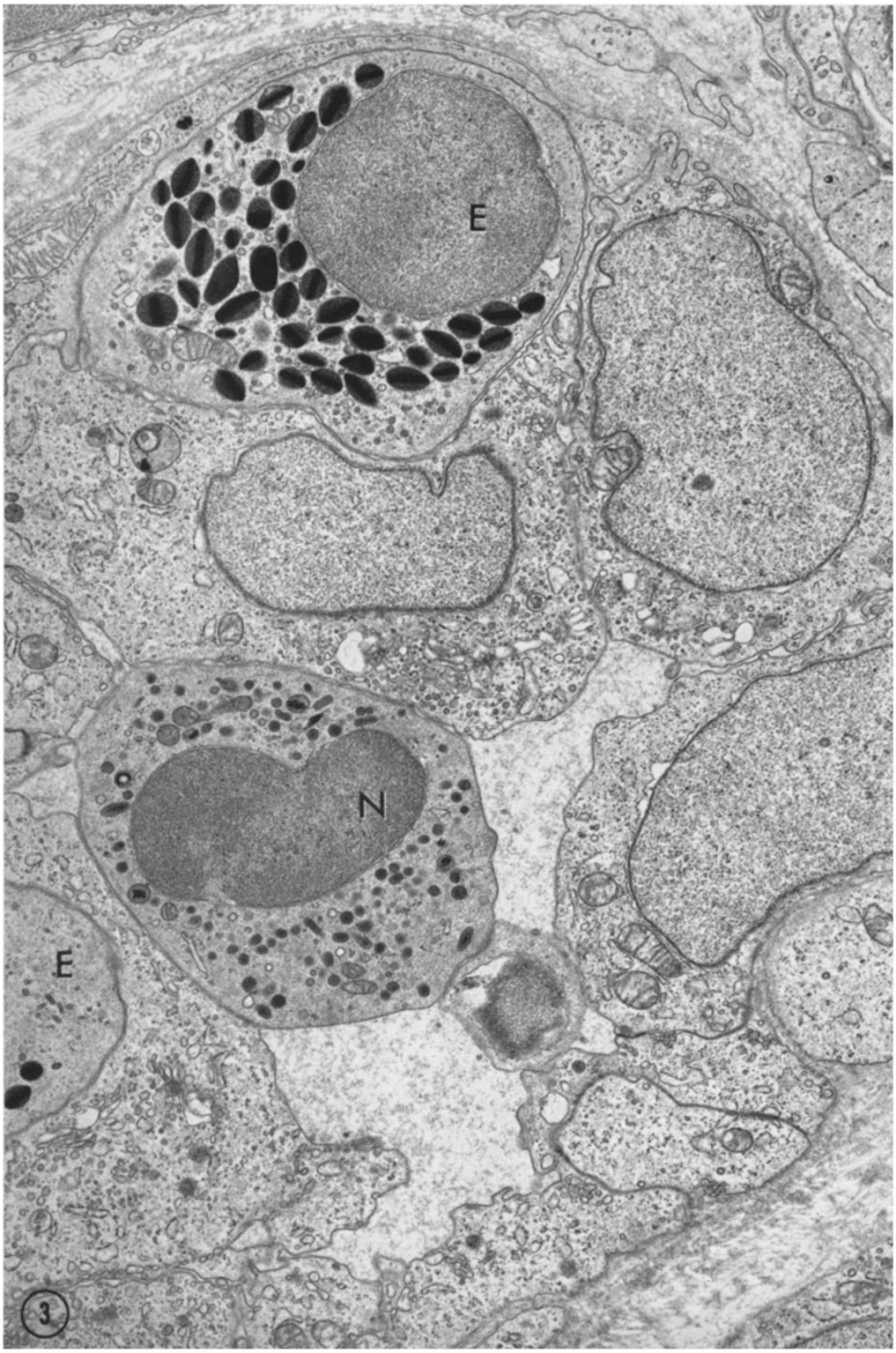
FIG. 2. An eosinophilic leukocyte can be seen in the process of emigration between the cells of a vessel located subjacent to the myometrium 1 day prior to estrus. This granulocyte is hourglass in shape and a relatively granule-free portion of the cell appears to precede a granule-rich portion. Portions of two eosinophilic leukocytes (arrows) can be seen in the lower right hand corner apparently located between the cells of this same vessel. × 11,000.



(Ross and Klebanoff: The eosinophilic leukocyte)

PLATE 66

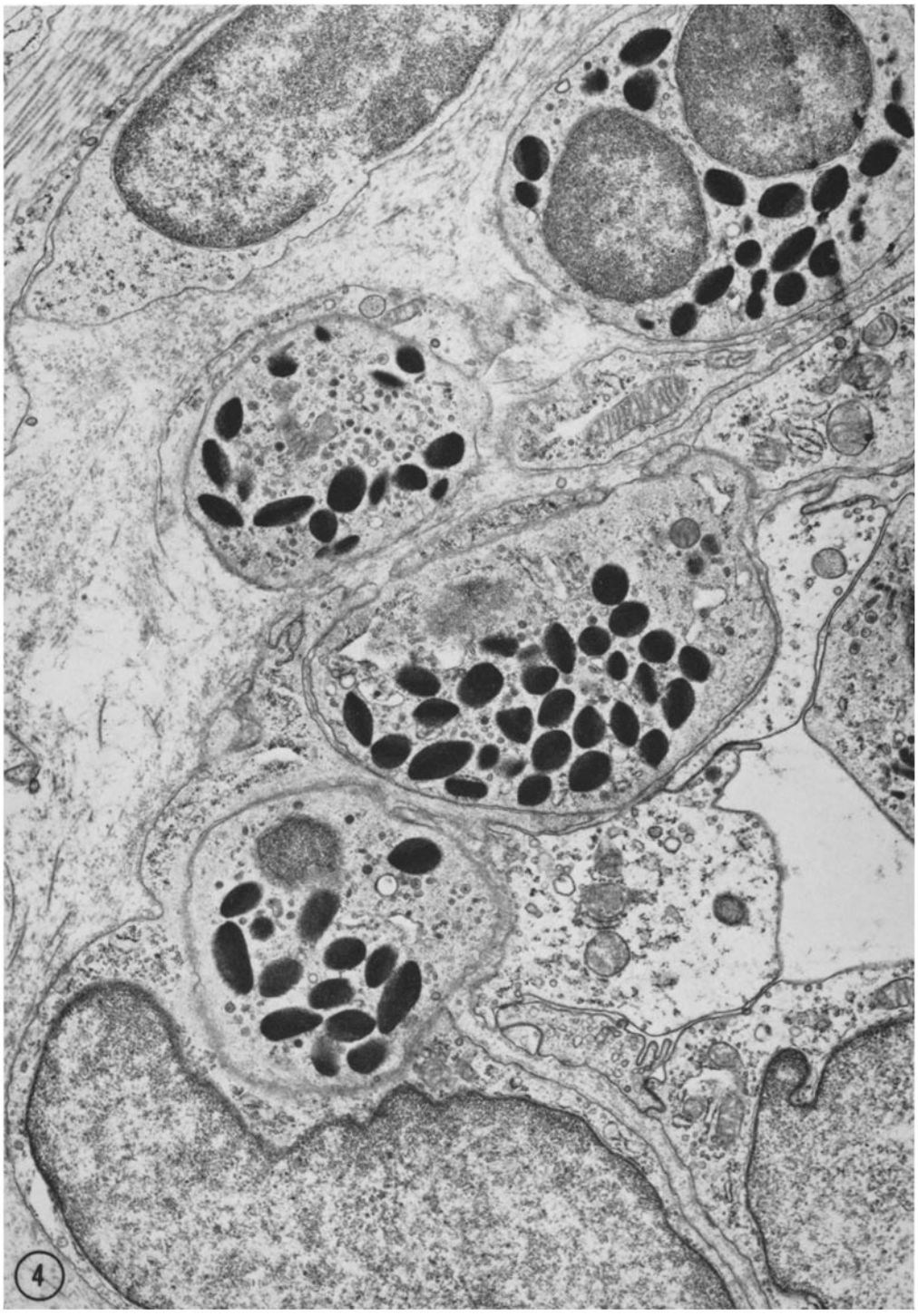
FIG. 3. This electron micrograph prepared from a rat 1 day preestrus contains a vascular channel subjacent to the myometrium. A neutrophilic leukocyte (*N*) is present within the lumen of the vessel and portions of two eosinophilic leukocytes (*E*) can be seen in the wall of the vessel. It was a common observation that the only granulocyte to emigrate under the influence of estrogen was the eosinophilic leukocyte. × 10,000.



(Ross and Klebanoff: The eosinophilic leukocyte)

PLATE 67

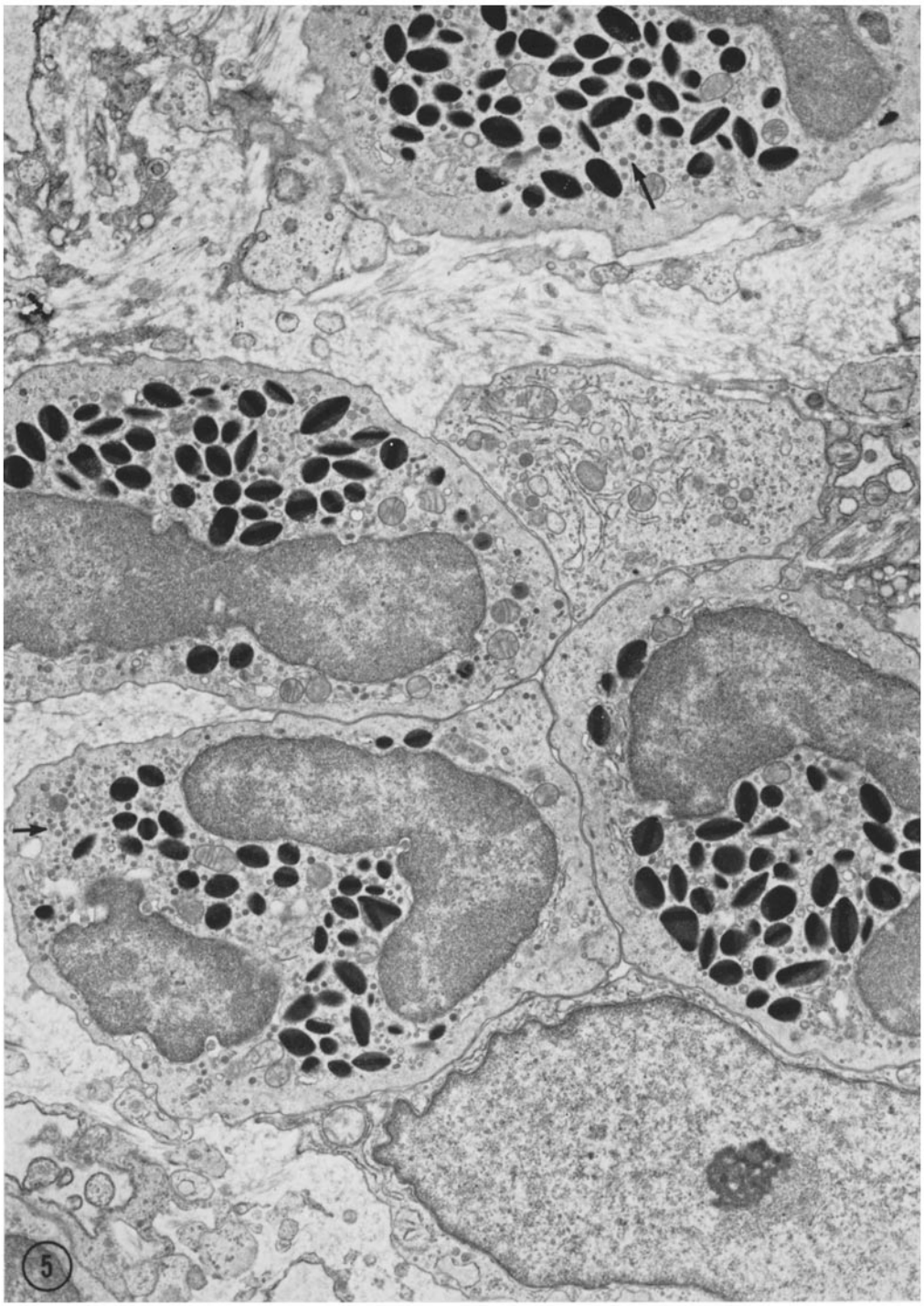
FIG. 4. In this blood vessel portions of eosinophilic leukocytes can be seen both within the vessel wall and in the extracellular connective tissue spaces immediately adjacent to the vessel. The animal was 1 day preestrus. × 11,000.



(Ross and Klebanoff: The eosinophilic leukocyte)

PLATE 68

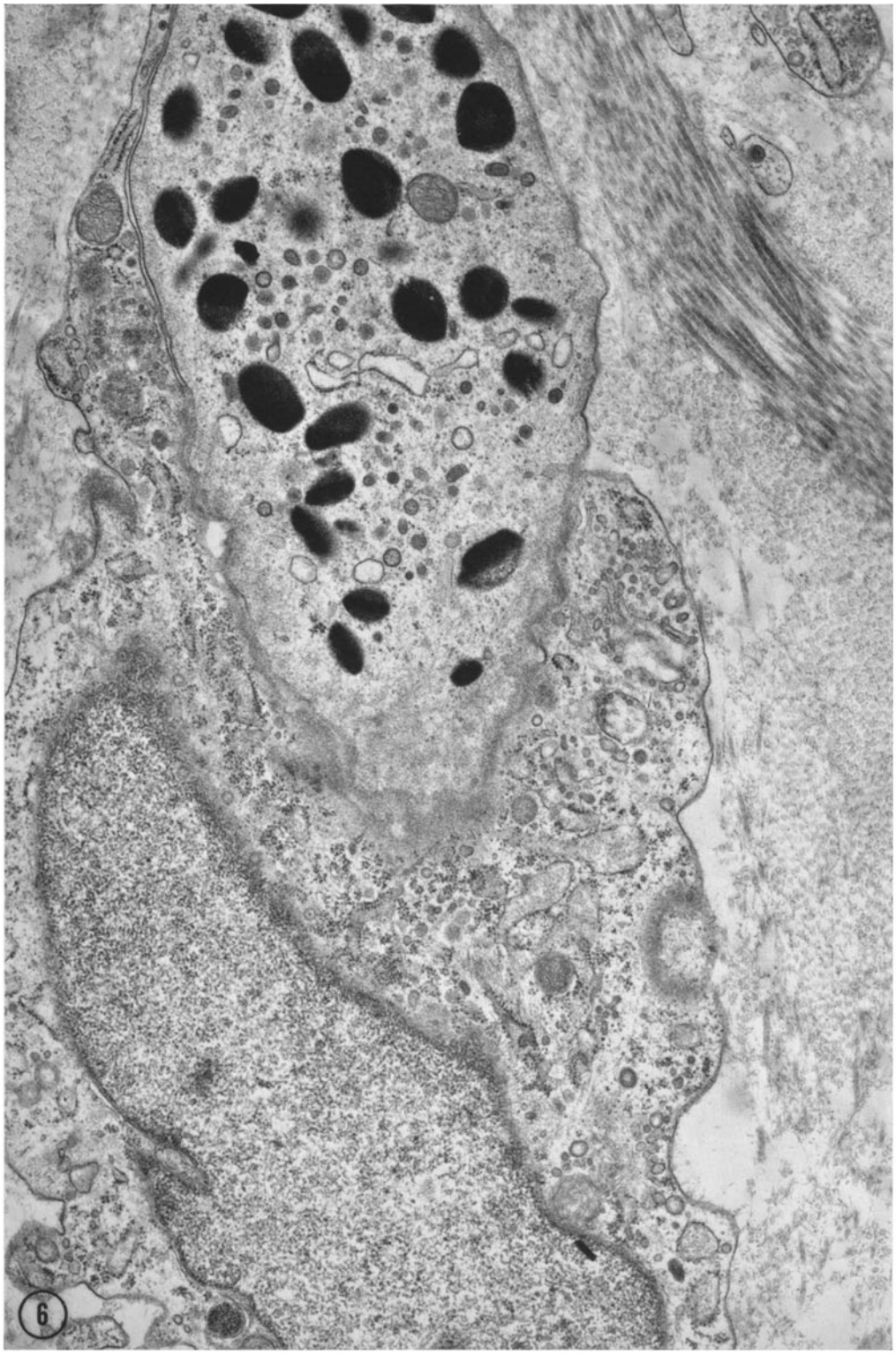
FIG. 5. This electron micrograph represents a typical region in the endometrium of an animal sacrificed just prior to estrus in which four eosinophilic leukocytes can be seen in the connective tissues. Their characteristic large granules containing central bars as well as small granules (arrows) are evident. × 10,000.



(Ross and Klebanoff: The eosinophilic leukocyte)

PLATE 69

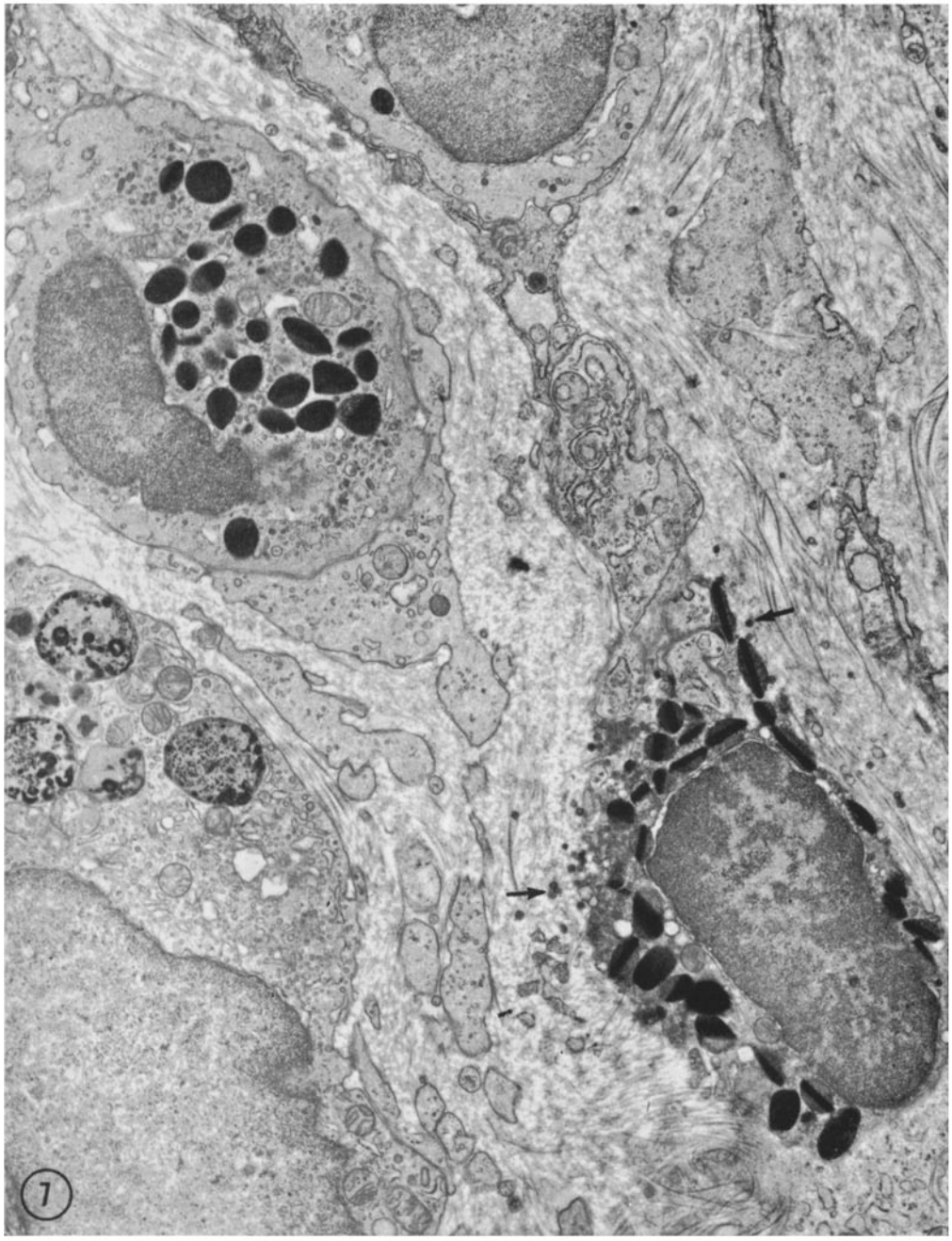
FIG. 6. Eosinophilic leukocytes are often seen in close juxtaposition to both macrophages and fibroblasts. In this micrograph of the uterus of an animal in estrus, the eosinophil appears to be partially surrounded by a connective tissue cell. It is not possible to state whether this represents early phagocytosis or simply intimate contact between these two cells. × 13,000.



(Ross and Klebanoff: The eosinophilic leukocyte)

PLATE 70

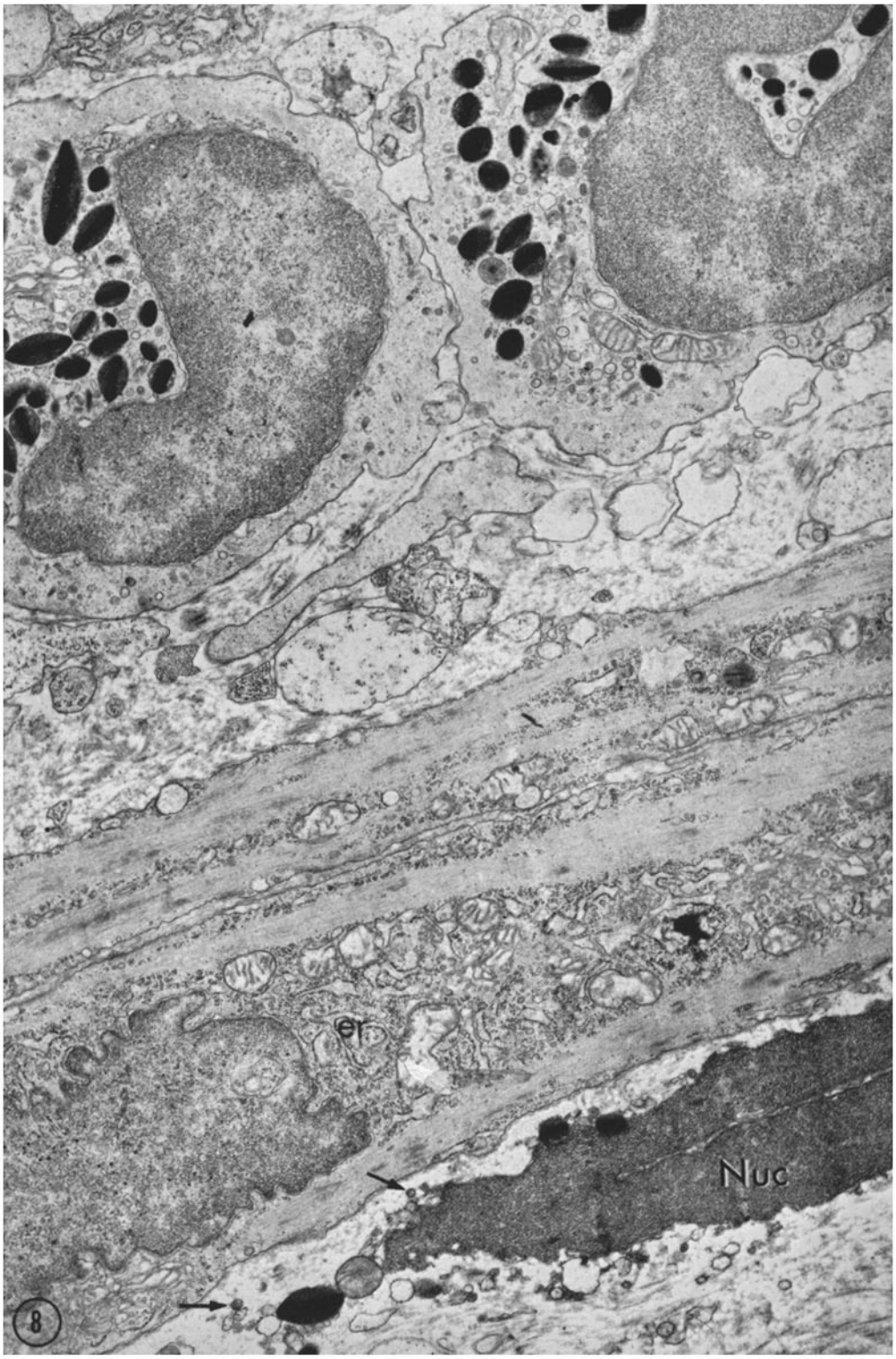
FIG. 7. In this micrograph of a uterus during estrus an intact eosinophilic leukocyte can be seen adjacent to an eosinophil in the process of lysis. Both the large and small granules (arrows) are found within the connective tissue spaces. The cell membrane of the lysed cell is no longer apparent. A macrophage containing nonidentifiable dense material within large vacuoles also can be seen. × 9000.



(Ross and Klebanoff: The eosinophilic leukocyte)

PLATE 71

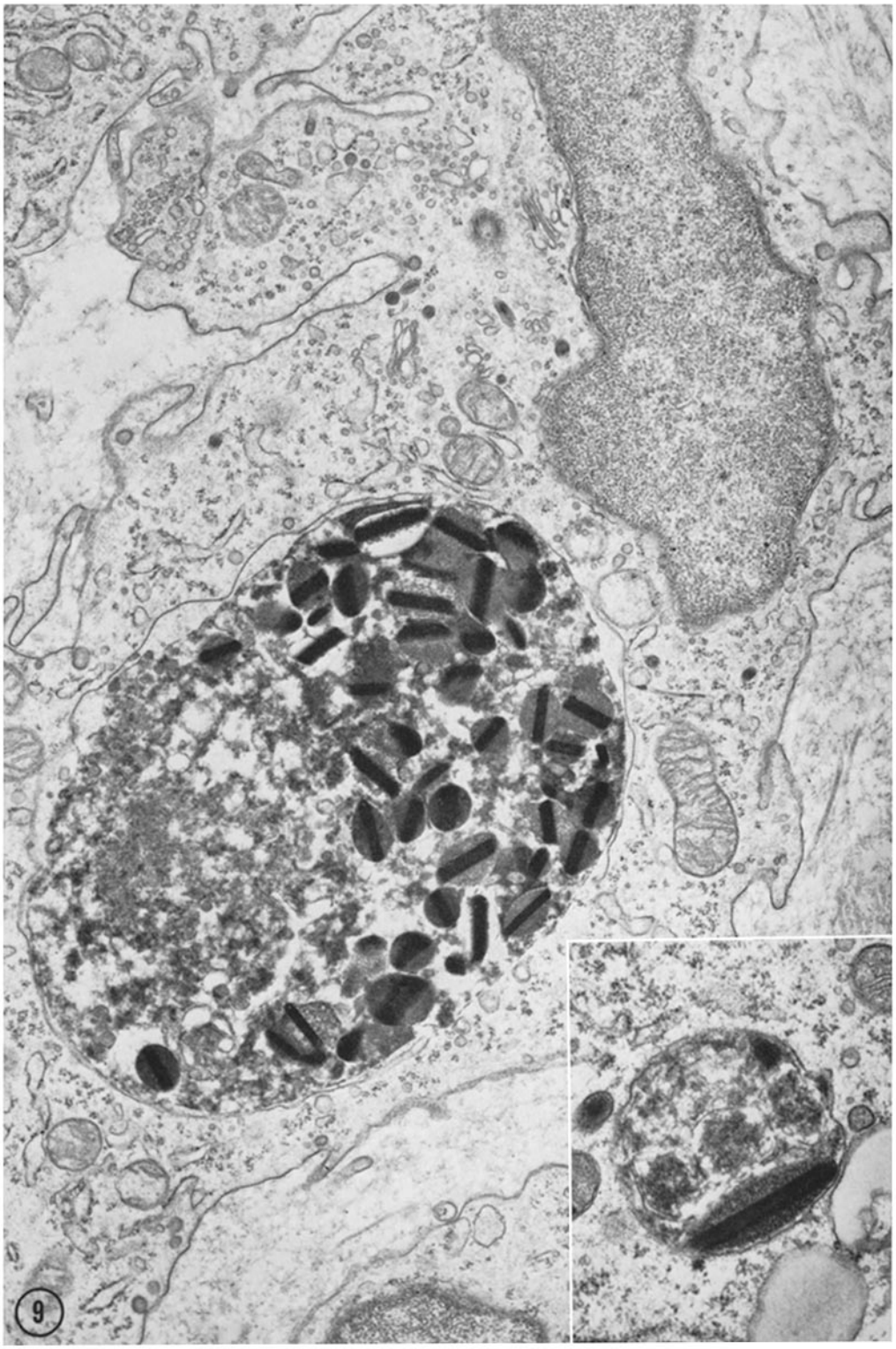
FIG. 8. Two smooth muscle cells can be seen adjacent to an eosinophilic leukocyte which has undergone lysis The remnants of the nucleus (*Nuc*) and the large and small granules (arrows) can be seen in the connective tissue spaces. Above the smooth muscle cells are two intact eosinophilic leukocytes. The smooth muscle cells demonstrate a marked increase in the amount of rough endoplasmic reticulum (*er*) within their cytoplasm. The animal was in estrus. × 10,500.



(Ross and Klebanoff: The eosinophilic leukocyte)

PLATE 72

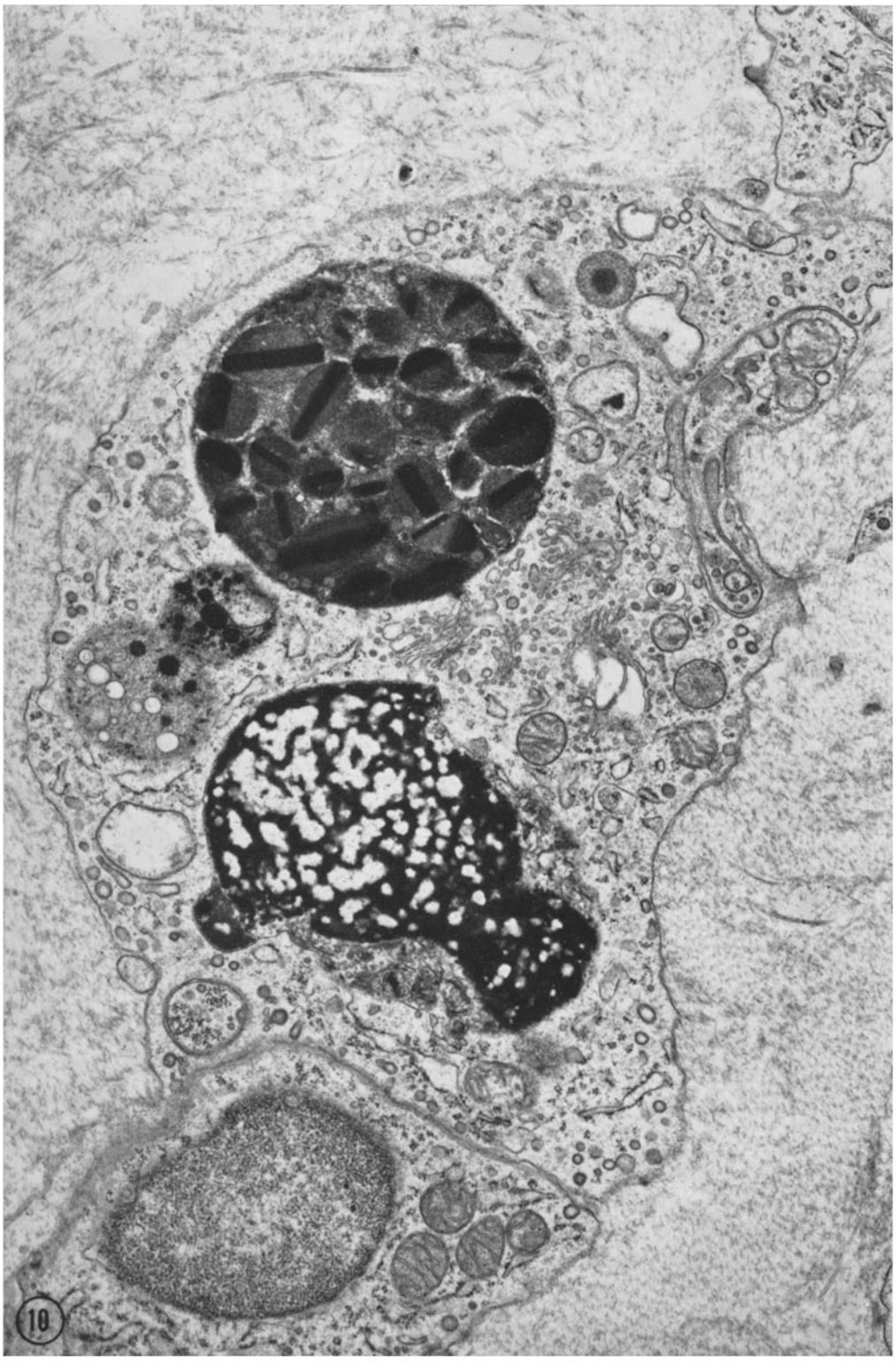
FIG. 9. This micrograph demonstrates a macrophage in the uterus of an animal 1 day postestrus. It contains the remnants of an intact eosinophil within a digestive vacuole in which the large characteristic granules are still identifiable. The degenerated nucleus of the eosinophil is represented by fine granular material. The insert demonstrates a phagocytic vacuole from another macrophage in which one intact large eosinophilic granule and nonidentifiable debris can be seen. × 15,000. Insert, × 30,000.



(Ross and Klebanoff: The eosinophilic leukocyte)

PLATE 73

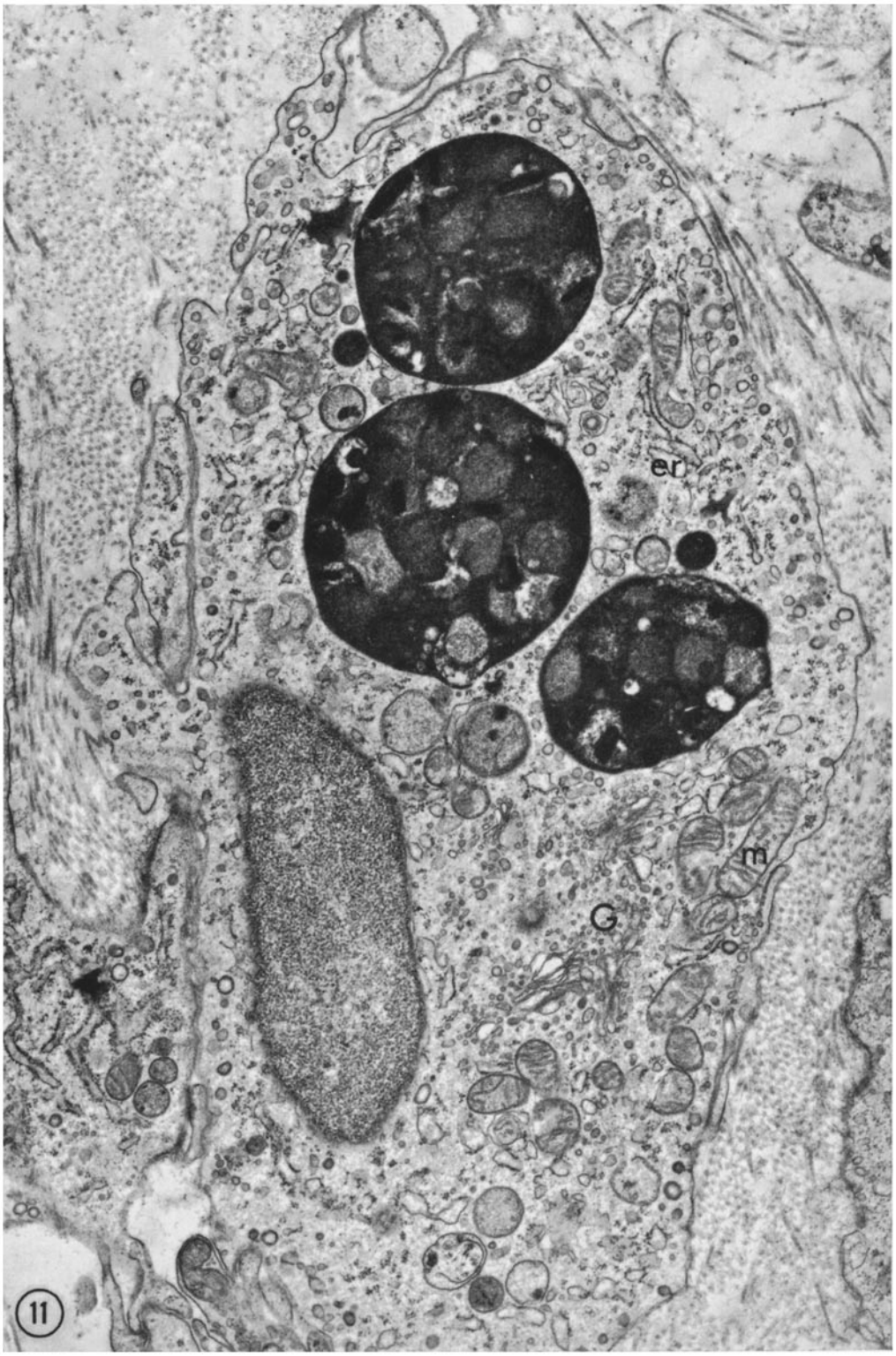
FIG. 10. This macrophage was present in the endometrial connective tissues of a rat uterus removed 1 day postestrus. The eosinophil granules within the large phagocytic vacuole appear to have undergone more dissolution than those seen in Fig. 9, however, they are still recognizable by the characteristic bar in the granule matrix. Several other phagocytic vacuoles are present within this cell whose contents are not identifiable. × 16,000.



(Ross and Klebanoff: The eosinophilic leukocyte)

PLATE 74

FIG. 11. This electron micrograph was taken of a rat uterus removed 2 days post-estrus. It is still possible to see the central bars suggestive of eosinophil granules within the phagocytic vacuoles. However, degenerative changes have proceeded beyond the stage seen in Figs. 9 and 10. This macrophage has a relatively poorly developed rough endoplasmic reticulum (*er*), a well developed Golgi (*G*), and numerous mitochondria (*m*). × 14,000.



(Ross and Klebanoff: The eosinophilic leukocyte)