INVOLVEMENT OF MACROPHAGES IN COLLAGEN RESORPTION

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INTRODUCTION

Although relatively stable in most tissues, collagen is rapidly turned over in several cases of morphogenesis (27). During anuran metamorphosis (7), postpartum uterine involution (9), and wound healing (6), large amounts of collagen are resorbed in a short time. The enzyme responsible for collagen degradation is thought to be a collagenase (13). Early attempts to demonstrate a collagenase in systems in which active remodeling is taking place were unsuccessful. Recently, however, Gross and his coworkers have shown that, when pieces of tissue undergoing resorption are grown on reconstituted collagen gel, they secrete a collagenase capable of lysing collagen at neutral pH and physiological temperatures (6–8).

Biochemical investigations have shown that during remodeling of tissues hydrolytic enzymes (such as acid hydrolases) increase many times (24, 26). On the basis of the well known association of hydrolytic enzymes with lysosomes, Woessner postulated a lysosomal theory of collagen resorption (27). According to this theory, the collagenase secreted extracellularly breaks up the long collagen fibers, which are then capable of being taken into phagocytic cells where they will be further degraded by the lysosomal enzymes. Several light microscopic investigations suggest that the osteoclast, fibroblast, foreign body giant cell, macro-

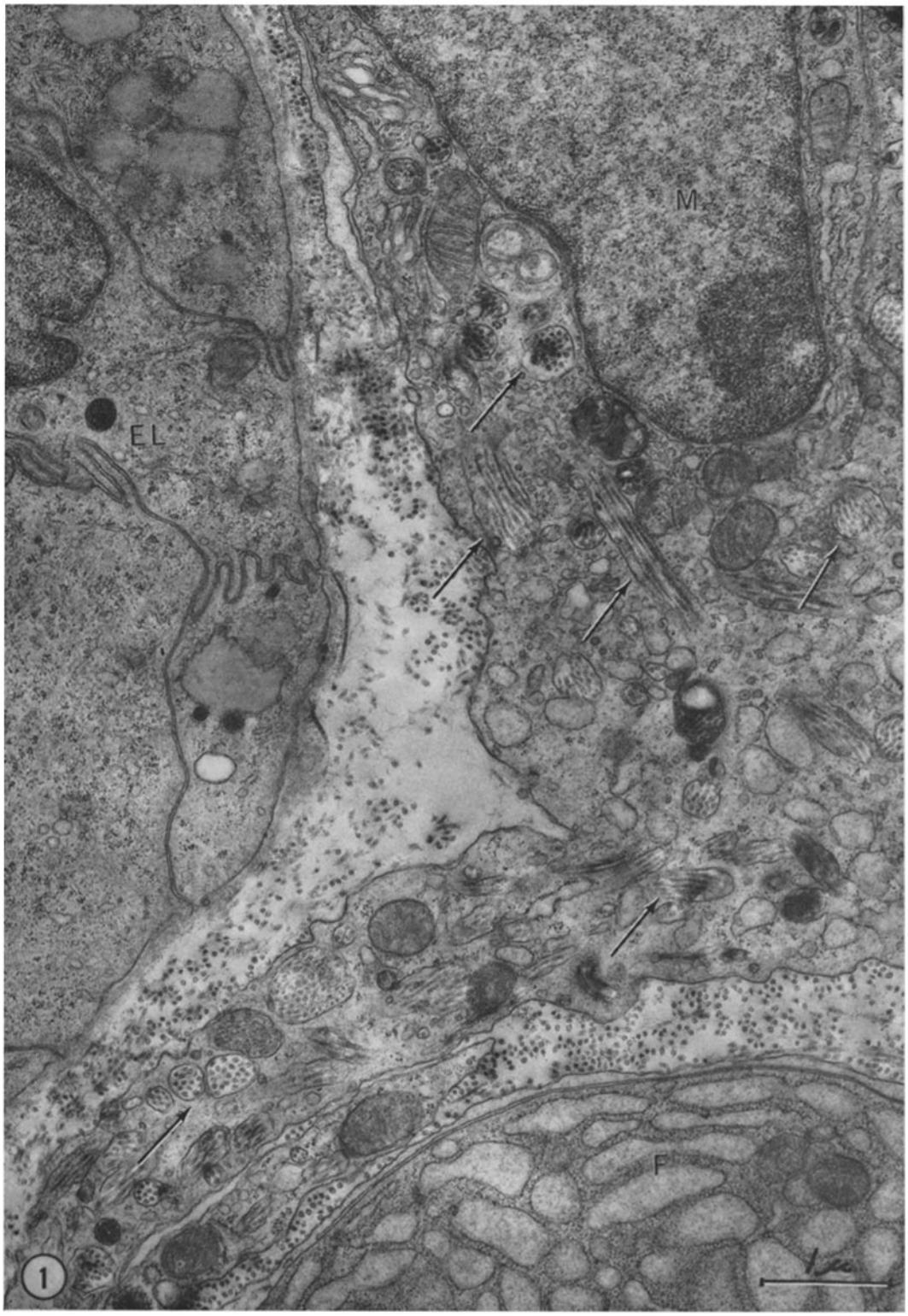


FIGURE 1 Electron micrograph. The connective tissue region immediately below the endometrial lining (*EL*) of the mouse uterus. The macrophage (*M*) contains numerous vacuoles (arrows) filled with either longitudinal, oblique, or cross-sectioned collagen fibers. There are many smooth-walled vesicles, but few profiles of rough-surfaced endoplasmic reticulum. The fibroblast (*F*) has a well developed, rough-surfaced endoplasmic reticulum with dilated cisternae. × 19,000.

phage, and endothelial cell are involved in phagocytosis of collagen during resorption (27). However, Woessner, in his most recent review (27) on the biological mechanisms of collagen resorption, remarks that "there are few electron microscope observations to date which could be interpreted as showing the attack of endocytic cells on collagen fibers."

In electron microscope studies of the involuting rat uterus, Luse and Hutton (12) found the fibroblast responsible for the resorption of collagen. Unfortunately, they have not elaborated these findings, reported in an abstract. On the other hand, Schwarz and Güldner (21), also working on involuting rat uterus, found histiocytes actively involved in the breakdown of collagen. In view of this discrepancy about the nature of the cell type involved in collagen resorption, I studied the involuting mouse uterus and found numerous cells, identifiable as macrophages because of their ability to pick up vital stains, in the connective tissue of both the myometrium and endometrium. Under the electron microscope, these macrophages were seen to have numerous vacuoles containing collagen fibers in various stages of disintegration. The fine structural details related to this process are reported below.

MATERIALS AND METHODS

For the demonstration of macrophages, 1 cc of 5% trypan blue in sterile, normal saline was administered intraperitoneally into mice 12 and 48 hr after parturition. 24, 48, and 60 hr later, samples of uteri were fixed in 10% formalin and embedded in paraffin. Virgin and pregnant mice served as controls.

For the demonstration of acid phosphatase activity, frozen sections of uteri from animals 24 and 40 hr after parturition were incubated by the Gomori method (5). The control sections were incubated without substrate. Some of the frozen sections were also stained with oil red O.

For the demonstration of acid phosphatase activity in the electron microscope, small pieces of uteri from animals 24 and 40 hr after parturition were fixed in 4.5% glutaraldehyde in cacodylate buffer at pH 7.4

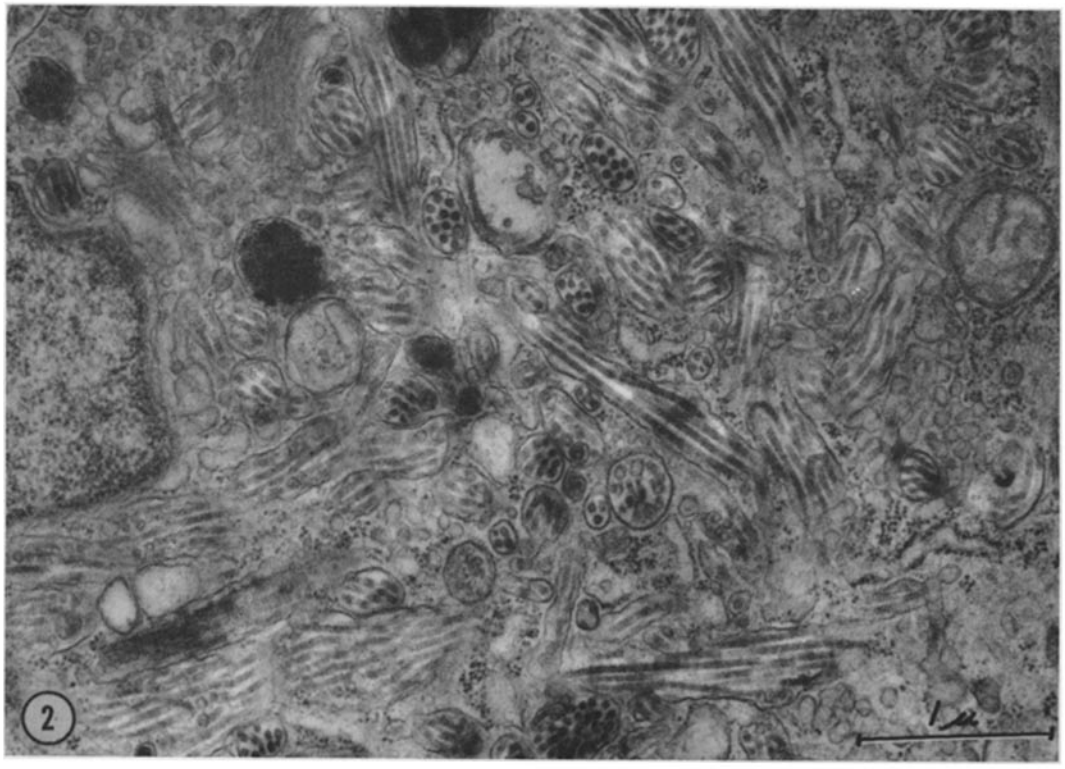


FIGURE 2 Portion of a macrophage depicting the extreme activity during phagocytosis. The cytoplasm is filled with vacuoles containing collagen fibers. × 25,000.

for 4 hr (20). The tissues were washed in several changes of cacodylate buffer for 4 hr and then incubated in Gomori's acid phosphatase medium for 1 hr. The incubated pieces of tissues were washed and postfixed in 1% OsO_4 in cacodylate buffer for 2 hr and processed for electron microscopy.

Samples of uteri taken from animals 24, 36, 60, and 72 hr after delivery were fixed for 2 hr at 4°C in 1% osmium tetroxide either in veronal acetate or in phosphate buffer at pH 7.5 (16). The tissues were later dehydrated in ethanol solutions of increasing concentrations and embedded in Araldite (11). Sections 1 μ thick were stained in 1% toluidine blue with borax for orientation purposes. Thin sections, cut with a glass knife on a Porter-Blum microtome, were doubly stained with aqueous uranyl acetate and lead citrate (17) and examined in a Philips 300 electron microscope.

RESULTS

Light Microscopy

The uterus of the virgin mouse was only sparsely populated with macrophages. On the other hand, the number of macrophages in the involuting uterus increased significantly soon after parturition. Most of these macrophages, which can be stained vitally by trypan blue, were distributed in the connective tissue of both the endometrium and myometrium. Numerous lipid droplets, demonstrable by oil red 0, characterized the macrophages of the involuting uterus.

During involution, the acid phosphatase activity increased rapidly until the 4th day after parturition and was localized mainly in the macrophages and, to a lesser extent, in the endothelial lining cells.

Electron Microscopy

The uterine macrophages of the virgin and pregnant animals were comparatively quiescent. Both types of macrophages had a moderately developed Golgi complex consisting mostly of a few electron-lucent smooth vesicles. The rough endoplasmic reticulum was inconspicuous, but large numbers of free ribosomes were sometimes present in the cytoplasm. The nucleus was irregularly shaped.

24 hr after parturition, the macrophages of the

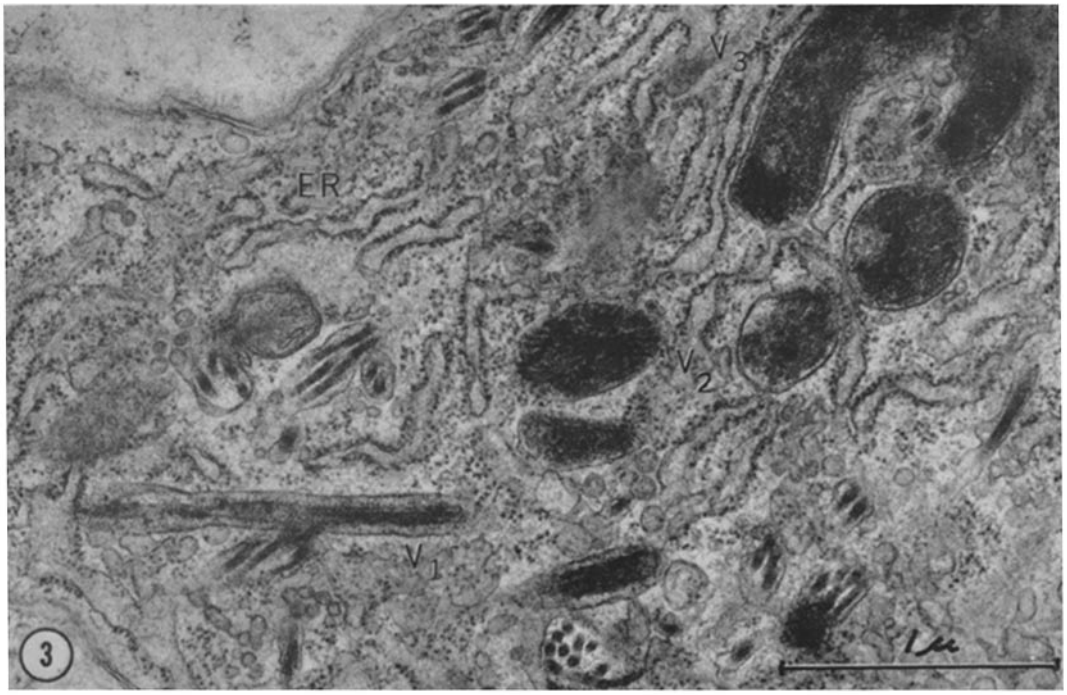


FIGURE 3 Electron micrograph. Portion of a macrophage showing early stages in the degradation of collagen in the vacuoles. V_1, newly engulfed collagen. V_2 and V_3 can be considered later stages in the process of collagen resorption. Note well developed, rough-surfaced endoplasmic reticulum (*ER*). × 36,000.

involuting uterus had undergone marked morphological alterations (Fig. 1). The elements of the Golgi complex, which now consisted of numerous smooth-walled tubules and vesicles, had increased strikingly. Several profiles of rough endoplasmic reticulum with attached ribosomes were distributed throughout the cytoplasm. The cells also contained many smooth-walled vacuoles of various sizes.

Macrophages that had begun to engulf collagen had elongated cytoplasmic processes (Fig. 1). The plasma membranes of these cells were comparatively smooth with only a few invaginations. In the cytoplasm, several vacuoles of various sizes contained collagen fibers varying in number from one to many. Depending upon the arrangement of the collagen, the vacuoles contained either longitudinal, oblique, or cross-sectioned fibers (Fig. 1).

As the macrophages became actively phagocytic, the entire cell was filled with vacuoles that contained collagen fibers undergoing degradation (Figs. 2–4). During this stage, it was almost impossible to follow the cell boundaries because of the extreme invaginations which they had undergone during phagocytosis. This frenzy of activity is reflected in Fig. 2.

Along with the phagocytic activity, the cell concomitantly had begun to degrade the engulfed collagen. Newly engulfed collagen when sectioned longitudinally exhibited the characteristic banding of collagen (Figs. 1–4). Many of the vacuoles contained collagenous material that had already lost its banding pattern (Figs. 3, 4). Thus, it was possible to identify in the vacuole morphology a series of stages that ranged from those containing newly engulfed collagen to those containing an amorphous material, presumbly degraded collagen (Figs. 3, 4).

Several of the vacuoles containing collagen had acid phosphatase activity, the reaction product always being located within the vacuoles (Fig. 5). Some of the adjacent vacuoles, however, lacked re-

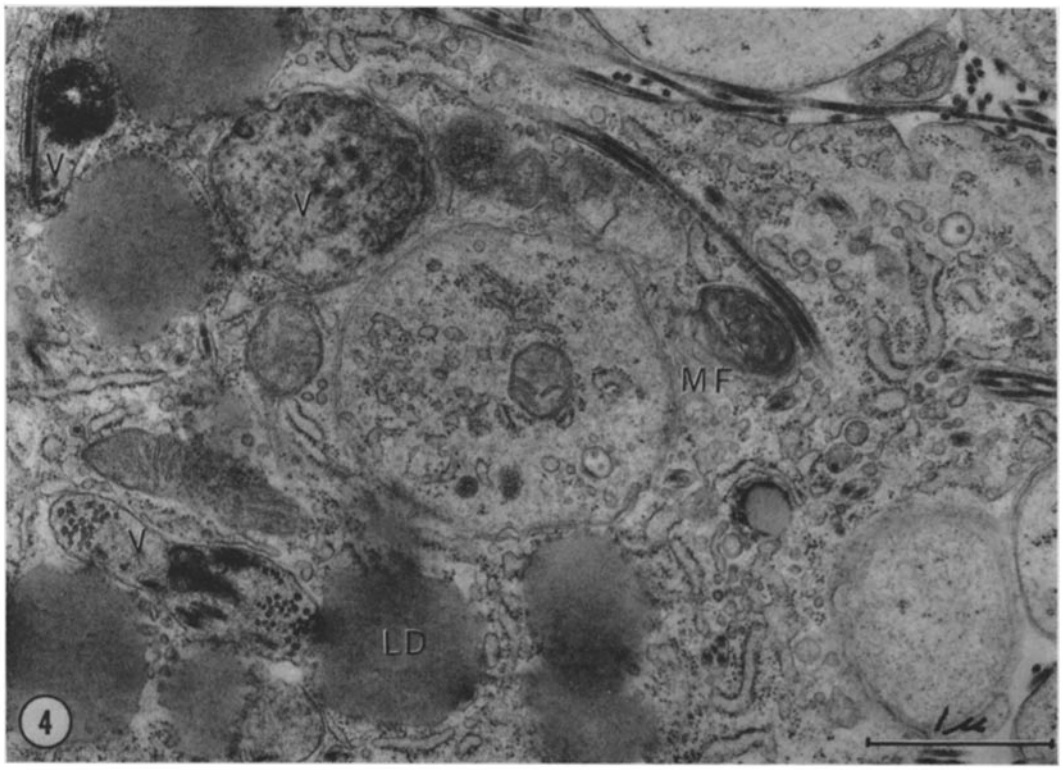


FIGURE 4 Electron micrograph. Some of the later stages in the resorption of collagen. Some vacuoles (*V*) contain collagen that has already lost its characteristics. In one of the vacuoles, beginnings of a myelin figure (*MF*) can be seen. Several lipid droplets (*LD*) and smooth-walled vesicles are distributed throughout the cytoplasm. × 24,000.

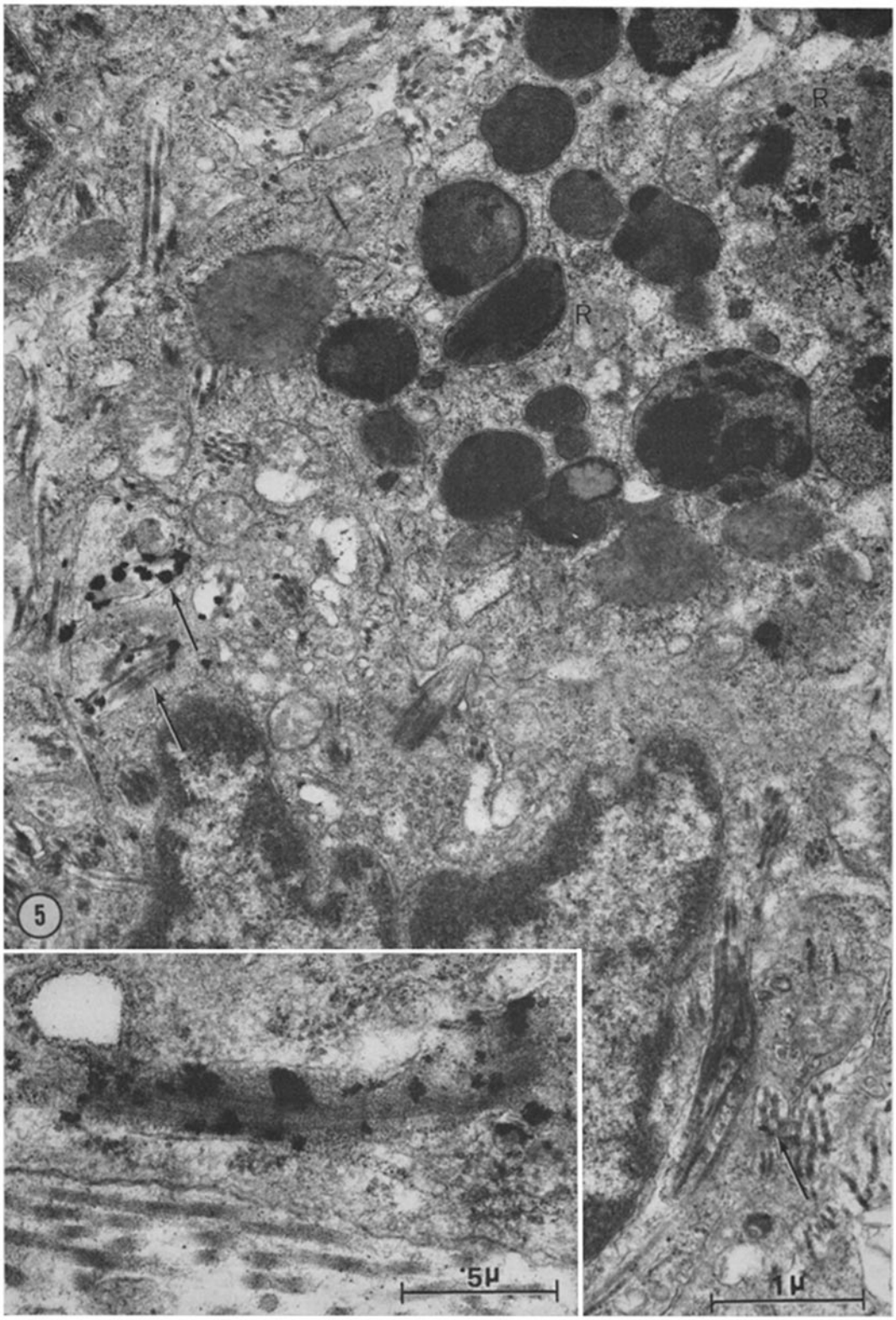


FIGURE 5 Portions of macrophages showing the location of acid phosphatase. The reaction products are localized exclusively in the vacuoles containing collagen (arrows) and in the residual bodies (*R*). Note the complete absence of reaction products outside the cells. × 24,000. *Inset.* An enlarged view of a vacuole containing collagen, showing acid phosphatase confined within the membranes of the vacuole. × 50,000.

action product and could be newly formed ones that had not yet acquired acid hydrolases. Reaction product was also found on many of the residual bodies.

Macrophages from uteri that had undergone 60 hr of involution contained many membrane-bounded structures of various sizes (Figs. 6, 7). Some of these structures appeared as myelin figures with whorls of membranes, others as typical residual dense bodies.

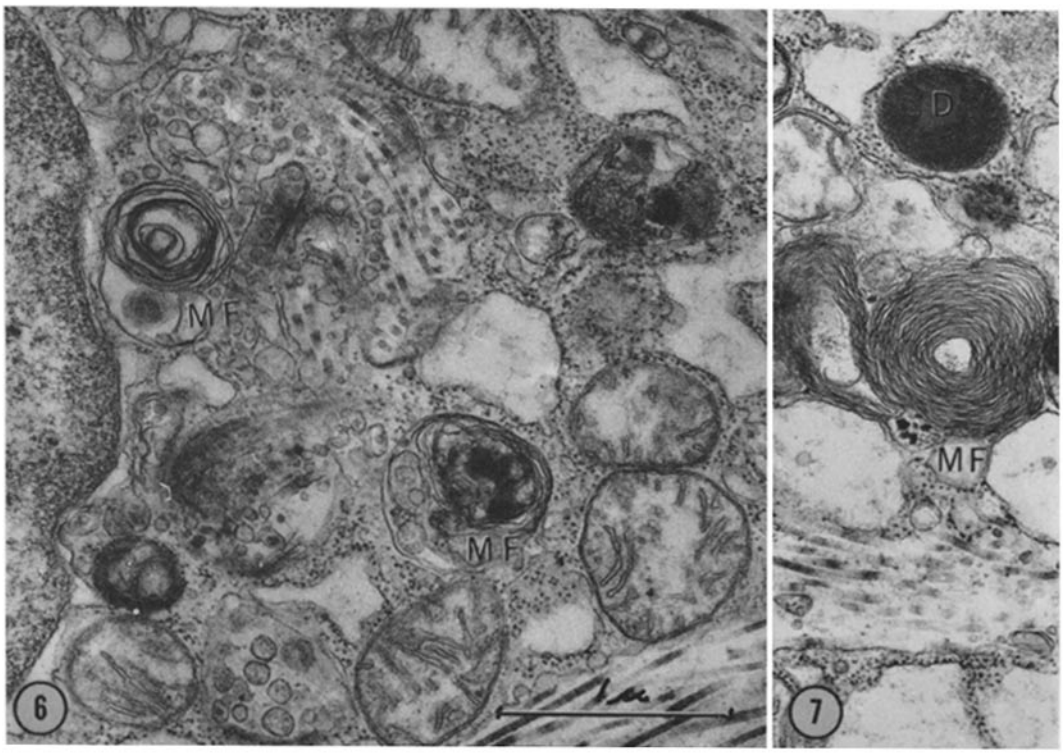
72 hr after parturition, the macrophages had numerous residual dense bodies but very few membrane-bounded myelin figures (Fig. 8). Apart from the above characteristics, the macrophages at this stage were morphologically similar to those from virgin or pregnant animals.

DISCUSSION

During the postpartum involution of the uterus, macrophages increase in number in the connective tissue of the myometrium and endometrium (4, 10). Lobel and Deane (10) have demonstrated that the macrophages of the involuting rat uterus, besides being markedly reactive for acid phosphatase, contain large accumulations of lipids. The present study on the involuting mouse uterus not only confirms these findings, but also identifies the invading cells as macrophages stainable with a vital dye such as trypan blue.

Although the origin of uterine macrophages during involution is uncertain, evidence shows that similar cells arise from the blood monocytes (4, 25). Studies of tissue culture have demonstrated that blood monocytes transform into macrophages (22, 25). During this transformation, the macrophages acquire their identifying characteristics. However, the possibility cannot be ruled out that the increase in the number of macrophages during involution is due to the increased mitotic activity of the resident population of macrophages in the uterus.

Several biochemical investigations have shown that, during involution of the uterus, acid hydrolases increase severalfold (9, 26) and are related



FIGURES 6 and 7 Electron micrographs. Portions of macrophages that have been actively phagocytic for at least 60 hr. Many membrane-bounded bodies contain myelin-like figures (*MF*). Occasionally, residual bodies (*D*) are also seen. × 30,000.

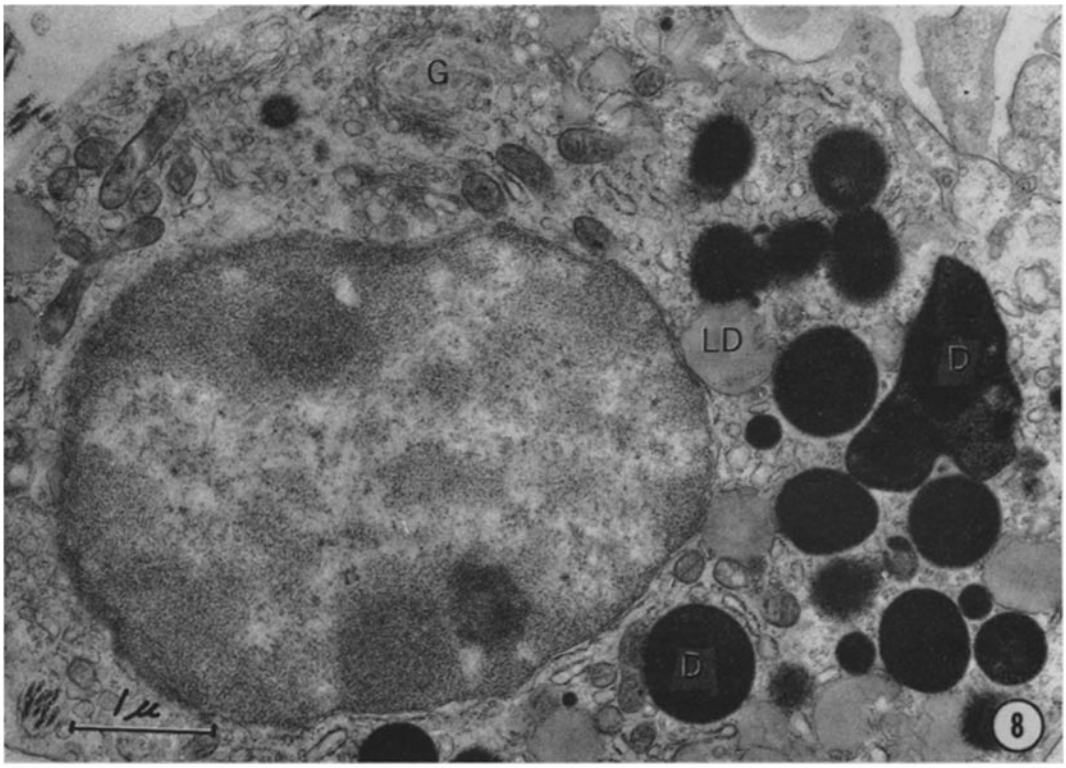


FIGURE 8 A macrophage from a uterus that has undergone 72 hr of involution. There are several residual dense bodies (*D*) and lipid droplets (*LD*) in the cytoplasm. The Golgi complex (*G*) and the rough-surfaced endoplasmic reticulum are relatively inconspicuous. × 17,000.

to collagen breakdown. The histochemical demonstration of acid phosphatase and of its precise localization in the vacuoles containing collagen in the macrophages of the involuting mouse uterus establishes the active role of acid hydrolases in the resorption of collagen. A similar increase in acid phosphatase activity has been shown to characterize actively phagocytic macrophages from other locations, such as the lung and peritoneum (1, 14, 15).

Recent studies of the fine structure of stimulated macrophages in culture (2, 3, 22) and during wound healing (19) have shown that, when macrophages begin to be actively phagocytic, the elements of both the rough-surfaced endoplasmic reticulum and the Golgi complex increase. The hypertrophied, synthesizing organelles are thought to be responsible for the elaboration of hydrolytic enzymes. In the present study, a similar increase in the synthesizing organelles of the actively phagocytic uterine macrophages indicates the role that these organelles play in synthesizing lysosomal enzymes. The histochemical demonstration of increased amounts of acid phosphatase in the macrophages during involution lends further support to these data.

The functional significance of numerous macrophages in the postpartum uterus has remained obscure (10, 27). However, the findings of the present study—the presence in the macrophages of numerous vacuoles containing collagenous material in different stages of degradation—indicate that the macrophages are involved in collagen resorption. This result concurs with that of Schwarz and Güldner, who have shown vacuoles containing collagen in the involuting rat uterus (21). After continuous ingestion of collagen, macrophages of the mouse uterus become loaded with numerous large membrane-bounded myelin figures. These whorls of membranous material probably are the undigested end product of collagen breakdown stored by the cell. Similar myelin-like

figures have also been reported in the monocytes of healing wounds (19) and in other phagocytic cells after ingestion of extracellular protein.

Very few electron microscope investigations have been able to demonstrate collagen fibers inside cells (21, 23). Lack of resolution of the electron micrographs imposed serious limitations on such studies and left two possibilities unresolved: (*1*) that the collagen fibers are synthesized inside the cells; (*2*) that the vacuoles containing collagen fibers are continuous with the outside. The first possibility can be ruled out by overwhelming evidence that collagen is polymerized extracellularly from the secreted tropocollagen molecules (18); the second, by the demonstration in the present study of acid phosphatase reaction products in many of the vacuoles containing collagen.

These morphological findings support the lysosomal theory of collagen resorption postulated by Woessner (27) and based mainly on biochemical evidence. Furthermore, they establish that the cell type involved in the resorption of collagen in the involuting mouse uterus is a macrophage. However, Gross et al. have demonstrated collagenase during the involution of the rat uterus (8). This enzyme may be involved only in breaking up the long collagen fibrils into smaller bits so that they can be readily phagocytosed by the macrophages. In this connection, it should be pointed out that animal collagenase, unlike bacterial collagenase, is incapable of completely degrading collagen (27).

SUMMARY

Uteri from mice were examined in the electron microscope at 24, 36, 60, and 72 hr after parturition. During uterine involution, numerous macrophages were found in the connective tissue of the myometrium and endometrium. The cytoplasm of the macrophages has numerous vacuoles filled with collagen in various stages of degradation. Histochemical studies showed that the macrophages during involution were highly reactive for acid phosphatase which was localized in the vacuoles containing collagen. Data from the present study support the lysosomal theory of collagen resorption postulated by Woessner.

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