FINE STRUCTURAL CHANGES IN UTERINE SMOOTH MUSCLE AND FIBROBLASTS IN RESPONSE TO ESTROGEN

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ABSTRACT

The fine structure of the estrogen-primed uterus was examined in two series of rats, with emphasis upon the alterations in smooth muscle cells and fibroblasts. The first series of animals were mature animals that were sacrificed at diestrus or estrus. The second series consisted of prepubertal rats (57–70 g) that received subcutaneous injections of estradiol-17 β in 20% alcohol. Four groups of animals received the hormone twice daily for 3 days for a total dose of 0.06, 0.6, 6.0, or 60.0 μg, respectively. An estrogenic response was observed in all groups as indicated by an increase in uterine weight. Control groups consisted of either untreated animals or animals receiving 20% alcohol. All animals were sacrificed on the 4th day. The fibroblasts and smooth muscle cells in the controls were similar to their counterparts in the mature animal in diestrus. They were small, contained relatively little rough endoplasmic reticulum, and the connective tissue cells appeared like fibrocytes. All of the estrogen-treated animals were similar in appearance and were comparable to their counterparts in the mature animal in estrus. Both the smooth muscle cells and the fibroblasts were increased in size, demonstrated a marked enlargement and dilation of ergastoplasmic cisternae, and contained increased numbers of attached and free cytoplasmic ribosomes. The presence of an extensive rough endoplasmic reticulum in the smooth muscle cells of the stimulated uterus is in marked contrast to the appearance of these cells in other tissues. These observations correlate with previous biochemical studies by other workers, in which estrogens have been shown to promote the synthesis of uterine RNA, collagen, and noncollagenous protein, and suggest that smooth muscle cells may participate in the synthesis of connective tissue proteins.

INTRODUCTION

In a previous paper (1), we reported on the fine structure changes in the connective tissues of the uterus of the rat during the estrus cycle, with particular emphasis on the striking alterations in the tissue eosinophils. In the course of this study, it was observed that during estrus there was an extensive enlargement of the rough endoplasmic reticulum of the smooth muscle cells.

Recent biochemical studies have emphasized the role of estrogens in the promotion of uterine RNA and protein syntheses. Administration of estrogen to the ovariectomized animal (2–4) induces an increase in the number of uterine ribosomes, followed by an increase in the amount of cellular and extracellular protein. Smooth muscle cells do not usually contain an extensive rough

endoplasmic reticulum, and, therefore, have not been considered to have, as an important function, the synthesis of protein for export. The marked development of the organelle in the smooth muscle cells of the uterus in estrus suggested that these cells might play such a role under these circumstances since these changes paralleled the biochemical observations previously noted. These considerations prompted a more extensive examination of the relationship of the ergastoplasmic changes in the smooth muscle cells and fibroblasts to the presence of endogenous or exogenous estrogen.

MATERIALS AND METHODS

Animals

MATURE RATS: 38 female Simonson albino (Sprague-Dawley) rats weighing between 190 and 250 g were examined during various phases of the estrus cycle (1). Vaginal smears were obtained daily from each animal to determine the regularity and status of the cycle, and at predetermined times 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.

IMMATURE RATS: 31 prepubertal female Simonson albino (Sprague-Dawley) rats weighing between 57 and 70 g were divided into six groups. These were:

A. An uninjected group.
B. A group injected with the 20% alcohol carrier in which estradiol-17 β was solubilized.
C. A group receiving a total dose of 0.06 μg of estradiol-17 β.
D. A group receiving a total dose of 0.6 μg of estradiol-17 β.
E. A group receiving a total dose of 6 μg of estradiol-17 β.
F. A group receiving a total dose of 60 μg of estradiol-17 β.

Each of these animals was injected twice daily for 3 days. At the end of the period of injections, each animal was sacrificed by decapitation, and the uterus was dissected out; one-half of the uterus was weighed and the remainder prepared for examination by light and electron microscopy.

Tissue Preparation

The horn of the uterus was cut into short, cylindrical segments. Some were fixed in neutral buffered formalin for paraffin embedding, and others were placed in *s*-collidine (5) buffered osmium tetroxide (pH 7.3) at 4°C. These tissues were fixed in osmium tetroxide for 1 hr, rinsed in buffer, then fixed again in neutral buffered formalin for 1 hr. They were subsequently dehydrated through a graded series of alcohols and embedded in epoxy resin (Epon 812) (6).

All tissues prepared for electron microscopy were double-stained with lead and uranyl acetate and examined in an RCA EMU 3G. Thin sections (1 μ) were also prepared and stained with azure II methylene blue for examination by light microscopy (7).

OBSERVATIONS

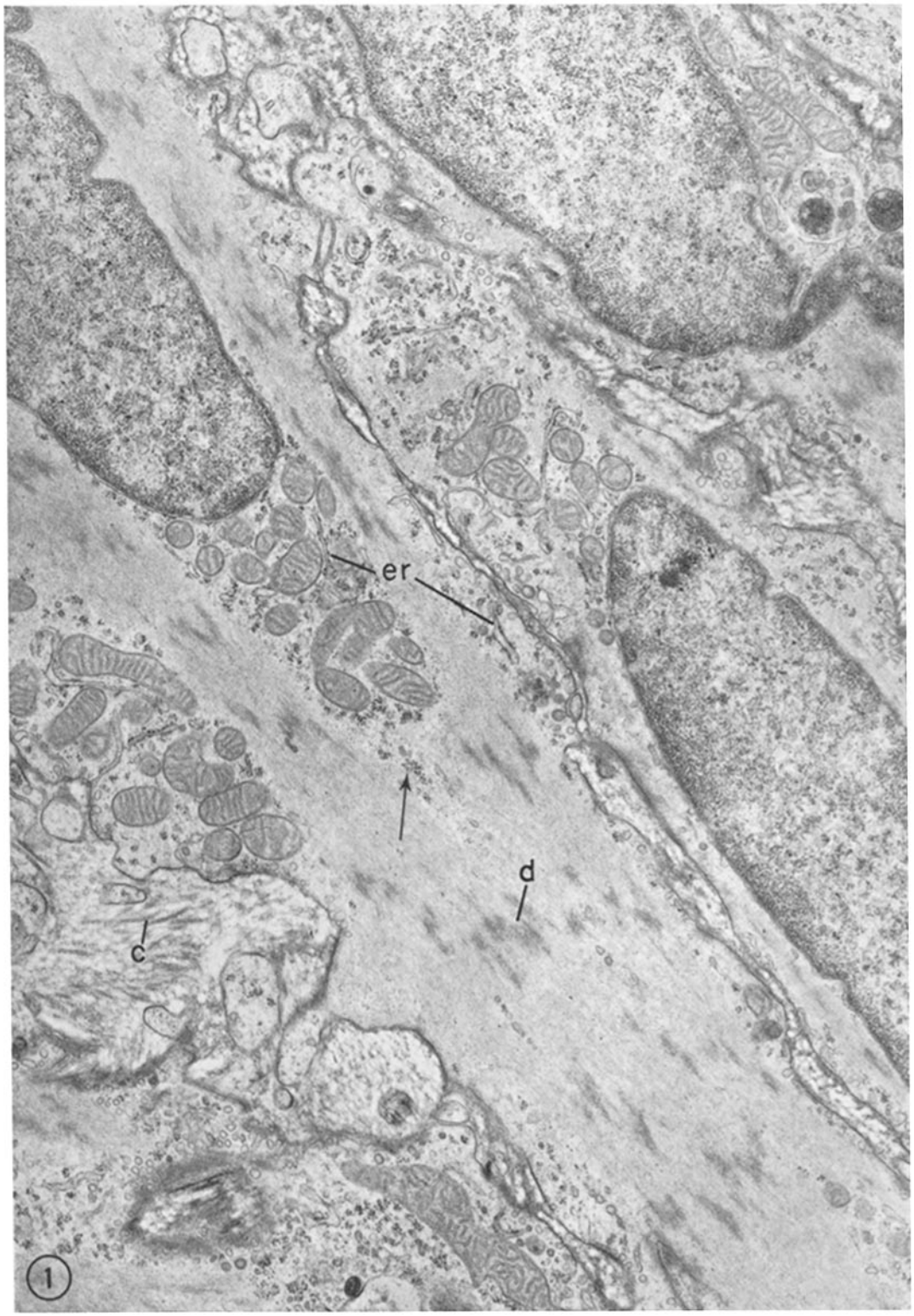
Smooth Muscle Cells

MATURE ANIMALS

DIESTRUS: During diestrus, the smooth muscle cells of the rat uterus are long, somewhat spindle-shaped, and contain a large ellipsoid nucleus. Most of the cell organelles are located adjacent to the nucleus or in small clusters in the cell periphery. Myofilaments occupy the largest portion of the cytoplasm and run parallel to the long axis of the cell. These filaments often come together in dense regions sometimes referred to as dense bodies or "attachment sites" (8–11, 29). Similar densities can also be seen at the surface of the cell immediately beneath the plasmalemma (Fig. 1).

In the regions in which organelles such as mitochondria and rough endoplasmic reticulum are

FIGURE 1 This electron micrograph displays parts of several smooth muscle cells from the myometrium of an adult rat uterus which was removed 2 days postestrus. As in smooth muscle cells from other tissues, the majority of the cytoplasm consists of myofilaments with interspersed dense bodies (*d*). Most of the organelles are located either in the juxtanuclear zone or at the cell periphery. In both of these regions cytoplasm is pale, free of myofilaments, and contains numerous mitochondria and a few cisternae of poorly developed rough endoplasmic reticulum (*er*). The extracellular spaces contain collagen fibrils (*c*) and numerous fine filaments. A few aggregates of free ribosomes (arrow) are also present in the regions in which these organelles are located. Numerous caveolae and small vesicles are present along the cell periphery. × 17,000.


er
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located, the ground cytoplasm is pale and devoid of myofilaments. The rough endoplasmic reticulum consists of a few cisternae with small numbers of randomly dispersed ribosomes attached to their membranes. Long stretches of membrane were often seen to be devoid of ribosomes. These cells have a well developed Golgi complex, and numerous rosettes of free ribosomes can be seen as well. The smooth muscle cell characteristically contains numerous small vesicles at the surface of the cell adjacent to the plasma membrane. Many of these form caveolae or small infoldings at the cell surface. The uterine smooth muscle cells of the rat have a poorly organized, often discontinuous basal lamina and they are surrounded by numerous collagen fibrils. In many regions, adjacent cells approach each other within distances of several hundred angstroms.

ESTRUS: At estrus marked changes occur in the smooth muscle cells of the myometrium (Fig. 2). These changes are largely restricted to the rough endoplasmic reticulum and Golgi complex. Both of these organelles become markedly enlarged and extensive. The rough endoplasmic reticulum consists of numerous complexly folded cisternae, the membranes of which are lined by large numbers of ribosomes. Mitochondria are randomly dispersed between these organelles. The cisternae of both the rough endoplasmic reticulum and Golgi complex contain relatively dense flocculent material.

During estrus, the surface of the smooth muscle cell becomes ruffled with numerous outpouchings and infoldings. This may be related to contraction of the uterine smooth muscle cells since Lane (30) has observed invagination of the borders of intestinal smooth muscle cells during mechanically stimulated contraction. It was common to see numerous elements of rough endoplasmic reticulum and Golgi complex in these outpouchings, and cisternal membranes approximated the plasma membrane at many of these regions, whereas other cytoplasmic projections were pale and organelle-free (Fig. 3). Although these cells contain abnormally large amounts of rough endoplasmic reticulum and Golgi complex, they are clearly recognizable as smooth muscle because of the abundance of myofilaments (Figs. 2, 3). If these myofilaments were absent, it would be difficult to distinguish this cell from cells such as fibroblasts that synthesize and secrete the proteins of the connective tissue (12).

IMMATURE ANIMALS

The uterine weight response of immature animals to injected estrogen is shown in Table I. Animals receiving a total dose of 0.6 to 60 μg of estradiol-17 β in six divided doses had a greater than threefold increase in uterine weight as compared to the uninjected or alcohol-injected control animals, and the enlarged uteri contained a large amount of luminal fluid. The uterine weight was intermediate in animals receiving 0.06 μg of estradiol-17 β.

CONTROL ANIMALS

In their fine structure, the uteri from uninjected controls and the alcohol-injected controls were similar in appearance. The smooth muscle cells (Fig. 4) were identical in structure to those seen in the mature rat during diestrus (Fig. 1). The cells were long and spindle-shaped with a relatively smooth surface. Most of the cytoplasm contained numerous myofilaments, and the organelles were located in a juxtanuclear zone. In this region the cytoplasm was pale, contained numerous mitochondria, a few cisternae of rough endoplasmic reticulum, and a small Golgi complex. An irregular basement membrane surrounded these cells, and numerous small vesicles and caveolae lined the surface (Fig. 4).

ESTROGEN-TREATED

The smooth muscle cells of the estrogen-treated animals (Fig. 5) were similar in appearance after all doses of estrogen and closely resembled the cells of the mature animal at estrus (Figs. 2, 3). There was a marked enlargement and dilation of the cisternae of rough endoplasmic reticulum.

TABLE I

Uterine Weight Response to Injected Estrogen

Animal group	No. of animals	Body wt.	Uterine wt.
		(g ± SD)	(mg ± SD)
A*	5	61.4 ± 3.9	27.8 ± 5.8
B	5	63.4 ± 1.4	34.8 ± 4.7
C	6	64.5 ± 3.8	60.7 ± 12.4
D	6	61.7 ± 4.0	127.3 ± 20.0
E	6	64.7 ± 2.6	114.6 ± 14.5
F	6	65.0 ± 5.0	119.6 ± 10.8

* Animal groups are described in Materials and Methods.

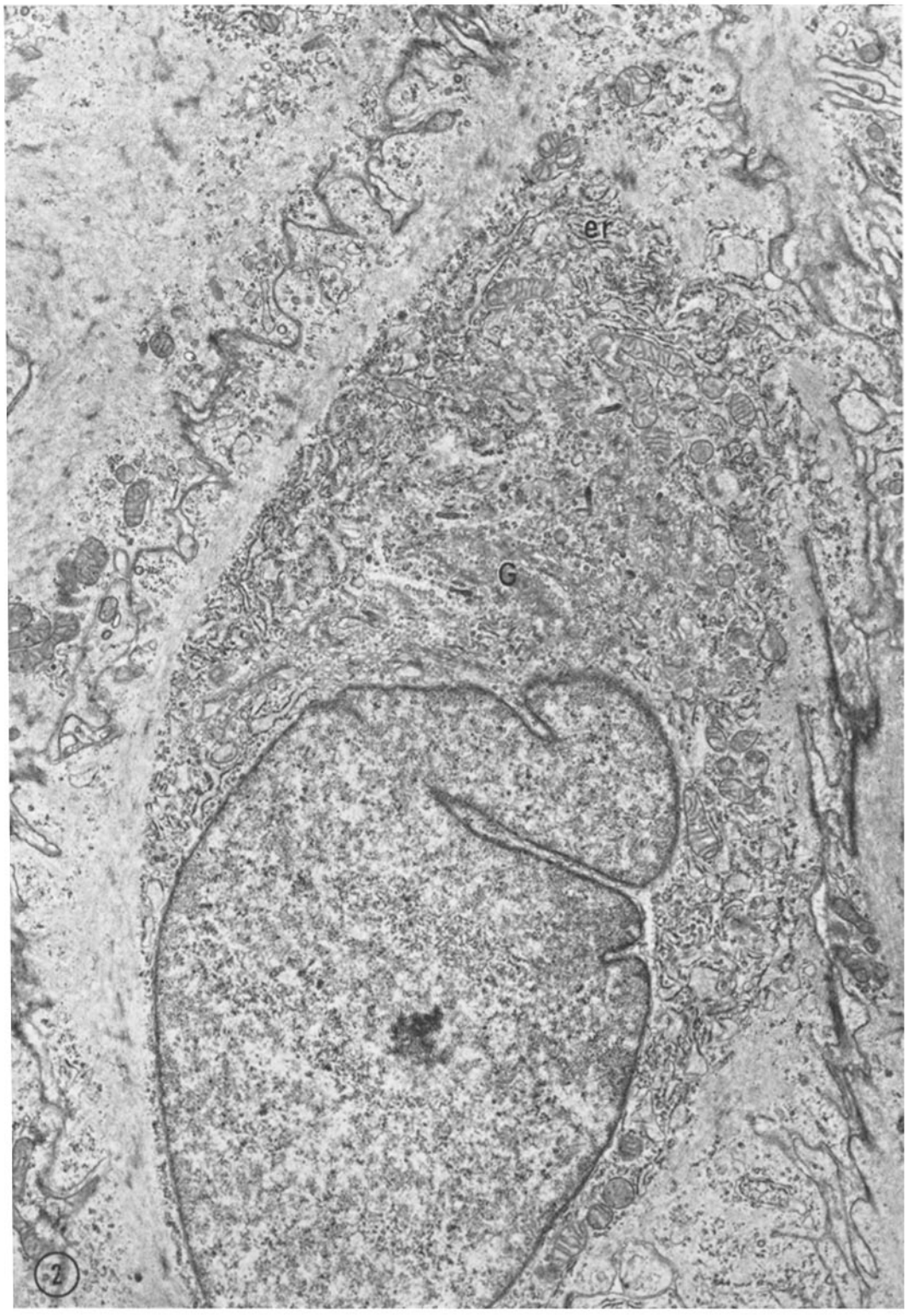

FIGURE 2 In this micrograph can be seen parts of several smooth muscle cells from the uterus of a mature animal in estrus. The cells are enlarged, contain numerous folds at the surface, and display an extensive enlargement and dilation of both the Golgi complex (*G*) and the rough endoplasmic reticulum (*er*). Myofilaments are present within the remainder of the cytoplasm. The rough endoplasmic reticulum contains many more ribosomes attached to the surface of the membranes than does the rough reticulum in the animals in diestrus. × 12,500.

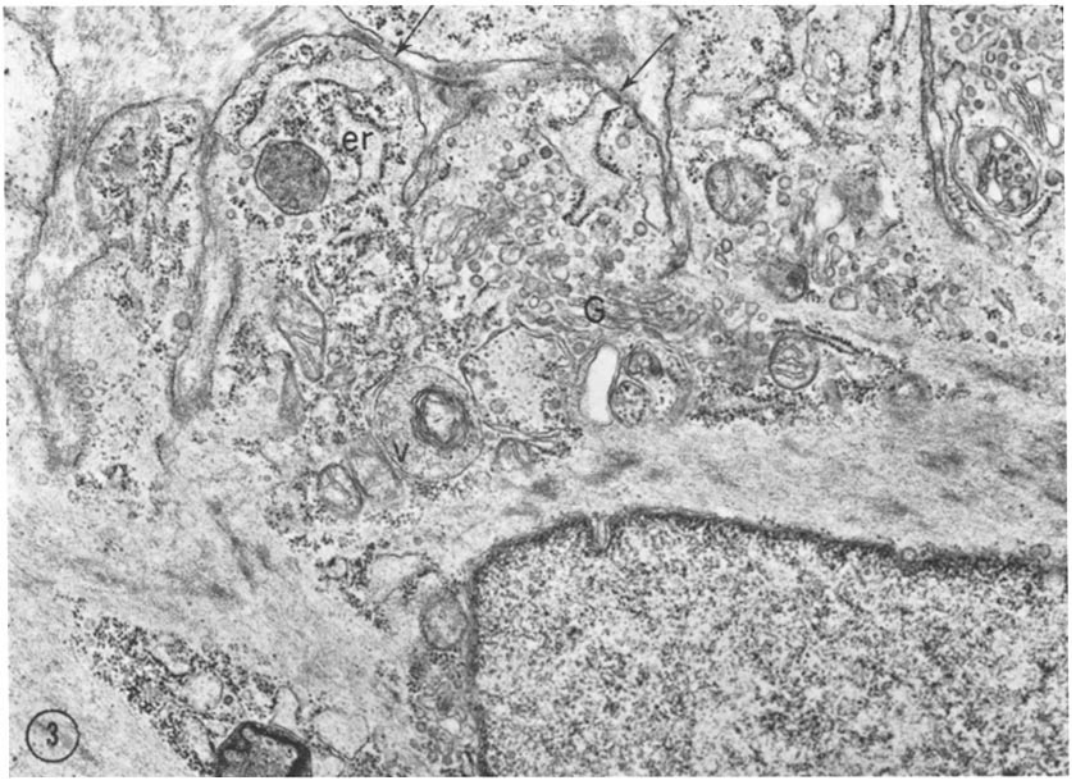

FIGURE 3 In this micrograph is a portion of the periphery of one smooth muscle cell from a uterus removed at estrus. At higher magnification, several cisternae of rough endoplasmic reticulum (*er*) together with a portion of the Golgi complex (*G*) are seen to be located in peripheral folds of cytoplasm. The membranes of the cisternae of the rough endoplasmic reticulum approximate the plasmalemma of the smooth muscle cell in several regions (arrows). This is a common finding in these cells during estrus. Numerous myofilaments, dense bodies, a vacuole containing whorls of membranes (*v*), and a portion of the nucleus are also apparent. × 22,500.

The membranes of the rough endoplasmic reticulum cisternae contained large numbers of attached ribosomes, and the cisternae were sufficiently dilated so that intercommunications between neighboring cisternae were clearly apparent. The Golgi complex was also markedly enlarged and randomly dispersed in many regions of the cell (Fig. 5). These organelles were located in the perinuclear zone and were also found close to the cell surface in outpouchings of the cell. Numerous collagen fibrils surrounded the cells in these regions, and the basement membrane was apparent but again appeared to be discontinuous. Many small smooth-surfaced vesicles were located at the cell surface. In addition, larger, coated vesicles were occasionally seen. Occasionally, groups of particles, larger than ribosomes, were found in the cytoplasm, many of which were similar in appearance to glycogen (Fig. 5).

Fibroblasts

Among the connective tissue cells of the endometrium of mature animals in diestrus, and immature control animals, were cells similar in appearance to fibrocytes described in other systems (13). These were long, narrow, spindle-shaped cells with a few cisternae of rough endoplasmic reticulum, a small Golgi complex, numerous mitochondria, and fine aggregates of filaments located at the surface of the cell (Fig. 6).

These cells were markedly increased in size in the mature rat during estrus and in the immature rat following estrogen administration and were identical in appearance to fibroblasts. The most

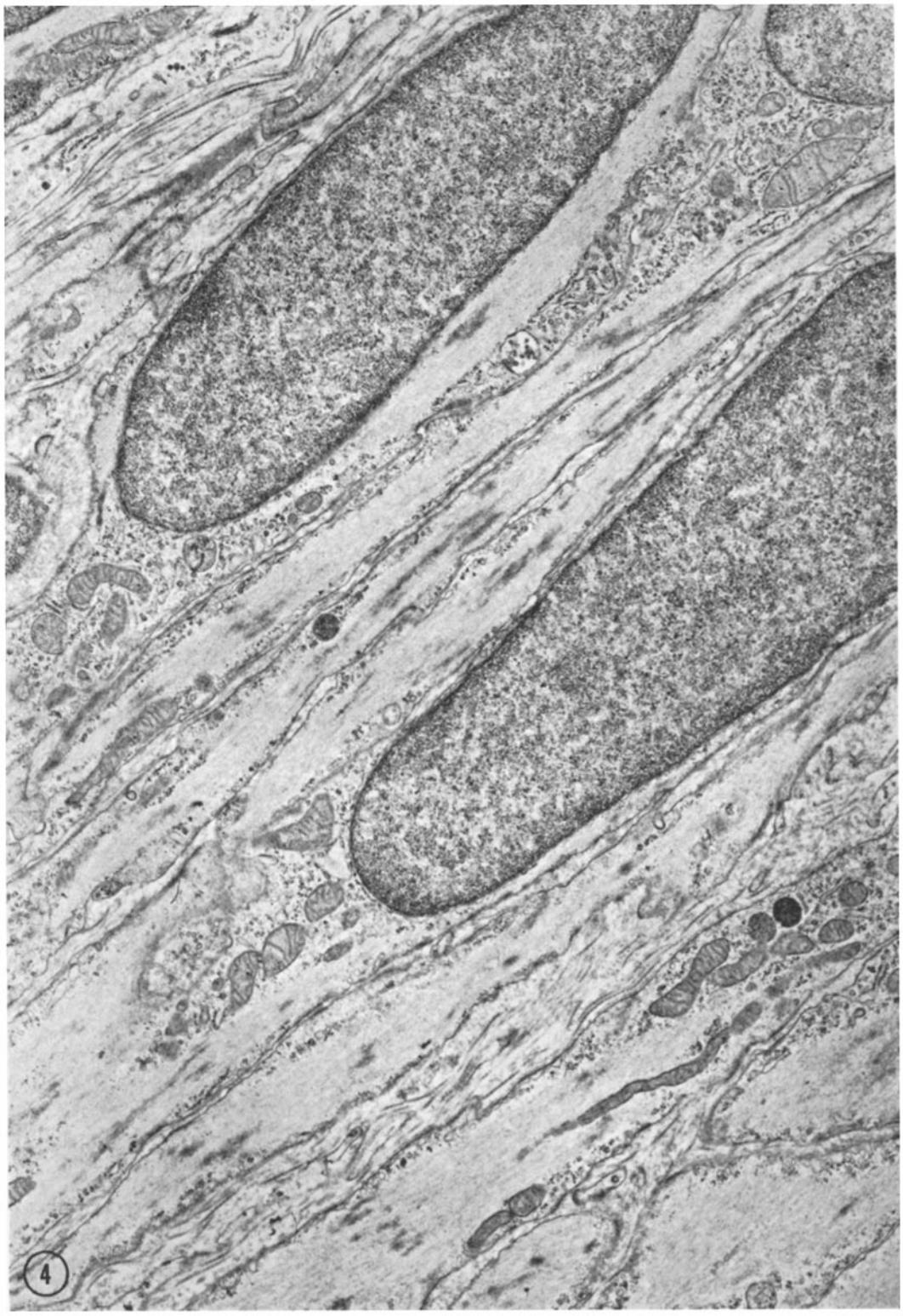

FIGURE 4 The smooth muscle cells in this electron micrograph are from the uterine myometrium of a prepubertal rat that was injected with the 20% alcohol carrier. These cells appear similar to the cells seen in Fig. 1. They contain mitochondria and a few cisternae of poorly developed rough endoplasmic reticulum located in pale, juxtanuclear or peripheral, cytoplasmic regions. The appearance of myofilaments, dense bodies, peripheral vesicles, and intercellular collagen fibrils is similar to that previously described for an animal seen in diestrus. × 12,500.

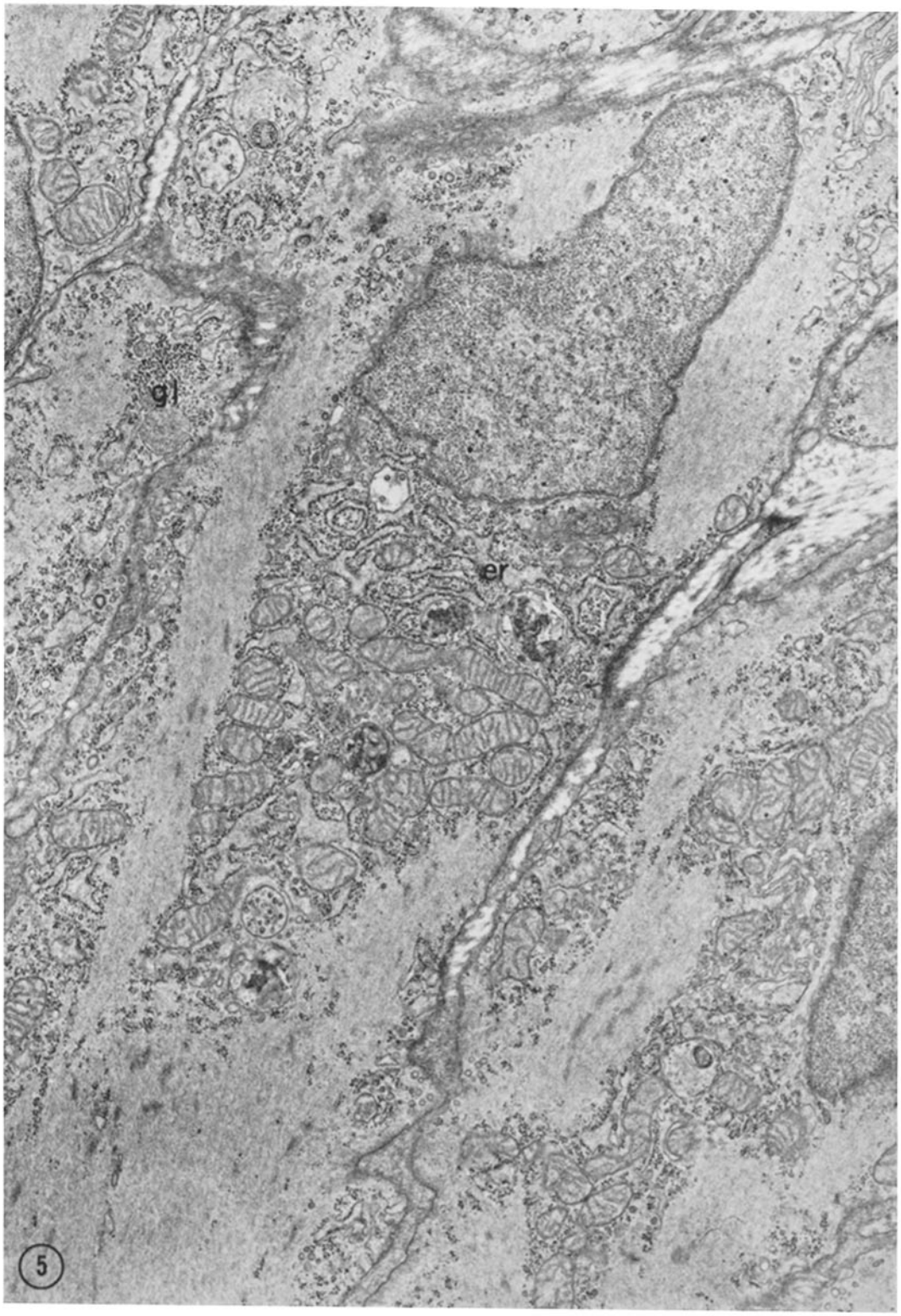

FIGURE 5 The smooth muscle cells in this electron micrograph were taken from the uterus of a prepubertal rat following the injection of a total dose of 60 μg of estradiol-17 β. The rough endoplasmic reticulum (*er*) in this cell is similar in appearance to that seen in smooth muscle from mature animals at estrus. The cisternae are extensively enlarged and dilated, and their membranes contain large numbers of attached ribosomes. Numerous mitochondria, multivesicular bodies, and vacuoles containing dense materials and debris can also be seen. Occasional groups of particles similar in appearance to glycogen (*gl*) are seen. Peripheral vesicles dot the surface of this cell, and extracellular collagen fibrils are visible. × 16,500.

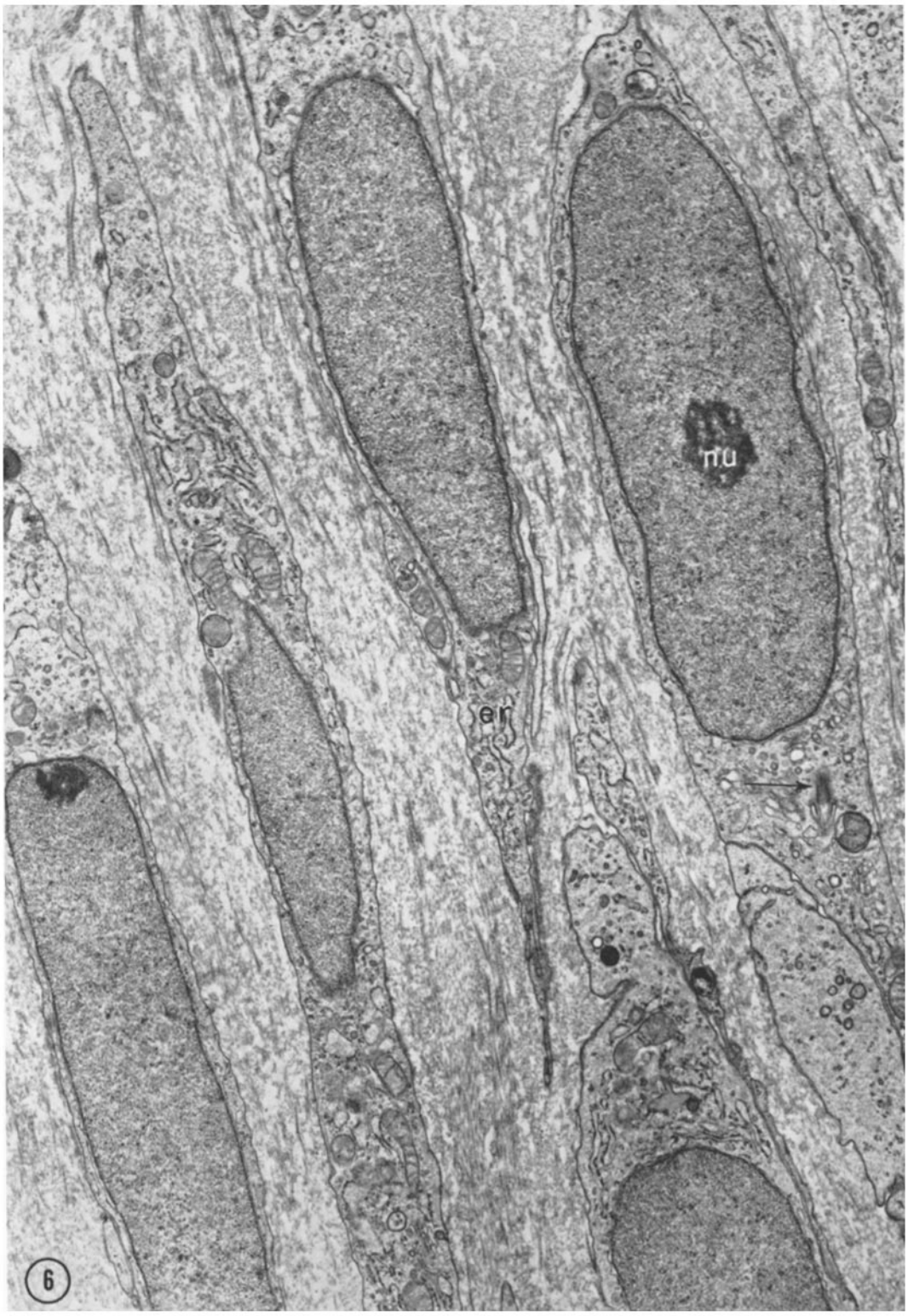

FIGURE 6 The fibrocytes in this electron micrograph were obtained from the endometrium of the uterus of an uninjected prepubertal animal. These cells are characteristically long and spindle-shaped, and contain a few cisternae of rough endoplasmic reticulum (*er*). Their nuclei are ellipsoidal and often contain prominent nucleoli (*nu*). Numerous poorly organized extracellular collagen fibrils can be seen. One of these cells contains part of a ciliary process (arrow). The endoplasmic reticulum is relatively poorly developed in these cells in contrast to that of the cells seen in Fig. 7. × 12,000.

prominent change in these cells was reflected in the rough-surfaced endoplasmic reticulum and Golgi complex. The cisternae of the endoplasmic reticulum became extensive, dilated, and ramified throughout the cell, and the Golgi complex was markedly increased in size (Fig. 7).

DISCUSSION

The smooth muscle cells of rat uteri from both mature animals in diestrus and immature animals were identical in appearance and were similar to the smooth muscle cells of the intestine, vas deferens, and human nonpregnant uterus (8–11). Striking changes in the numbers and distribution of the rough endoplasmic reticulum and Golgi complex of the uterine smooth muscle cells occurred both during estrus, when high levels of circulating estrogens were present, and following the administration of estradiol to immature animals. The rough endoplasmic reticulum was markedly enlarged and contained many more attached ribosomes, and the Golgi complex was also increased in size. These alterations are similar to those reported in smooth muscle cells of the human myometrium in nonpregnant and pregnant uteri by Laguens and Lagrutta (14). These investigators noted an increase in the amount of rough endoplasmic reticulum and Golgi complex of the smooth muscle cells during pregnancy.

We have also observed changes in certain uterine connective tissue cells which in the unstimulated state have the appearance of fibrocytes or inactive cells. On estrogen stimulation, these cells have a markedly enlarged rough endoplasmic reticulum and Golgi complex and appear like fibroblasts, cells commonly associated with the synthesis and secretion of connective tissue proteins (12, 13). Iversen and Christensen (19) previously noted that in the estrogen-stimulated, ovariectomized guinea pig uterus the "synthetic activity" of interstitial cells showed an apparent increase as evidenced by an increase in the amount of rough endoplasmic reticulum in these cells. The interstitial cells in their study were similar in appearance to the fibroblasts in the estrogen-stimulated uteri in the present report.

It is now well documented that the stimulation of uterine RNA synthesis is an early major response to estrogen administration (15–18). The largest proportion of the cellular RNA is ribosomal RNA, and a number of investigators have reported an increase in uterine ribosome formation upon estrogen administration. Greenman and Kenney (2) found that the ribosome content of a uterine system capable of cell-free protein synthesis was decreased by ovariectomy and restored after the administration of estrogen to the ovariectomized animal. Moore and Hamilton (3) noted an increase in the synthesis of ribosomal RNA up to 24 hours after a single injection of estrogen into the ovariectomized animal, as indicated by the incorporation of tritiated uridine into ribosomal RNA, and by an increase in the cytoplasmic level of 78S ribosomes. Gorski and Nelson (4) observed that 1 hr after estrogen administration there was an 88% increase in cytidine-H^3 incorporation into RNA, an increase which involved all RNA fractions including ribosomal RNA. The morphological findings reported here are in agreement with these biochemical data, which indicate an increase in ribosome formation in the estrogen-primed uterus. They further localize the increased ribosome formation to at least two cell types, the smooth muscle cells and the fibroblasts.

In the smooth muscle cell of the estrogen-stimulated uterus the cisternae of the rough endoplasmic reticulum were frequently in close approximation to the plasmalemma. Similar findings were reported by Laguens and Lagrutta (14) in the pregnant human uterus. They suggested that these membrane approximations were related to a stimulatory phenomenon analogous to the role played by the T-system and various tubular systems in skeletal muscle. However, it appears that the enlargements of membranous systems in the smooth muscle cells in the rat uterus in response to estrogen are largely associated with the endoplasmic reticulum and Golgi complex, organelles well known to be responsible for the synthesis and secretion of extracellular protein. Fibroblasts have also been observed to contain a similar topographic relationship between the rough endoplasmic reticulum and the cell surface, and it has been suggested that these may serve as sites for the secretion of sequestered material from the ergastoplasm (12, 20).

Several investigators (21–24) have noted a marked increase in the total amount of uterine collagen of castrate rats following the administration of estrogen, as indicated by an increase in the concentration of protein-bound hydroxyproline. In a light microscopic study of uteri of castrated rats following administration of estradiol-17 β, Fainstat (25) noted an increase in the number of

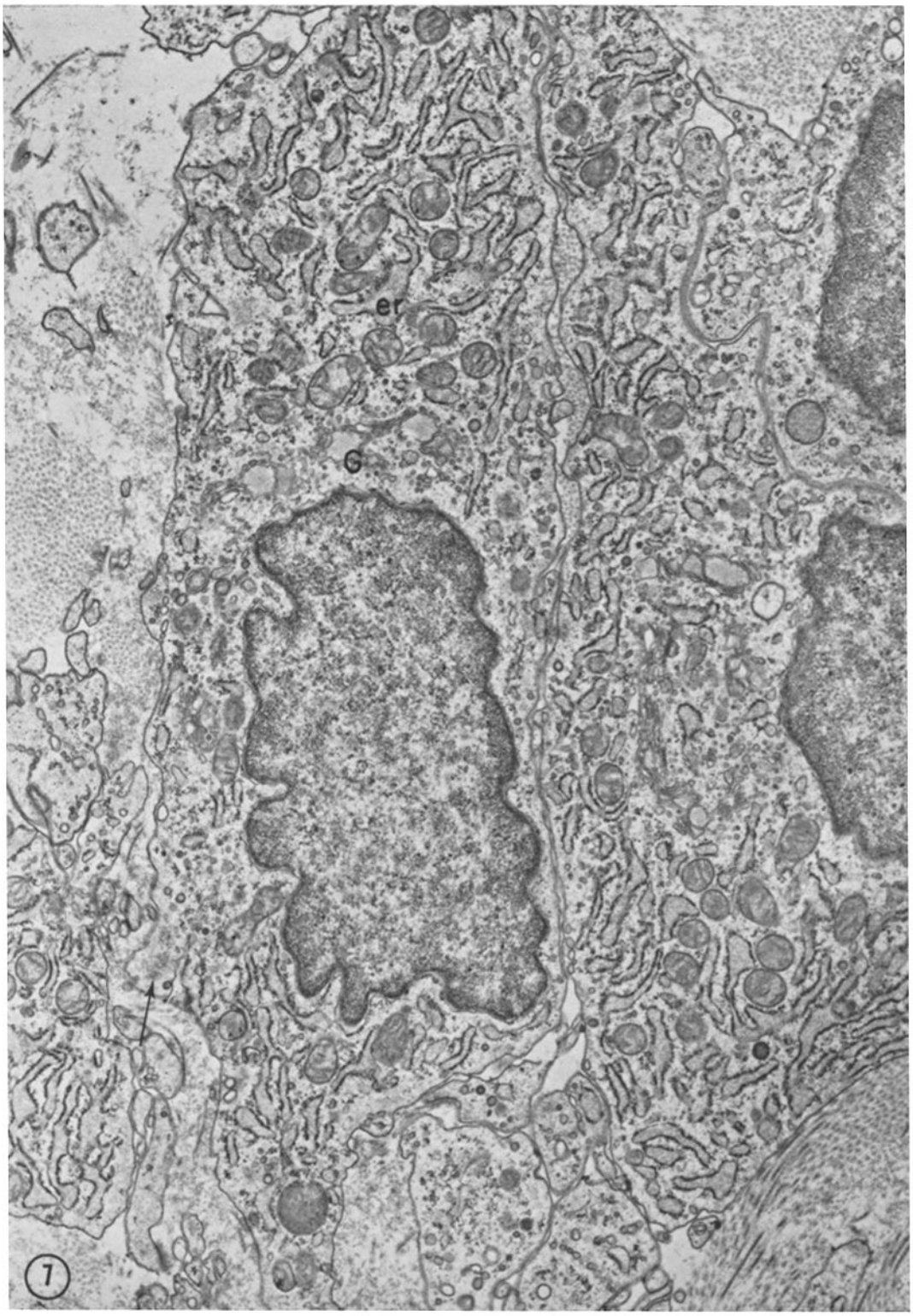

FIGURE 7 The fibroblasts in this figure are from a similar region of the endometrium of a prepubertal rat uterus following estradiol-17 β injection (total dose 0.6 μg). These cells appear to be typical fibroblasts in that they contain an extensive rough endoplasmic reticulum (*er*), the cisternae of which ramify throughout the cell, and contain dense, flocculent material. The Golgi complex (*G*) contains several dilated cisternae and numerous flat saccules and many vesicles. Collections of fine filaments can be seen in the cell periphery (arrows), and numerous collagen fibrils sectioned both transversely and longitudinally appear in more discrete bundles than those seen in Fig. 6. × 15,000.

collagen fiber bundles in the endometrial stroma. His observations correlate nicely with the chemical studies previously cited. More recently, Kao et al. (26) have studied the formation of collagen in uteri from castrated and normal rats in vivo and in vitro. They studied the incorporation of tritium-labeled amino acids into both collagen and noncollagenous protein in uterine slices and noted a marked increase in both the specific activity and the total amount of collagen after estrogen stimulation, as well as an increase in the specific activity of noncollagenous protein.

The smooth muscle cell has been previously implicated in collagen synthesis in the atherosclerotic plaque. Changes in the configuration and distribution of the rough endoplasmic reticulum of smooth muscle cells similar to the changes reported here were observed in the rabbit atherosclerotic plaque by Parker and Odland (27) and by Thomas et al. (28). Since no other cells with an extensive rough endoplasmic reticulum were found, it was suggested that the smooth muscle cells might be responsible for the marked increase in collagen synthesis associated with the atherosclerotic plaque.

In view of the striking changes in the concentration and distribution of rough endoplasmic reticulum and Golgi apparatus in the uterine smooth muscle cells and fibroblasts following estrogen therapy, it is possible that both the smooth muscle cells and the fibroblasts of the estrogen-primed uterus participate in the synthesis and secretion of collagen, as well as noncollagenous protein.

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