10 Ultrastructure of the Myometrium

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I. Introduction

Since the description by Keye (1923) of the specific influence of the two ovarian hormones, estrogen and progesterone, upon the contractility of the myometrium, many biochemical and physiological investigations have been carried out to explain this phenomenon. The morphologic basis for these various kinds of contraction, however, remains largely unknown. The older studies with light microscopy concerned either the cellular architecture of the uterine wall (Goerttler, 1931) or the problem of growth (hyperplasia, hypertrophy, and metaplasia; Reynolds, 1949). An attempt at functional cytomorphology of the smooth muscles failed because of the difficulty of fixation of definite functional stages and because of lack of knowledge of the artifacts arising during removal and fixation. The various partially contradictory findings are probably attributable to these difficulties.

McGill (1909) showed that contracted smooth muscles consist mainly of dark cells. Henneberg (1900), too, found light and dark muscle cells, but he thought that the light cells were the contracted elements, shorter and thicker than the dark cells. According to Roskin (1936), shortening of the muscle cells is related to a helical twist of the whole cells or of individual nuclei. Fischer (1944) attributed these helical shapes to passive obstructions related to the active contraction of neighboring masses of muscle. Heidenhain (1900) observed changes in the shape of the nuclei in contracted cells; he assumed that the contracted masses of the cell exert pressure upon the nuclear poles, and that in extreme cases they could cause even a break in the nucleus. The multinuclear cells observed in sections were, therefore, interpreted as tangential cuts of these contracted muscle cells. Meigs (1912), who did not receive immediate attention, described a decrease in volume of the muscular cells during contraction, and he explained the finding as a "squeezing out" of cellular fluid into the extracellular space. This extrusion theory has just recently been revived (Greven, 1951; Bailey, 1954; Flory, 1957; Gansler, 1960). With the introduction of the electron microscope and of improved methods of fixation and preparation, the morphological problems of smooth muscle were attacked anew and agreement was reached in the observation that there appears, on the basis of its diameter, only *one* kind of filament in the cells of smooth muscle, in contrast to those of striated muscle (Gansler, 1956, 1960; Mark, 1956; Moore and Ruska, 1957; Shoenberg, 1958, 1962). Investigations of the influence of the ovarian hormones upon the muscle cells of the myometrium (Gansler, 1956) have shown that under the influence of estrogens there appear light muscle cells with only narrow zones containing filaments, whereas under the influence of progesterone the muscle cells are darker and uniformly permeated by filaments. Finally, we (Gansler, 1960) have been able to show, in agreement with the observation of Meigs, that in isolated muscle strips taken from frog stomach the contraction and relaxation are probably related to transfer of fluid between intracellular and extracellular spaces. Similar observations, meanwhile, have been presented concerning muscle cells of the human myometrium also (Jaeger and Pohlmann, 1962). A decrease in the parallel orientation of the filaments, furthermore, has been observed by us in isotonically contracted muscle cells. It seems to be related to the decrease in anisotropism during contraction.

Further improvement in methods of fixation and preparation, mainly intravascular fixation (fixation by perfusion; Palay and associates, 1962; Nemetschek and Becker, 1964) has resulted in confirmation and supplementation of our observations to date.

II. Organized Muscle Bundles

The specific functions of smooth muscle—tension, contraction, and relaxation—are the result of the coordinated activity of muscle cells, rather than the behavior of the single cell. A functional syncytium is also postulated on electrophysiological grounds, at least for certain functional states. More recently, Barr

(1963) described the fusion of membranes of neighboring muscle cells at certain points as a nexus, and expressed the belief that this connection represented the morphologic basis for the functional syncytium.

It must be pointed out, however, that the number of observed points of contact is small in proportion to the cell surface. Dewey and Barr (1964), Lane and Rhodin (1964), and Evans and Evans (1964) believe, furthermore, that different forms of cellular contacts are characteristic of the smooth muscles of

Fig. 1. Light and dark muscle cells from the intestine. In the lower part of the picture are three sectioned spiny cells, which in part are not yet closely interdigitated (arrows). Light muscle cells with clear cell borders are seen at the top. × 7500.

different organs or of different species. On the basis of our own investigations (Gansler, 1961), however, we are led to assume that different forms of cell contacts are attributable to different functional states of the same muscle. Muscle bundles thus exist as interdigitated cells over broad areas with ill-defined cell membranes or as discrete single cells, which either interlock by means of pseudopodia or have a smooth surface. Occasionally, all forms can be observed adjacent to one another in the intestinal tract (Fig. 1), if fixation is effected *in situ;* otherwise, the muscle cells undergo uncontrollable changes on mere touching of the

FIG. 2. Light cell with smooth surface (before ovulation). Filaments are markedly reduced. The small channels of the endoplasmic reticulum are dilated and denser than the myoplasm. × 45,000.

unfixed muscles. As Fig. 1 shows, there is a correlation between the appearance of the various cell surfaces and the density of the myoplasm: interdigitated cells are lighter than spiny cells. It appears as though the interlocked light cells become the dark spiny cells through release of liquid into the extracellular space. It seems unlikely that this finding results from variations in the affinity of the myoplasm for heavy metal salts, because differences of density are observed

Fig. 3. Muscle bundles interdigitated over wide areas typical of the effect of estrogen (before ovulation and after delivery). The cell membranes are barely recognizable because of the identical density of the myoplasm and the extracellular space. The filaments are arranged loosely with little orientation. In the extracellular space, amorphous ground substance. × 30,000.

also after fixation with aldehydes alone. It should rather be assumed that the contractile proteins in the dark cells are packed more tightly, as the cell volume is reduced through the release of water, and accordingly scatter the electron beam more markedly. Under the influence of the ovarian hormones, too, various forms of muscle bundles are found in the myometrium (Nemetschek, unpublished) characteristic of the effects of estrogen or progesterone.

Under the influence of *estrogen*, even within a single section, three distinct types of cellular groups may be observed simultaneously: (1) light cells lying

FIG. 4. Spiny cell (after ovulation). Nucleus is lighter than the myoplasm. Free ribosomes and endoplasmic reticulum at the nuclear poles and in the ameboid projections. The filaments exhibit special orientation only in the central cellular areas. An apparent intercellular bridge is shown by the arrow. Collagenous fibrils in the extracellular space. × 30,000.

Fig. 5. Incipient spiny cells from the same section as shown in Fig. 3. The distance between membranes has increased; the extracellular space is lighter than the myoplasm. The endoplasmic reticulum reaches the cellular surface. The filaments are unoriented with the exception of the central area of the lower cell. In the extracellular space more collagenous fibrils may now be found. × 30,000.

closely together in approximately parallel orientation with more or less smooth surfaces (Fig. 2); (2) light cells in oblique arrangement, with their surfaces interlocked over broad areas (Fig. 3); and (3) darker spiny cells in similar oblique arrangement, differing from the former group by virtue of the greater density of the myoplasm and greater distances between the cells (Fig. 4 and 5).

The nuclei are looser, and plump cells with two nuclear incisions are found

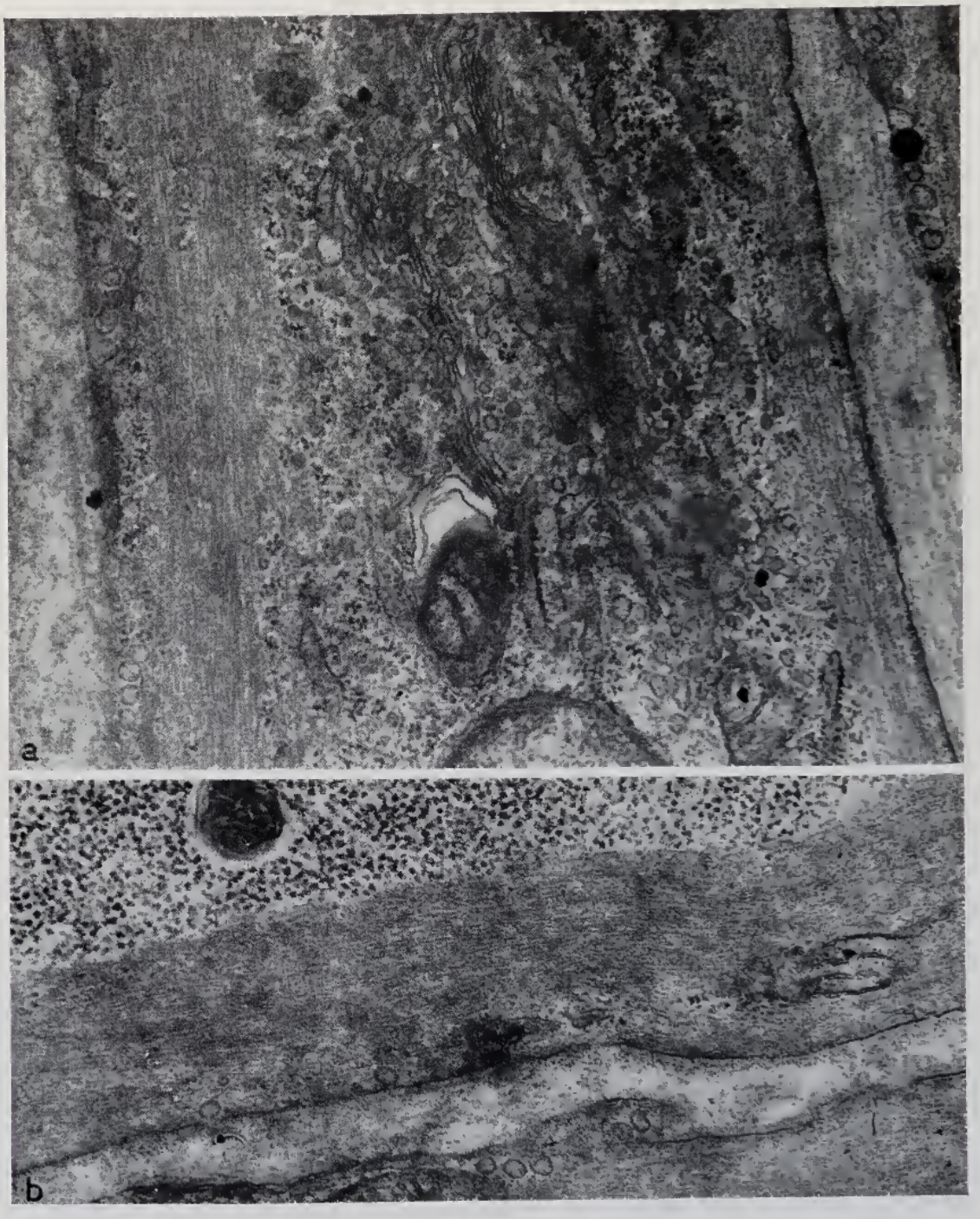

FIG. 6. Muscle cells from gravid myometria. (a) Muscle cells at the onset of pregnancy with greatly increased agranular reticulum, free ribosomes, and tubules. The cellular membranes are smooth and exhibit pinocytotic activity. In the extracellular space, distinct collagenous fibrils. (b) Sections from a muscle cell near the placenta. The central cellular area shows only one large deposit of glycogen. × 30,000.

frequently. This appearance may be interpreted as an indication of a nuclear twist as well as of a true nuclear fragmentation.

Under the influence of *progesterone*, after ovulation and during pregnancy, light cells can only rarely be found. Instead, markedly vacuolated muscle cells are conspicuous, especially after ovulation. Otherwise, the muscle bundles consist predominantly of dense cells (Figs. 6 and 7), and their appearance is determined

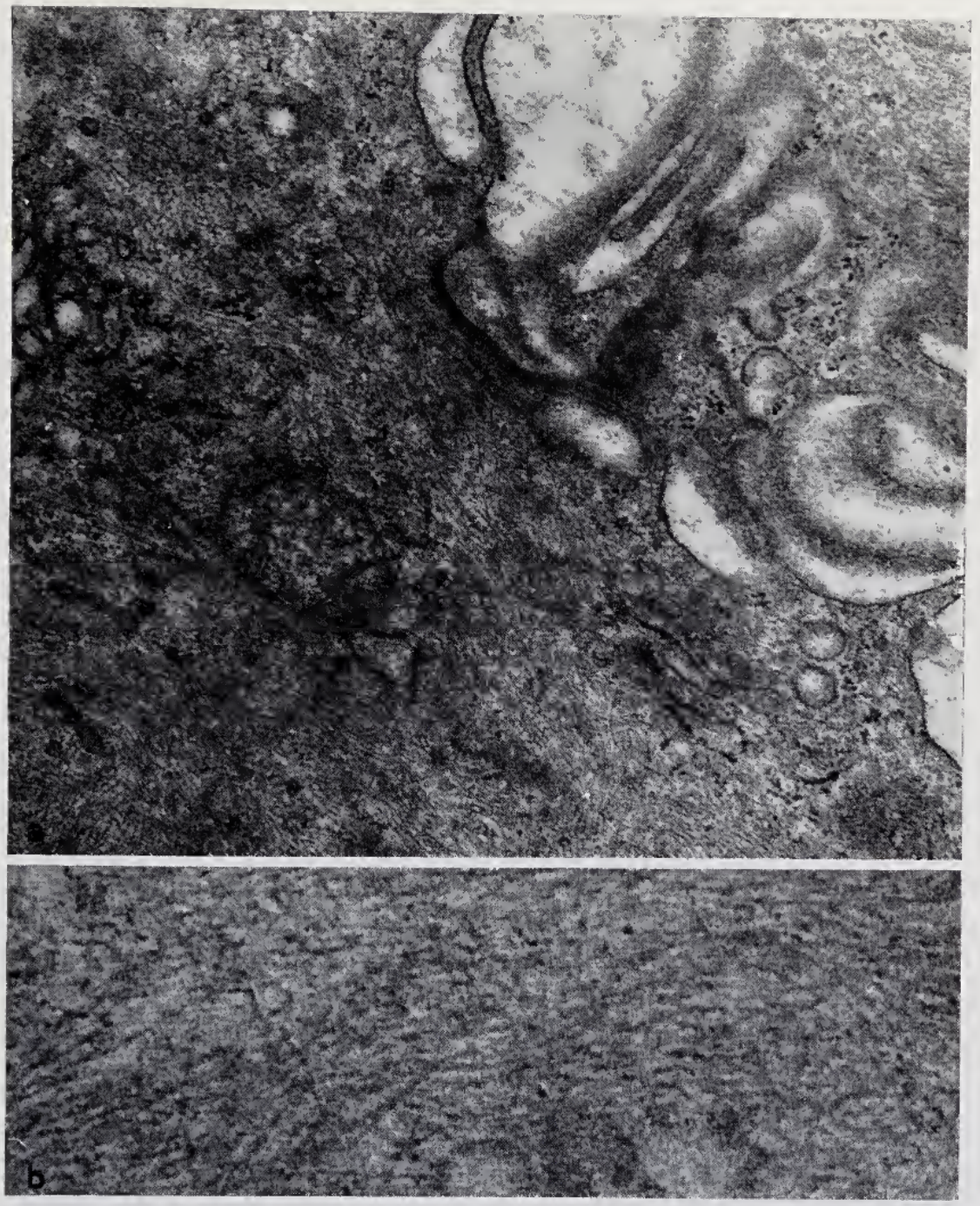

Fig. 7. Muscle cell near the placenta (second trimester). (a) Bizarre distortions of cell surfaces, unoriented filaments, and tubules. × 30,000. (b) Higher magnification of filaments. × 60,000.

mainly by the distances between the single cells. There are, in addition to the typical spiny cells (Fig. 4), muscle cells with a more or less smooth surface (Fig. 8). The structure of the nuclei is, once more, loose, but the surface is more greatly folded than before ovulation. In metestrus and diestrus the muscle bundles all consist of distinct single cells with a smooth surface, but the nuclei are plumper and lighter in metestrus than in diestrus. It is interesting that during delivery, or rather immediately before labor begins, broadly interdigitated cells reappear, typical of the effect of estrogen. Differences between muscle cells

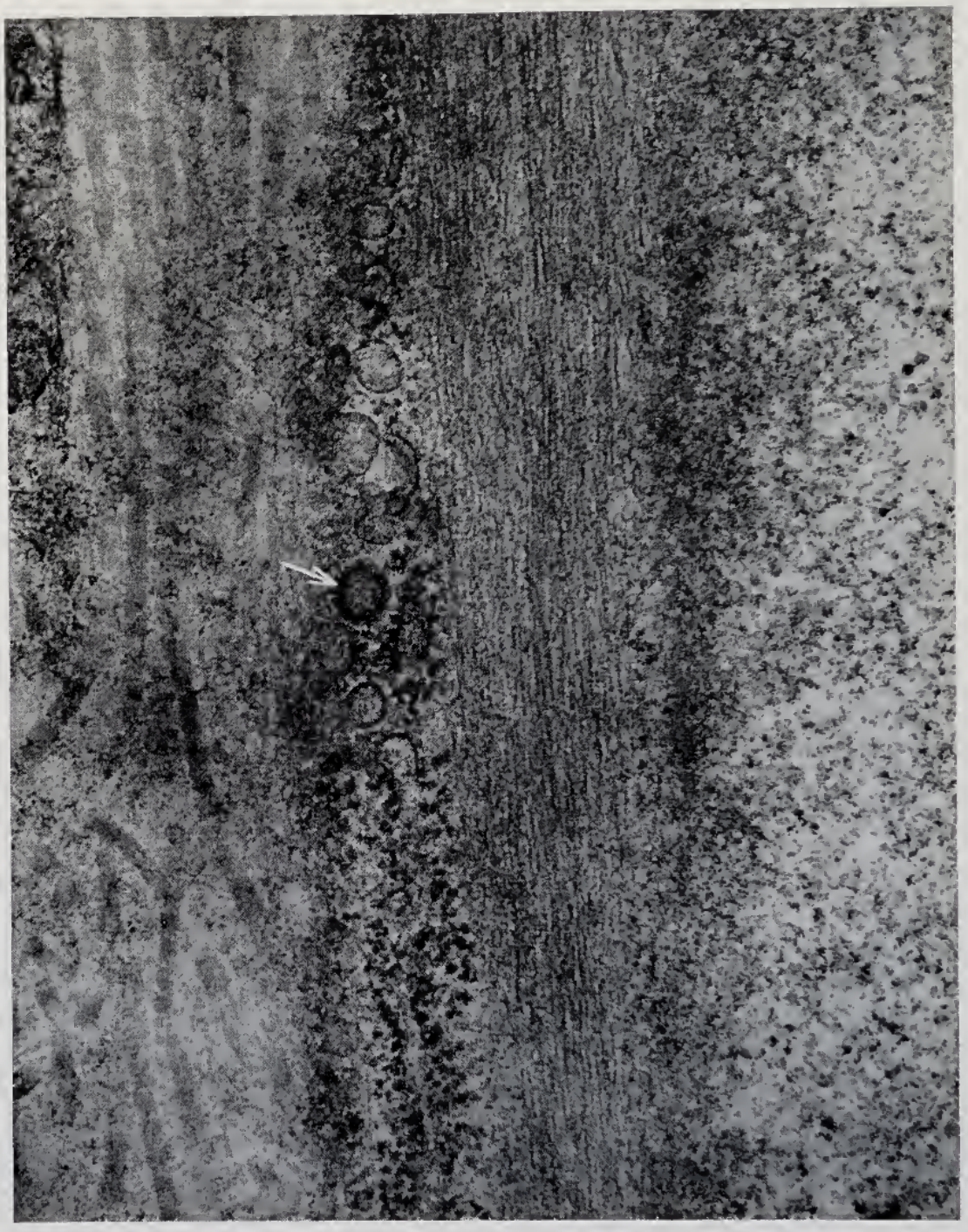

FIG. 8. Dark muscle cells with smooth surface (after ovulation). The cell surface merges, without clear outlines, with the extracellular space. The nuclear membrane, too, lacks clear definition. The small vesicle indicated by the arrow may represent intake of protein. Myofilaments are in parallel orientation. In the extracellular space are extremely fine filaments and transversely striated collagenous fibrils. × 60,000.

situated close to the placenta and those distant from it (Figs. 6 and 7) diminish at the onset of labor. Before such times the muscle cells close to the placenta are plump, exhibiting bizarre pseudopodia as well as perinuclear deposits of glycogen that frequently attain considerable size.

A comparison of these peculiarities characteristic of the effects of estrogen and progesterone on the cellular aggregates may be significant in explaining

the differences in contractility (Keye, 1923; Csapo, 1955; Bozler, 1941). The effective performance, or the extent of the single contractions of three-dimensionally interdigitated muscle cells, is probably greater than that of tissue in which areas of contact of the individual cells are farther apart from one another. The differing propagation of contraction waves and of action potentials (Bozler, 1941; Kao, 1959; Kuriyama and Csapo, 1961) may be explained similarly.

The various forms of muscle cells seem also to aid in answering the question whether myometrial contraction is isometric or isotonic. Csapo (1955) believes that the function of the myometrium consists in an isometric contraction. On the other hand, the morphological observations suggest a shortening of the individual cell groups resulting from the interlocking ("cross-shearing") of the single cells. The main cause seems to be the change in the shape of their nuclei, but it is still unknown whether this change occurs actively or passively. As this shearing mechanism can be observed in muscles fixed *in situ* as well as in isolated strips of muscle, artifacts of preparation may be excluded. Our observations suggest, furthermore, that in the myometrium and the smooth muscles of other hollow organs, isometric and isotonic contractions are but different phases of a complex cycle of contraction, with the exception perhaps of the smooth muscles of the vascular system. The function of these muscles seems, according to morphologic observations, to be a rhythmic change from relaxation to isometric contraction and vice versa. In view of the fact that only the volume of the vascular system remains constant, the difference in function is easily understandable. It should be mentioned at this point that the *vascular* muscles of the myometrium in the various phases of the cycle also exhibit the same ultrastructure.

III. Hyperplasia, Hypertrophy, and Metaplasia of the Myometrium

A metaplasia of muscle cells from histiocytes in gravid uteri has been described by Stieve (1929) and Froböse (1932). A regressive metaplasia from muscle cells to histiocytes and macrophages is said to occur during involution, and electron microscopic observations seem to be confirmatory (Nemetschek, unpublished). At the start of pregnancy, between the circular muscles proper and the loose connective tissue of the endometrium there are numerous muscle cells unconnected with others; transitional stages between them and histiocytes can be observed. In addition, a hypertrophy of the muscle cells can be demonstrated. The mitoses described by light microscopy (Froböse, 1932) have not yet been observed by us with the electron microscope. Estrogen is known to produce a hyperplasia of the castrate's uterus, but no mitoses have been described in this connection, not even with the light microscope.

The appearance of granulocytes with or without eosinophilic granules is characteristic of stimulation by estrogen (Gander, 1930; Gansler, 1954; Bjersing and Borglin, 1964; Schaefer and Fischer, 1964). They appear in the castrate's uterus, upon estrogenic stimulation, first in the myometrium. After prolonged administration of estrogen, they are diffusely distributed also in the endometrial

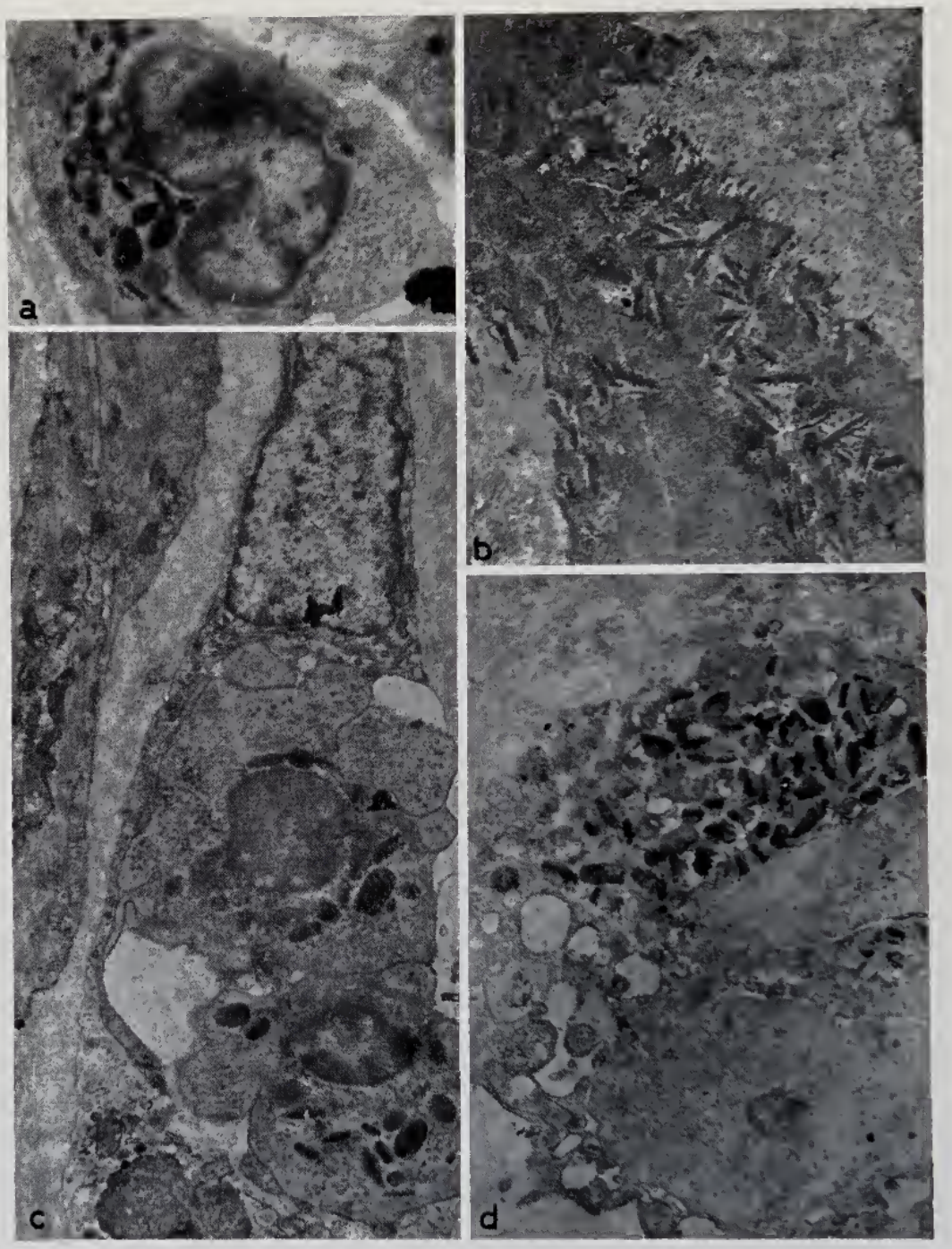

FIG. 9. Granulocytes from the myometrium under the influence of estrogen. (a) Typical eosinophilic granulocytes. (b) Fragmented nucleus of a muscle cell with adjacent eosinophilic granulocyte. (c) Cell fragment with numerous "crystals," some of which protrude freely into the extracellular space. (d) Cell with two nuclei. Lower part is markedly vacuolated without eosinophilic granules; upper part contains eosinophilic granules closely apposed to the cell nucleus. × 7500.

stroma. More recent investigations of this problem (Nemetschek, unpublished) suggest that there are atypical forms of division in histiocytes as well as in muscle cells, and that in these processes there may also appear granulocytes with and without eosinophilic granules (Fig. 9). The cause of these kinds of division may perhaps be found in the cellular edema characteristically produced

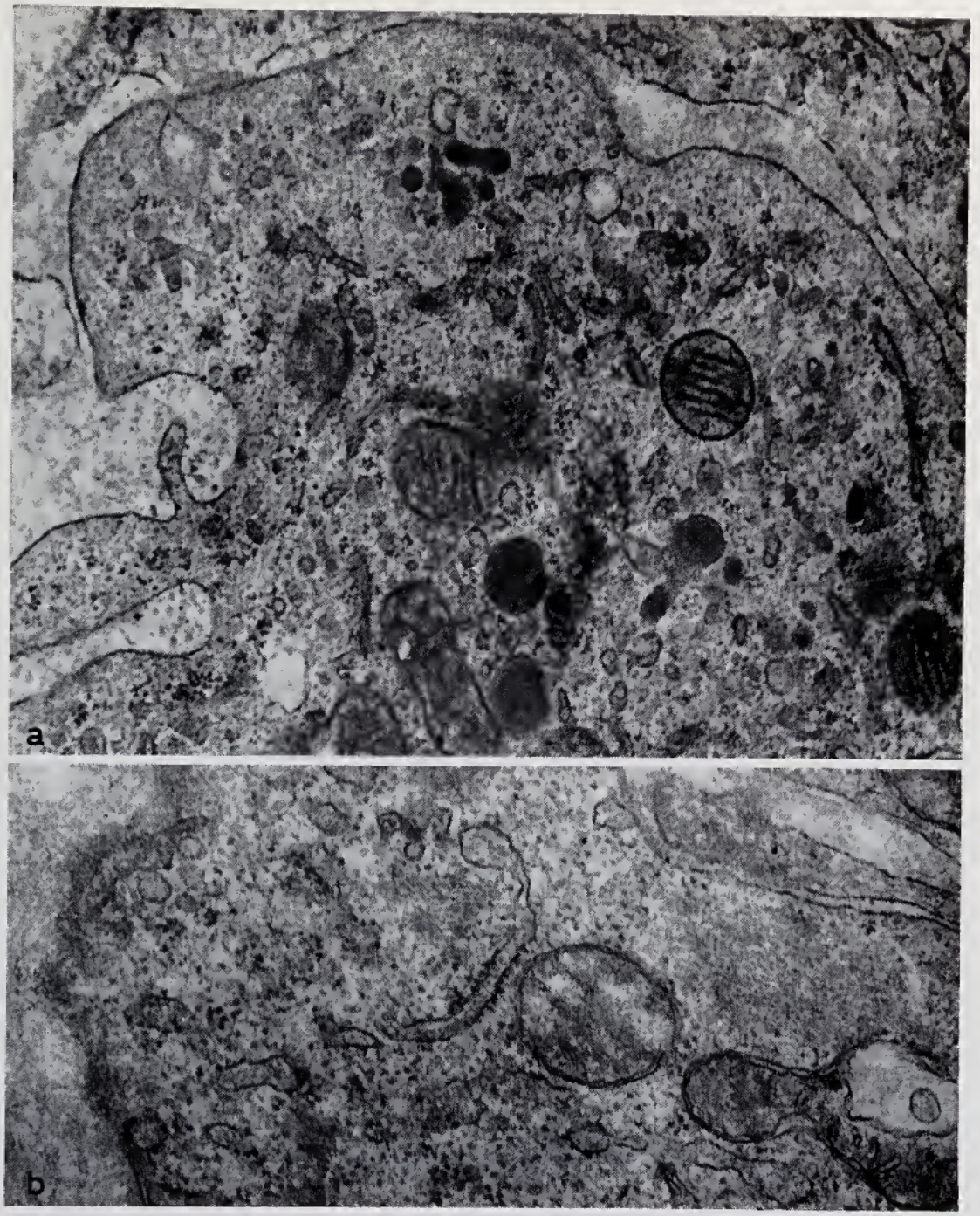

Fig. 10. (a) Section from a granulocyte (or muscle cell?) without eosinophilic granules, but with numerous "protein" granules lacking crystalline inclusions. (b) Surface of a light muscle cell that contains also numerous small vesicles of varying density. A reduction of the filaments is noticeable. × 30,000.

by estrogen (Reynolds, 1949; Bialy and Pincus, 1962). The polymorphism of these granulocytes is conspicuous, as is their spatial relation to the muscle cells and to the cells of the connective tissue; there are, furthermore, several transitional stages between them and the granulocytes (Fig. 10). The occurrence of centrioles and basal bodies in these cells seems to support the assumption that there is atypical mitosis or amitosis. The great number of small dense vesicles and the absence of invaginations of membranes (micropinocytosis) are conspicuous

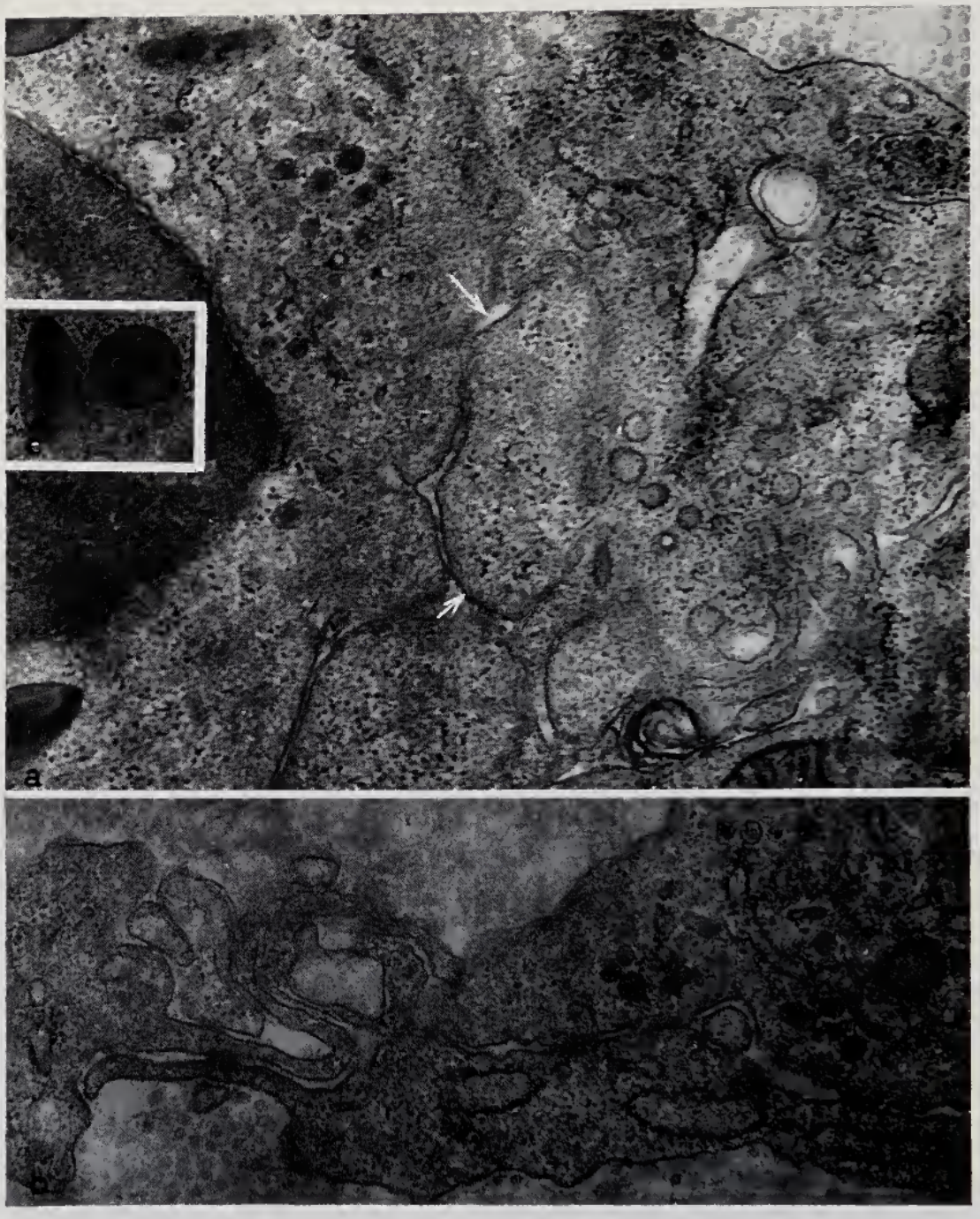

FIG. 11. Sections from a granulocyte. (a) Bizarre distortions of the cell surface (arrows). Many small vesicles with varying density can be seen in the finely granulated cytoplasm, as well as some sporadic eosinophilic granules. (b) Bizarrely interlocked pseudopodia of granulocytes. (c) (Small inset). Next to a granule with crystalline inclusions is a granule that does not yet exhibit small vesicles. × 30,000.

and typical of these cells. These small vesicles may perhaps be interpreted as indicative of intracellular storage. Granulocytes without typical eosinophilic granules exhibit (Fig. 10) numerous rather large osmiophilic granules, probably proteinaceous (Locke and Collins, 1965), which could have been formed by confluence of the denser small vesicles. There are intermediate stages (Fig. 11) between them and the eosinophilic granules with pseudocrystalline inclusions. When the estrogenic effect decreases, there appear only granulocytes without

cosinophilic granules, but with dense, small vesicles of varying diameter. We therefore assume that disintegration of the eosinophilic granules has taken place. The subsequent behavior of these cells is not yet known. In addition to these polymorphous granulocytes, there are in the connective tissue of the endometrial stroma typical eosinophilic granulocytes, which are not different from those in the circulating blood. Thus it was assumed that they could be hematogenous (Fischer and Schaefer, 1965) (Fig. 9a), for it was pointed out that the alkaline phosphatase is negative in tissue eosinophiles as well as in blood eosinophiles of normal mice, whereas it is positive in X-irradiated mice, after the injection of bone marrow from rats. Since the alkaline phosphatase is always positive in rat eosinophiles, the positive result in tissue eosinophiles of mice thus treated suggests their derivation from the blood. On the other hand, Nowell and co-workers (1956), in the eourse of similar speculations, have considered it possible that the injection of cells of a foreign species might cause an increase in alkaline phosphatase in the body's own cells. Gander (1930) has already pointed out that the discrepancy between the small number of blood eosinophiles and the excessively large quantity of tissue eosinophiles renders improbable the origin of these cells from the blood.

The role of these tissue leukocytes in the myometrium and in the loose connective tissue of other organs is not yet known. Archer and Hirsch (1963) were able to observe phagocytic activity cinematographically in eosinophiles from the blood, but there is to date no indication of a similar significance of the tissue leukocytes. Bjersing and Borglin (1964) believe that the tissue eosinophiles in the myometrium are somehow related to growth, but it is not clear why the eosinophiles disappear under the influence of progesterone. We have the impression that the excess of tissue eosinophiles in the myometrium after oophorectomy and administration of estrogen can be defined more aptly as lack of progesterone. True growth of the uterus can take place only if both hormones are present. If progesterone is absent, the increase in synthesis of RNA characteristically produced by estrogen (Gorski and Mueller, 1963) could be important in causing the appearance of the granulocytes. Thus, there may perhaps be some relation also to the high content of hydrolytic enzymes in the eosinophilic granules (Archer and Hirsch, 1963).

IV. The Fine Structure of the Smooth Muscle Cell

A. *The Contractile Substance*

The fact that in the smooth muscle cells only one kind of filament can be observed with the electron microscope was at first difficult to understand, because biochemical investigations seemed to point to the identity of the contractile proteins of both smooth and striated muscles (Csapo, 1955). Further biochemical investigations, however, revealed several differences (Needham and Shoenberg, 1964). These studies led to the designation of the contractile proteins of the smooth muscle as tonoactomyosin (TAM). Laszt and Hamoir (1961) assume that it is "dissolved" in the sarcoplasm in the form of asymmetrical aggregates and that its solubility depends on the concentration of adenosinetriphosphate

FIG. 12. Section from a light muscle cell (proestrus). Filaments in parallel orientation occupy only a small area of the cytoplasm. In the cytoplasm without filaments a finely granular substance can be seen, in addition to increased numbers of free ribosomes, tubules, and granular and agranular reticulum. × 60,000.

(ATP). The increase of potassium ions in the muscle cell, which causes an increase in muscular tonus, is said to result in formation of a firmer network and the phenomenon of syneresis. Electron microscopic observations of isolated muscular strips (Gansler, 1961) seem to be in good agreement with this concept. The question remains open, of course, whether the electron microscopically visible filaments are actin or actomyosin. As the diameters of the filaments are the same as those of the I-filaments of skeletal muscle, Needham and Shoenberg

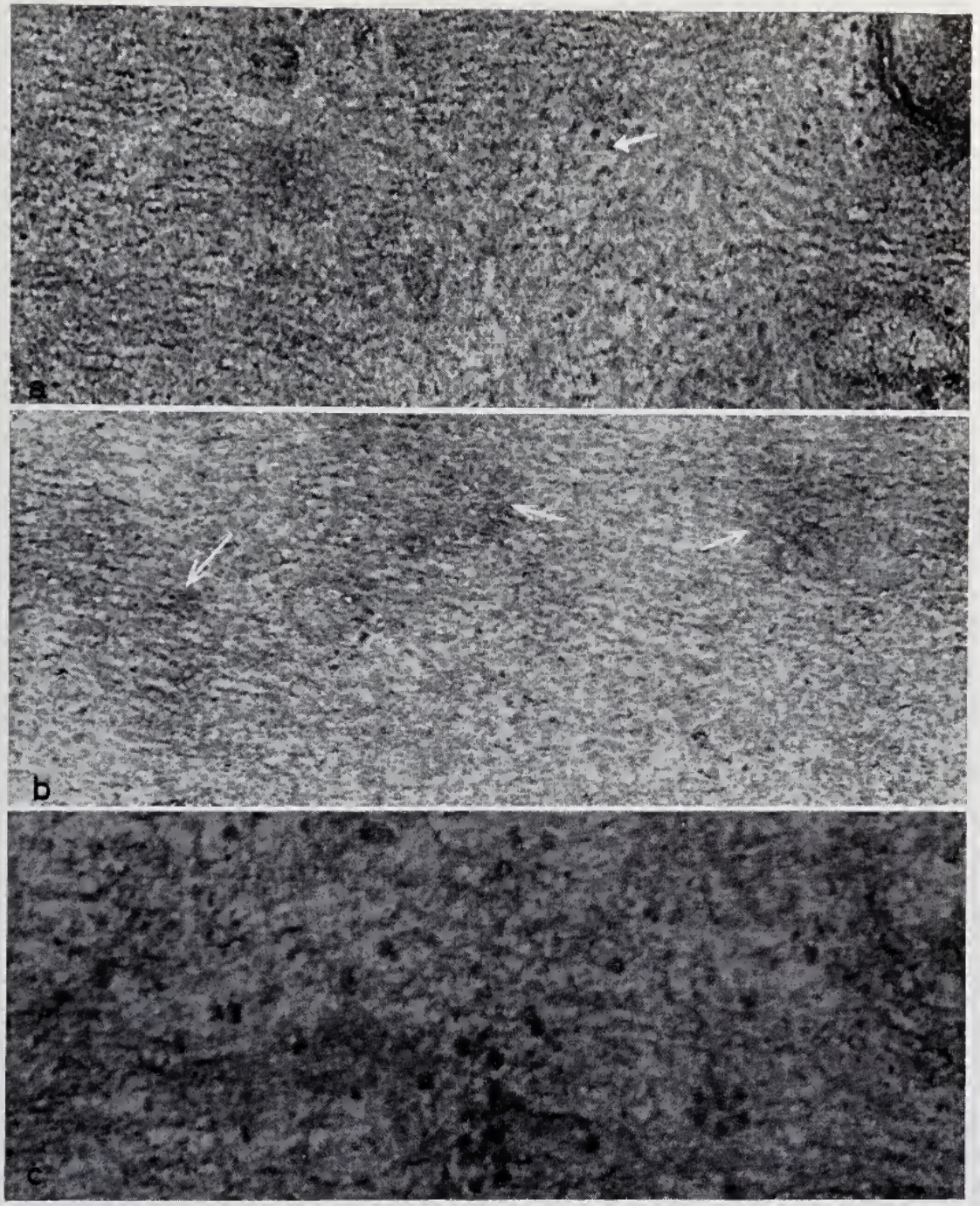

Fig. 13. Various filamentous arrangements in light and dark cells. (a) Almost completely oriented filaments merge, in the central area of the picture, into an unoriented network. A splitting up of filaments can occasionally be seen in this area (arrow). (b) Filamentous network that shows a certain preferential direction, and three denser areas (arrows). (c) Loose unoriented filaments from a lighter cell (proestrus). × 120,000.

(1964) assume that they are actin filaments. This idea is consistent with the fact that in slightly extended smooth muscle cells only actin could be observed by X-ray (Elliott, 1964). That observation, of course, does not preclude the presence of additional components, amorphous on X-ray examination. As to the influence of the ovarian hormones on the contractile proteins, Csapo has shown that an increase in contractile proteins in the castrate's uterus can be produced only by

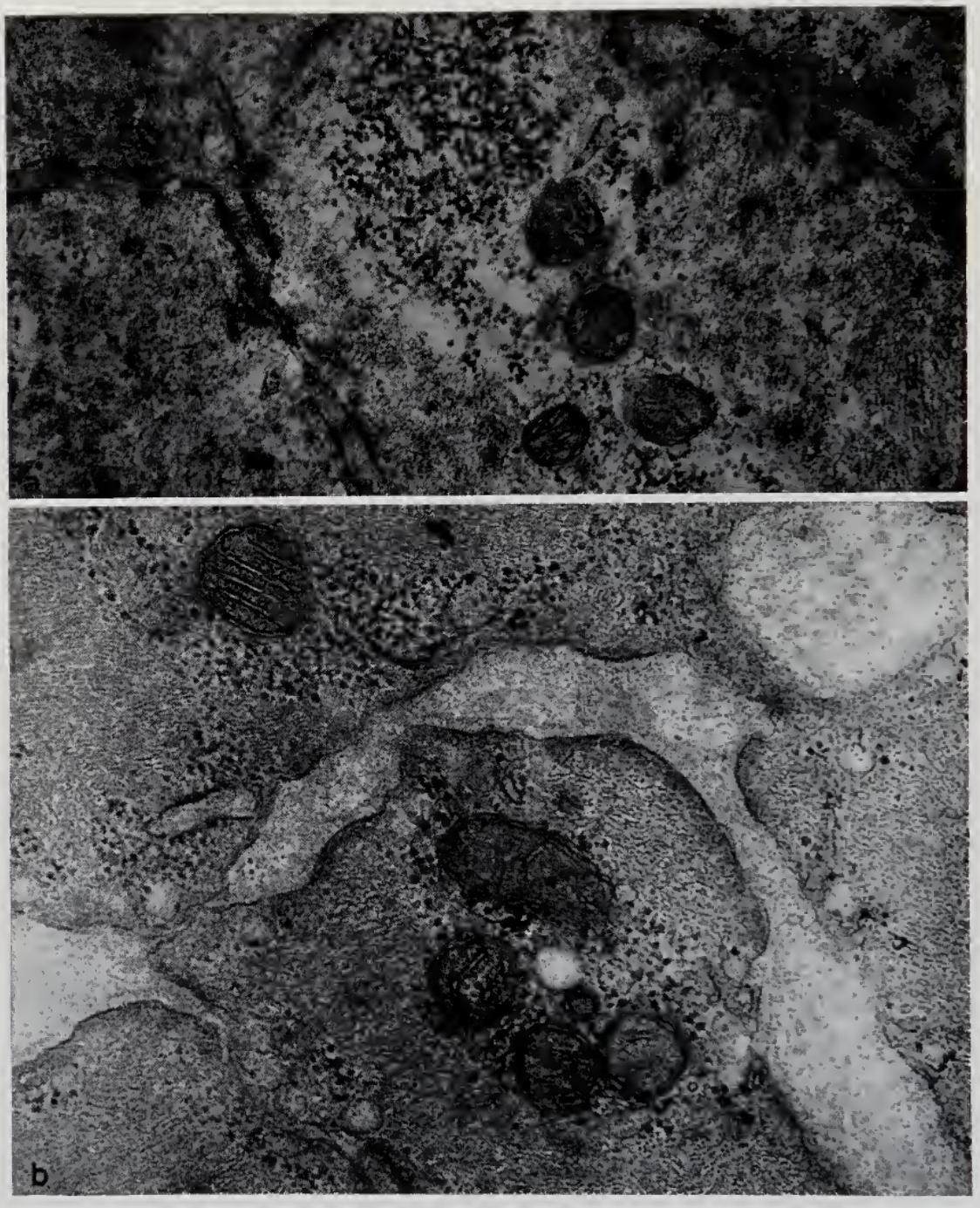

FIG. 14. Cross sections through muscle cells (a) before ovulation; (b) after ovulation. In (a) the distances between the cells are very small; the filaments are either in a uniformly loose arrangement or they form unhomogeneous areas of filaments. In the cell to the right an aggregate of particles of glycogen can be detected. In (b) the distance between the cells is greater; the filaments are tightly packed and particles of glycogen are finely distributed among the cells. × 30,000.

estrogen, whereas progesterone has no influence upon them. Lerner and Turkheimer (1965) describe an increase, after administration of estrogen, in the weight of the uterus. This increase can be suppressed by a protein-free diet. Papers by Wilson (1963), Gorski and Mueller (1963), and Gorski (1964) show that estrogen activates the synthesis of RNA in the uterine muscles. It may, therefore, be assumed that the estrogen exerts its effect primarily on the nuclear

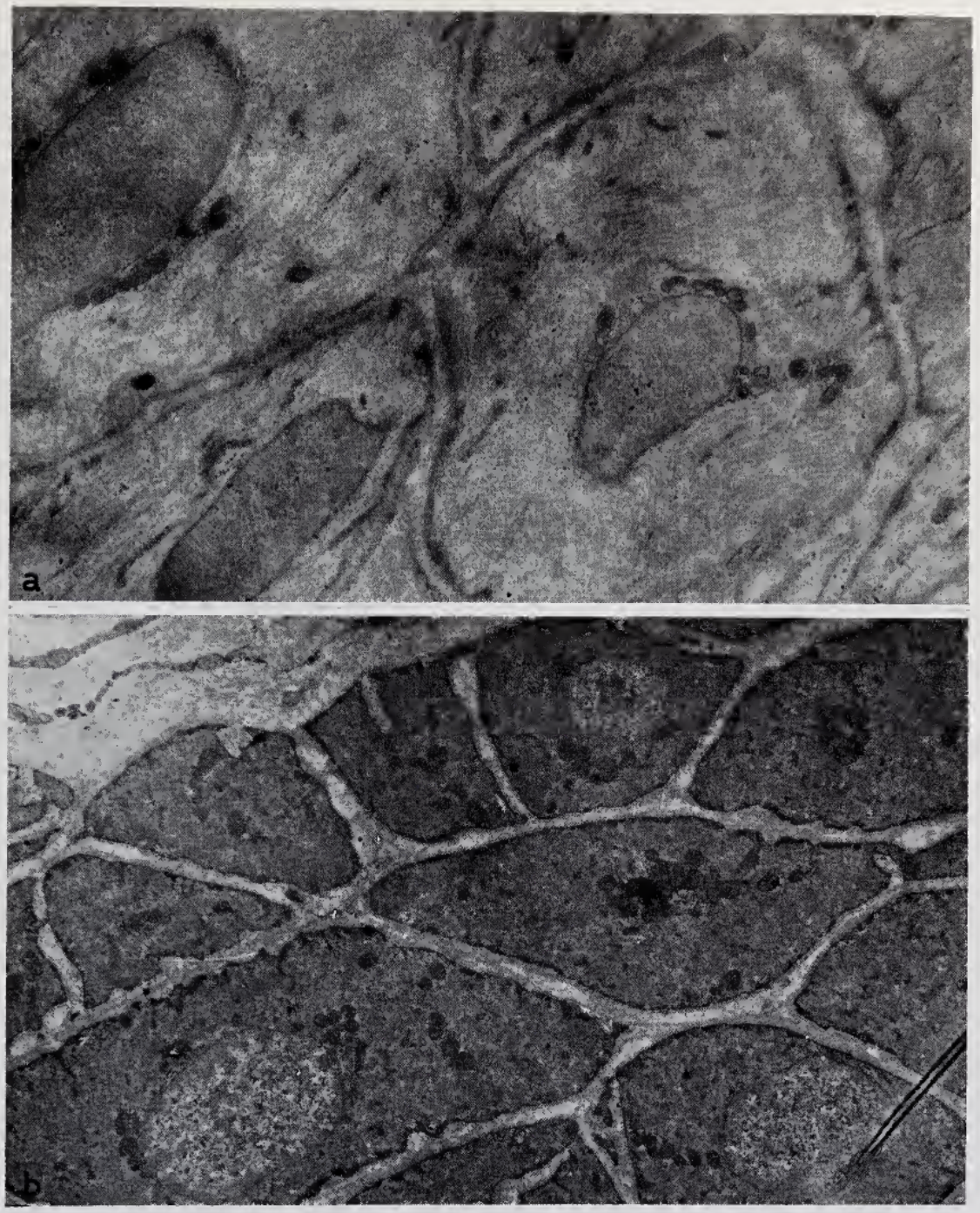

Fig. 15. (a) Relaxed muscle cells from the cerebral artery. (b) Contracted muscle cells from the middle cerebral artery. The relaxed cells are lighter and more voluminous; the nuclei of the relaxed cells are denser; those of the contracted muscle cells are lighter than the myoplasm. × 7500.

and cytoplasmic RNA, and only secondarily on the contractile proteins. The content of contractile proteins is said to double, approximately, during pregnancy (Csapo, 1955). (See Chapters 5 and 6 of this volume.)

Comparing the fine structure of the myoplasm under varying hormonal conditions, we notice that the muscle cells after oophorectomy contain many more filaments than do those stimulated by estrogen (Figs. 12 and 13c). On the other hand, after ovulation and during pregnancy they are quantitatively not

FIG. 16. Section from a spiny cell (proestrus). The cell surface exhibits ameboid projections with aggregates of small vesicles and channels. Unhomogeneous accumulations of a denser substance may be seen on the plasma membrane. The filaments form an unoriented network. × 60,000.

different from those after oophorectomy (Fig. 14). This observation seems inconsistent with the increase in uterine weight and muscular proteins described as an effect of estrogen. The explanation may be a higher rate of depolymerization of the filaments. It may, furthermore, be noticed that even in neighboring muscle cells the filaments are packed with varying degrees of density and that there is a correlation between the distances between filaments and the cell surfaces. If the filaments are packed tightly, the distances between the cells are greater and the

Fig. 17. Section from a contracted vascular muscle cell. Partly unoriented filaments appear indistinct because of the dense interfilamentous material. × 60,000.

surfaces are either smooth or ameboid. In a looser arrangement of the filaments the distances between the cells are smaller and the cell surfaces are smooth or interdigitated over a wide area (Figs. 3 and 5). This loss of volume may be discerned especially well in vessels (Fig. 15).

Two phenomena, therefore, appear to play a role in the electron microscopic demonstration of filaments: (1) a differential depolymerization of contractile proteins; and (2) a transfer of liquid between intracellular and extracellular spaces. The marked reduction of the filaments under the influence of

estrogen suggests a high degree of depolymerization of the contractile proteins. A second ultrastructural peculiarity concerns the arrangement of the filaments. In addition to filaments in approximately parallel orientation, there are bundles of filaments obliquely arranged, as well as totally unoriented filaments (Figs. 7b, 8, 16, and 17). Differently arranged areas are not infrequently seen adjacent to one another, even within a single cell. Filaments in the ameboid cellular processes, furthermore, are generally less well oriented than in the central areas of the cells. There are also, however, muscle cells with smooth surfaces and unoriented bundles of filaments (Fig. 17). The diameters of the filaments vary between 50 and 60 Å. For electron microscopic reasons, however, these small deviations cannot be considered real variations of thickness or interpreted as proof of the existence of different types of filaments. The differing positions of single filaments within the section can themselves produce differences of thickness; furthermore, the splitting of filaments (Fig. 13a), which may occasionally be observed with high resolution, cannot serve as proof of a combination of different contractile proteins. Even though we are as yet unable to say anything about the biochemistry of the filaments, we assume that both actin and myosin have a share in this structure, and that the reduction of filaments arises from further depolymerization of actin and myosin.

"Dense areas" are another characteristic of the contractile structure. They are arranged in the myometrium in pointlike or spindlelike shapes, parallel to the longitudinal axis of the cell; they occur in larger numbers in vessels, where they extend radially from the cellular surface into the myoplasm. With higher electron microscopic resolution it may be seen (Fig. 13b) that they consist of filaments between which a denser material is deposited. Our impression is that these dense areas are extremely labile and are eventually reduced during contraction. Tropomyosin is perhaps involved here; according to Elliott (1964) it should be interpreted as a special configuration of myosin.

In summarizing the electron microscopic findings, these factors should be considered for a scheme of contraction: (1) changes in the volume of the muscle cell; (2) differential polymerization of the contractile proteins; (3) oriented and unoriented arrangements of the filaments. A change in volume of the muscle cells has been described by Daniel (1963b) in cold-induced contraction. Burnstock, Holman, and Prosser (1963) observed that a muscle in an isotonic solution of sucrose releases liquid after addition of calcium, a phenomenon that cannot, according to these authors, be explained by osmosis alone, and that permits comparison with other agents capable of swelling. There also occurs after calcium uptake a reversal of swelling, together with the release of water by substances that originally were markedly swollen. The fact, too, that muscles in isotonic solutions lose weight during extension (Ernst, Tigyi, and Nagy, 1954), is explained by changes in the volume of the muscle cells. If we assume, furthermore, that the contractile proteins actin and myosin in the relaxed muscle cell or in the cell under influence of estrogen occur predominantly without orientation and without forming filaments, then the contractile proteins can no longer be detected electron microscopically in prepared sections. An interaction of these molecules, probably induced by calcium ions (Daniel, 1963a), could lead to the formation of fine polymers, which appear as filaments under the electron microscope. The

significance of the calcium ion in the interaction of the contractile proteins is suggested also in the previously mentioned paper by Burnstock, Holman, and Prosser (1963), who indicate that changes in volume and weight may be attained through the action of calcium.

There is no answer yet to the question whether the further dehydration of the myoplasm, resulting in a decrease in volume of the muscular cells, may be explained by an increasing interaction between filaments. As to the *arrangement* of the filaments, our observations suggest that under isometric conditions, too (as in vascular muscles), parallel orientation is of secondary importance (Fig. 17), whereas three-dimensional interaction of filaments with extrusion of liquid is the dominant factor, with contraction of the colloids (syneresis). In addition, the surfaces of the contracted muscle cells indicate formation of such contracted gels. The influence of the ovarian hormones can be defined thus: only estrogen causes a marked depolymerization of the contractile proteins, possibly associated with the greater relaxation. This effect probably stems from the altered permeability of the cell membranes. Under influence of progesterone, on the other hand, the contractile proteins are aggregated into filaments in relaxed cells, also. This observation is in good agreement with the higher resting tonus associated with progesterone. Other physiological observations suggest that reduction in the number of filaments under the influence of estrogen represents a depolymerization of the contractile proteins. Csapo (1955) observed that muscular strips from estrogen-stimulated myometria first became elongated and only upon attainment of a certain length exhibit large, slow, spontaneous contractions. On the other hand, muscular strips from progesterone-dominated myometria become shorter until a final value is reached. Then only do small, frequent, spontaneous contractions begin. This observation, apparently overlooked in earlier papers describing variations in contractility related to the ovarian hormones, is especially important, because the greater plasticity, described as an effect of estrogen, is consistent with the concept of protein solutions of a low degree of polymerization. Conversely, contraction under the influence of progesterone requires a higher degree of viscosity or polymerization of the proteins. This idea suggests also a basis for the differing amplitude of single contractions. If the viscosity differs only slightly between contraction and relaxation (progesterone dominance), the single contraction has a low amplitude. If, however, changes in plasticity and viscosity occur (effects of estrogen), then greater amplitudes of single contractions result. These differences are magnified if the single cells are, as mentioned at the outset, arranged in larger units, which probably influence the amplitude and especially the duration of the contraction.

B. *Surface Membrane Complex*

In the discussion of the muscle bundles, the various morphologic forms are correlated with differences in the surface membrane complex. A parallel orientation of neighboring membranes is shown in the light cells with smooth as well as with interdigitated cellular surfaces (Fig. 18). The cell membranes in these groups appear distinctly only with high resolution, because of the small difference between the densities of myoplasm and extracellular space. The distance between

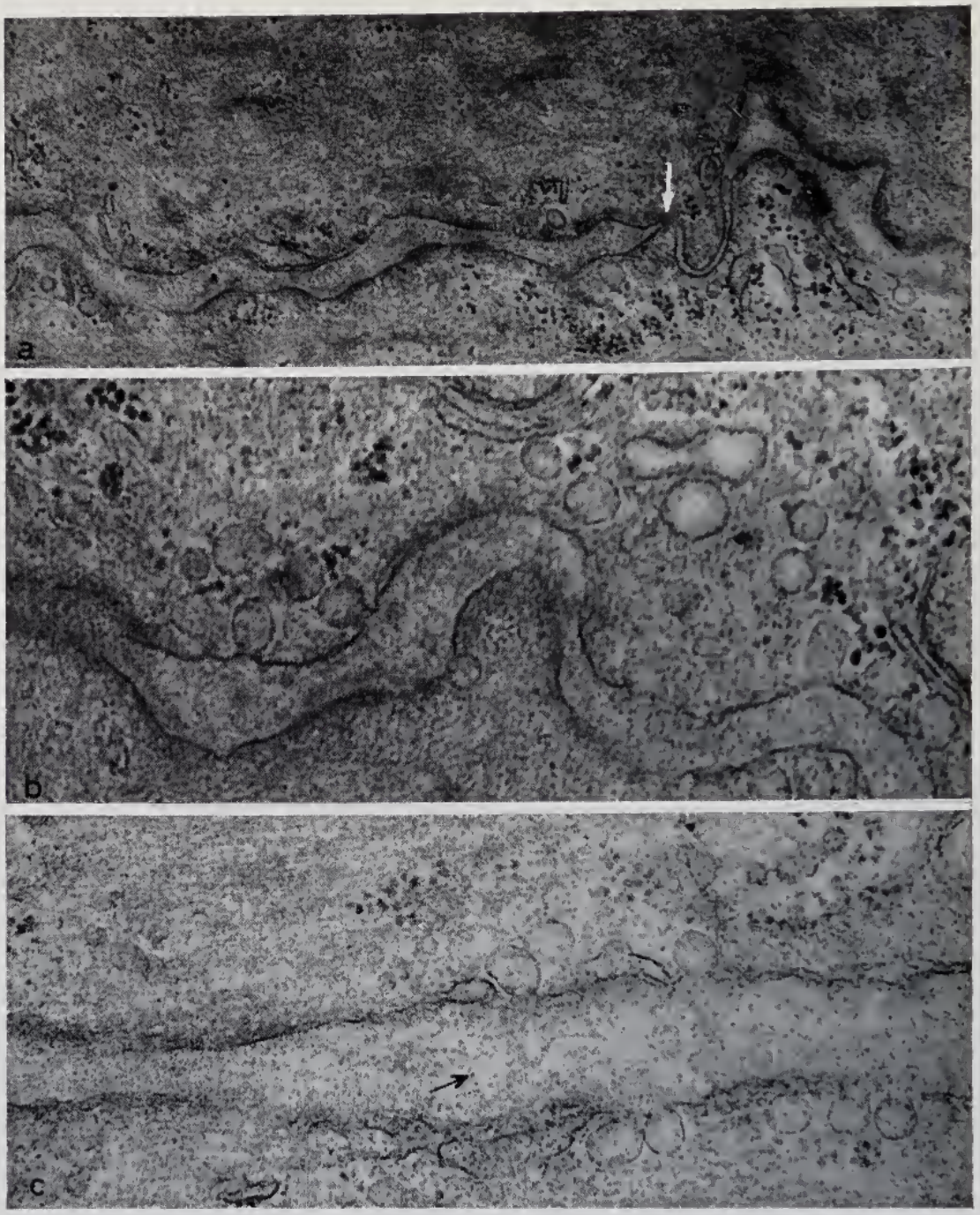

FIG. 18. Surface membranes of light cells. (a) Wavy membranes in parallel orientation; near arrow, apparent interruption of the extracellular space. Actually, the surfaces of the two cells are interdigitated here, and both membranes are clearly visible. (b) More markedly undulating membranes in parallel orientation and sporadic pinocytosis. In the left part of the picture the surfaces of both cells merge without sharp outlines. Amorphous ground substance in the extracellular space. (c) Smooth surfaces of light cells with pinocytosis. The point indicated by the arrow suggests release of material into the extracellular space. × 60,000.

these cell membranes in parallel arrangement varies between 300 and 2000 Å. Occasionally one gains the impression of intercellular bridges (Fig. 19a,b), but with good resolution it can be seen that in those areas the distance between membranes has merely become smaller (Fig. 18a) and the membranes are actually preserved. It seems to us that the membranes of these cellular tissues

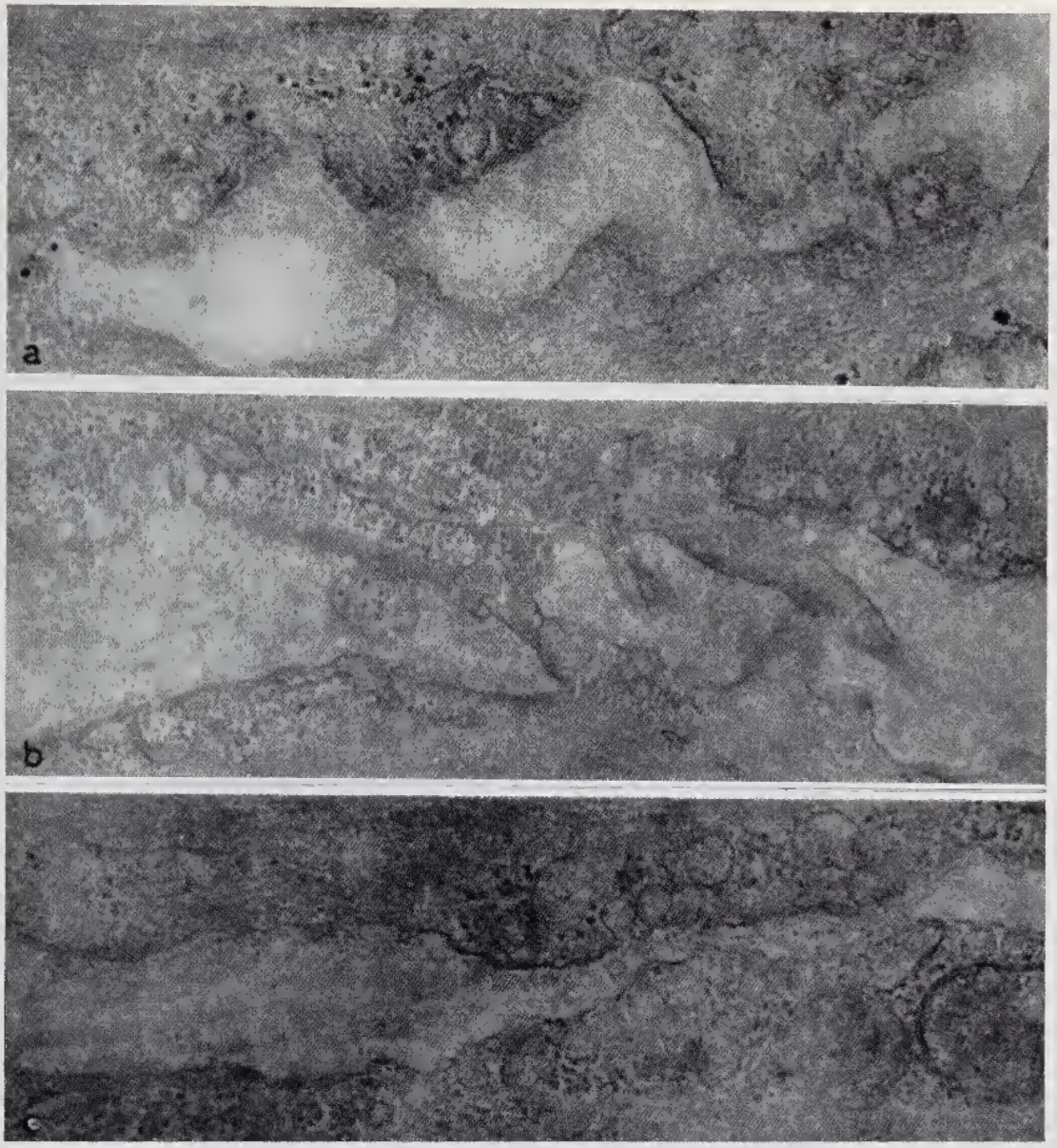

Fig. 19. Surface complex of dense cells. (a) Spiny cells with apparent intercellular bridges. In the extracellular space, only sporadic collagenous fibrils. × 60,000. (b) Spiny cells at greater distances from one another, and collagenous fibrils. × 30,000. (c) Smooth surface of discrete cells with many small membranous vesicles and distinct collagenous fibrils. × 30,000.

exhibit micropinocytosis much more rarely than the dark cells, which are farther away from one another. It can, furthermore, be easily seen that the extracellular space of these tightly packed cells (with smooth or interdigitated surfaces) only rarely shows collagenous fibrils, but contains a predominantly amorphous ground substance. The occurrence of distinct collagenous fibrils increasing with the distance between the cell membranes suggests differing degrees of aggregation of the collagen, possibly caused by some changes in the extracellular content of electrolytes (Nemetschek and Gansler, 1961). It is not yet clear whether there is in addition an enzymatic decomposition of collagen enhanced by disaggregation in the presence of cathepsin (Kao and co-workers, 1964). According to Woessner and Brewer (1963), cathepsin appears also in normal myometria

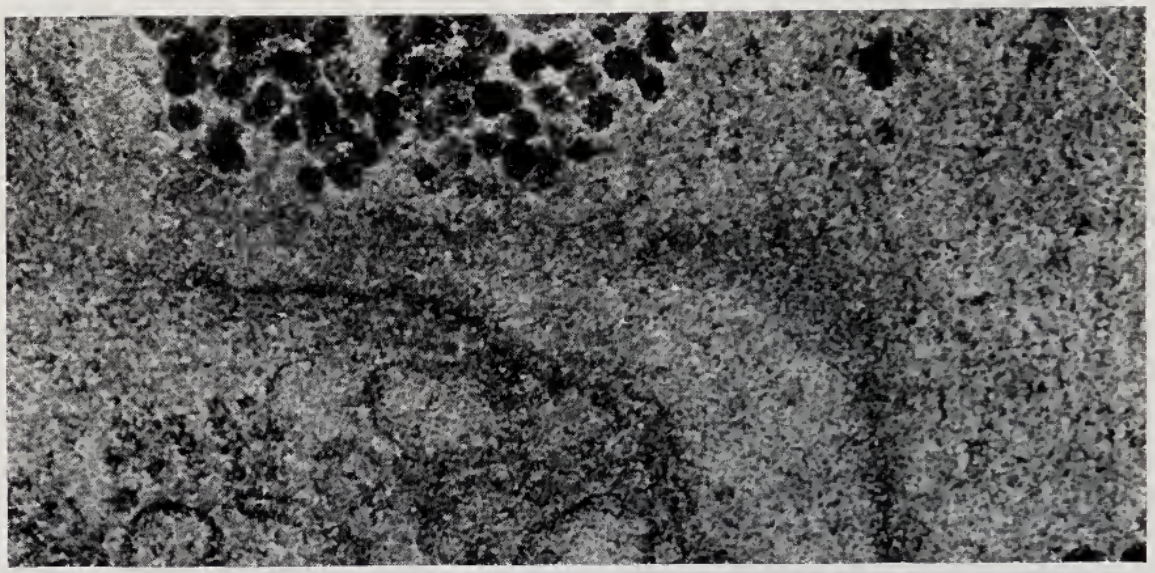

FIG. 20 (Above). Surface membrane diffusely widened, probably a tangential section. Particle of glycogen at the cell surface. × 120,000.

FIG. 21 (Below). Spiny cells interdigitated by means of pseudopodia with apparent areas of contact (arrows). × 60,000.

and is increased during involution. It should be stated here that the cellular membranes occasionally exhibit unhomogeneous areas of higher density (Fig. 16), which are seen especially well in cross sections, but an interpretation of this observation is not yet possible. Concentrating upon the surface membranes of those muscle bundles showing the typical effect of progesterone (Fig. 19), we see that the membrane complexes appear more clearly because of the greater differences in density between the myoplasm and the extracellular space. With lower resolution, in this case too, we gain the impression that there are intercellular bridges, most frequently among the spiny cells. In some of these cells no

membranous structure can be recognized even with high resolution (Fig. 19a,b). It is doubtful, however, whether that finding can be taken as an indication of a true syncytium, because the more or less distinct picture of a membrane depends essentially on its vertical or oblique orientation with respect to the electron beam. Thus, we see in Fig. 20 diffuse surface areas that are probably explained by the tangential sectioning. We believe, however, that the question of the existence of these intercellular bridges or of nexal communications (Fig. 21) is of only secondary importance for excitation and mechanical and electrical propagation. The membranes of the muscle cells, under the influence of progesterone, exhibit marked micropinocytosis. The extracellular space of the tissues in this area consists mainly of distinct collagenous fibrils. Two phenomena must be considered in the attempt at a functional interpretation of the surface membranes. First, there is a change in shape of the cellular surfaces, occurring apparently as a consequence of the increase in viscosity of the myoplasm during contraction. In this change of shape one has to consider a correlation between the increase in viscosity and the contraction, or excitation, as described previously by Winton (1926) and Gasser and Hill (1924). On the contrary, one might argue that the change in shape of the cellular surfaces is caused by a passive obstruction. An active role, however, is indicated by the fact that during delivery, the phase of maximal function, there occur either only interdigitated cells or only spiny cells. Artifacts of fixation appear unlikely with the introduction of glutaraldehyde (Page, 1964). The second phenomenon concerns the differences of the surface complex typical of the effects of estrogen and of progesterone. Their functional significance must be considered mainly in connection with the different mechanical and electrical propagation and conduction. Thus, the widely interdigitated cellular surfaces must be related to the quick and complete propagation under the influence of estrogen, whereas the spiny cells are characteristic of the effect of inhibition of propagation typical of progesterone. It follows that at the onset of contraction, there is a functional syncytium only under the influence of estrogen. We make the assumption that the basis for mechanical and electrical conduction must not be sought only in fusions of the surface membranes (Barr, 1963). The differences of the myoplasm and the extracellular space seem to be extremely significant in determining the properties of the membranes. In addition, the intracellular membranous systems (endoplasmic reticulum and pinocytotic vesicles) should also be considered in this connection (Huxley, 1964). In an attempt at an interpretation of physiologic states, the fact that myoplasm, membranes, and extracellular space form a functional unit should always be considered.

C. *The Endoplasmic Cytomembranes*

Granular and agranular reticulum and invaginations of the surface membrane (micropinocytosis) form a complex membranous system in the smooth muscle cell. These small intracellular channels and vesicles are important in the transport of macromolecules, in the conduction of impulses, and, finally, in secretory functions of the cells.

Invaginations of the surface membranes can be observed in most muscle

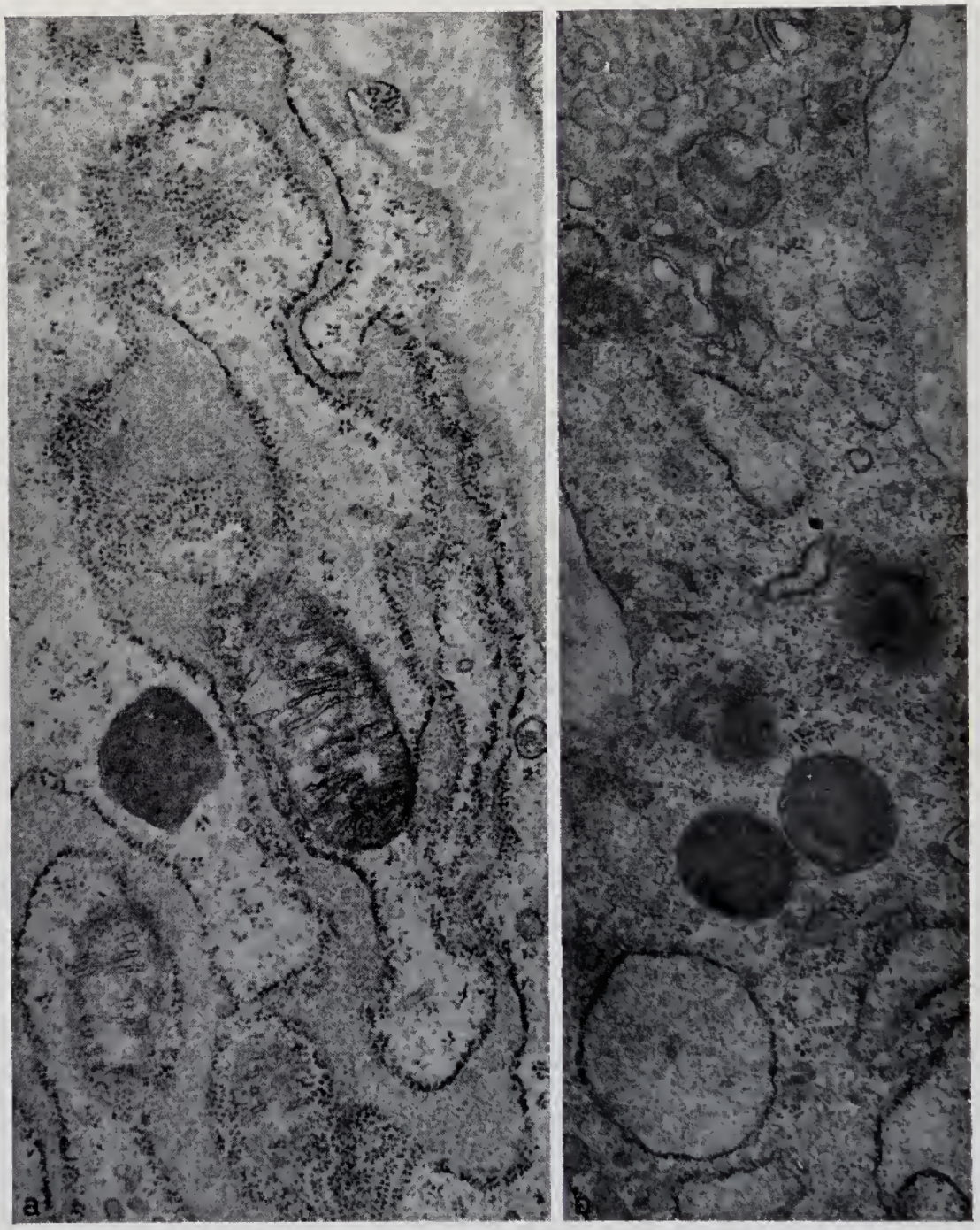

FIG. 22. (a) Histiocyte in which endoplasmic reticulum predominates. The small channels are dilated and denser than the ground substance. Typical arrangement of polysomes in rows. (b) Histiocyte in which the agranular reticulum and proteinaceous granules predominate. × 30,000.

cells, but the prevalent impression is that they are more common during relaxation than contraction. Micropinocytosis in cells with smooth surfaces appears mostly as small vesicles in rows, whereas it takes the form of groups of small vesicles or small channels in the pseudopodia of spiny cells. No sharp differentiation is possible between these membranous invaginations and the endoplasmic reticulum (Figs. 2 and 15b).

Roth and Porter (1962) were able to show that invaginations of membranes

in oocytes incorporate proteins from the extracellular space. In the muscle cell, too, similar dense accumulations of invaginated membranes can be observed (Fig. 8). They seem to play a role, furthermore, in the release of substances into the extracellular space, as shown in Fig. 18. Finally, a release of liquid into the extracellular space by way of membranous systems of this kind might also be considered. This idea is suggested by vacuoles of various sizes within those cells that show the typical early effeet of progesterone.

The granular reticulum and the free ribosomes are greatly increased under the influence of estrogen in muscle cells as well as in cells of the connective tissue (Fig. 22) and in uterine epithelia. At the same time, there not infrequently appears a dilatation of the granular reticulum, the contents of which are often more compact than the surrounding ground substance (Figs. 2 and 22a). These observations are in close agreement with the finding of increased synthesis of RNA under the influence of estrogen, as described by Gorski and Mueller (1963). As the agranular reticulum is clearly increased at the expense of the granular reticulum during pregnancy (Figs. 4 and 22b), it has been suggested that estrogen enhances the nonspecific synthesis of RNA, whereas synthesis of contractile proteins takes place in the agranular membranous systems only as an effect of progesterone. Siekevitz (1959) assumes, on the basis of biochemical differences between free and membrane-bound ribosomes, that, first, a marked increase in free ribosomes meets the need for RNA during increased cellular activity (induced, in the case of the muscle cells, by estrogen), and that only later are the "export proteins" formed on the membrane-bound ribosomes. Both membranous systems can be found in perinuclear as well as peripheral areas of the cell. According to Porter, Ledbetter, and Badenhausen (1964), microtubules, which up to the present have not been described in muscle cells, are the expression of cytoplasmic motion (Fig. 12). We have not yet been able to observe quantitative differences among these tubules in the various cellular forms.

D. *Glycogen*

Particles of glycogen with a diameter of 250 Å and a high density (Fig. 23) exhibit great differences in the myometrium under the influence of the ovarian hormones, quantitatively as well as in their localization. Before ovulation, glycogen-rich areas are found only in the external longitudinal muscles, but the circular muscles are free of them (Fig. 14). After ovulation, particles of glycogen are diffusely dispersed within the muscle cells of the longitudinal and circular layers of the myometrium. The greatest accumulation of glycogen occurs in the periplacental muscle cells (Fig. 6b). It is interesting that during delivery glycogen is amply dispersed throughout the cytoplasm of the interdigitated cells, whereas it is almost totally absent in the dark, spiny cells of the same preparation. There may possibly be a correlation between this observation and the reduction of visible glycogen under the influence of estrogen, on the one hand, and increased glycolysis, on the other (Walaas, Walaas, and Löken, 1952). According to Csapo and Gergely (1950), glycolysis is of particular significance in the myometrium in providing a transitory supply of energy. The accumulation of glycogen during pregnancy is of interest in this connection.

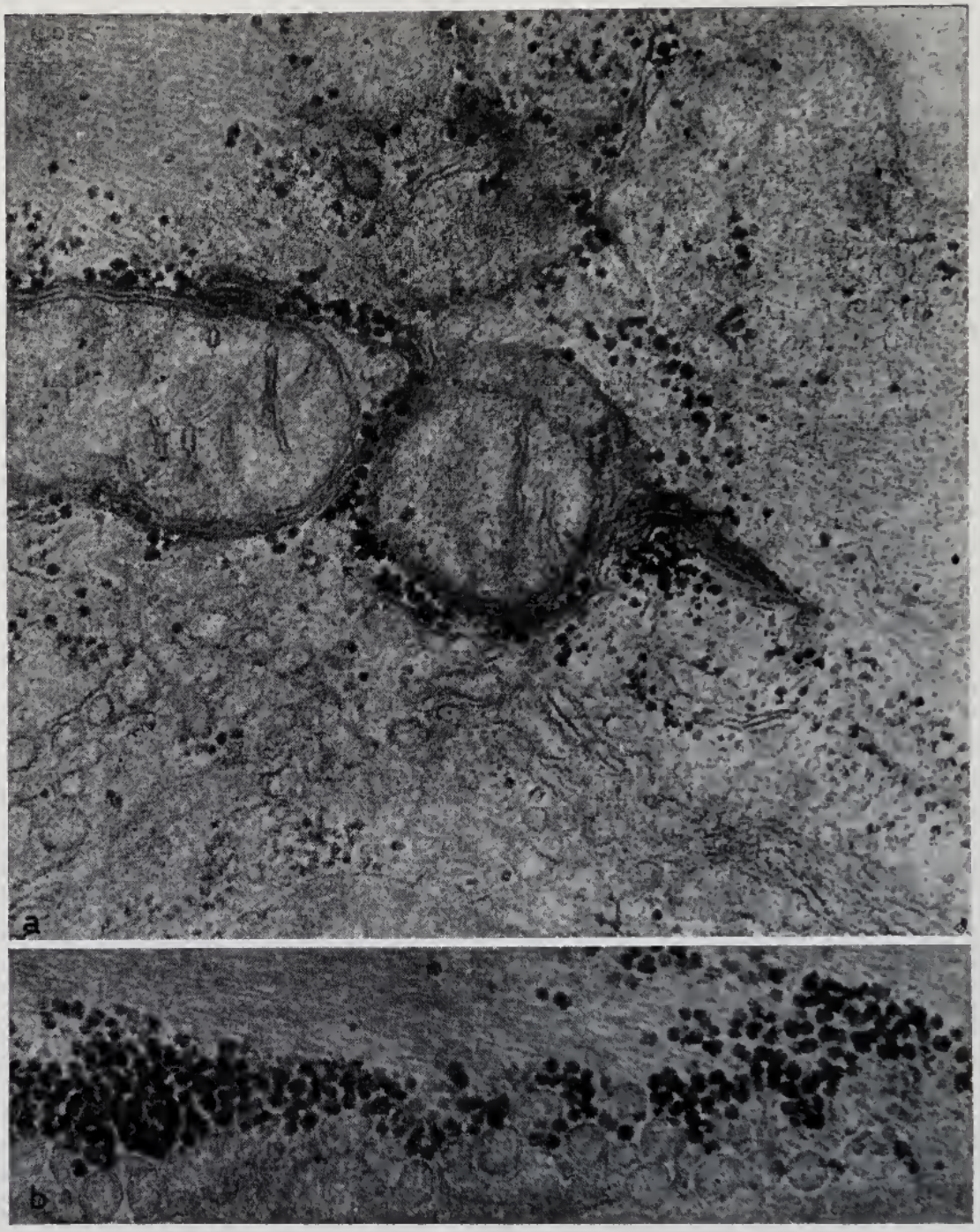

FIG. 23. Glycogen from muscle cells of gravid uteri. (a) Golgi complex free of glycogen, but massive deposits on the surface of mitochondria. (b) Accumulations of glycogen at the surface and, in part, apparently in small vesicles. × 60,000.

V. *Conclusion*

Electron microscopy of the myometrium is still in its earliest stages. The observations made to date, however, have shown that ultrastructural analysis is of aid in achieving a better understanding of myometrial function. With the development of current electron microscopic techniques, which make possible

almost instantaneous snapshots of tissue *in situ*, a closer correlation of biochemical, physiological, and ultrastructural observations becomes desirable.

In addition to the morphologic changes in the endometrium, which have been known for a long time, it is now possible to describe also the corresponding changes in the myometrium during the menstrual cycle and pregnancy. The electron microscopy of myometrium has been related to the varying states of uterine contractility, equally well known for a long time. The contractile proteins form less well-oriented structures in the smooth muscular cell than in the striated muscular cell. It is this very fact to which the greater range of variability of the types of contraction might be attributed.

Relatively little is known about the interaction of hormones and contractile proteins. As the morphologic observations of the myometrium do not differ basically from those of other hollow organs, however, it may be assumed that the hormone-specific action consists in a change of tonus from the resting state to spontaneous activity and back again. The changes are induced in the same way as in other hollow organs by means of other drugs. It is not known, however, why the described hormonal effects remain limited largely to the uterine muscles. Perhaps local increases in concentration of the ovarian hormones are involved, as described in the muscle cells of gravid myometria near the placenta (Barnes, Kumar, and Goodno, 1962). We lack knowledge also of why the atrophy that begins after oophorectomy concerns only the uterine muscles, and why it can be reversed only by estrogen.

The mechanism of contraction, too, is far from clear. It is our opinion, however, that the assumption, as presented here, of a sol–gel transformation of the contractile proteins may serve as a working hypothesis for further investigations. It offers the advantage of considering not only the filaments but all other structural phenomena, too. We believe this correlative approach to be particularly important because the function of biological systems must always be interpreted as a complex interaction of all cellular organelles, single cells and organized cell groups. One should, therefore, not succumb to the temptation to study just filaments, or membranes, or secretory granules. Such a restricted point of view renders the understanding of any function more difficult. The inherent disadvantage of our method is that too often the words "perhaps" and "probably" must accompany the interpretation of the observations.

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