Microtubule and plasmalemmal reorganization: acute response to estrogen

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SZEGO, CLARA M., BIRGITTA M. SJÖSTRAND, BARBARA J. SEELER, JUNE W. BAUMER, AND FRITIOF S. SJÖSTRAND. *Microtubule and plasmalemmal reorganization: acute response to estrogen.* Am. J. Physiol. 254 (Endocrinol. Metab. 17): E775–E785, 1988.—The acute ultrastructural effects of estrogen in endometrial epithelial cells were investigated by transmission electron microscopy (TEM), with special reference to the microtubule (MT) apparatus and the luminal surface. Ovariectomized rats anesthetized with pentobarbitol sodium were injected intravenously with estradiol-17β ($E_2\beta$), 0.5 μg/100 g body wt. At intervals from ~30 s to 30 min thereafter, 70–80 nm cross sections of a uterine horn were prepared for TEM. In placebo controls, cytoplasmic MT were conspicuous in length and number, whereas only a minimal population of short microvilli (MV) was evident. In contrast, the specimens subjected to $E_2\beta$ for only 35 s showed a significant decrease in MT number and length, with virtually complete depletion of these organelles by ~80 s. Concomitantly, the luminal MV exhibited striking enhancement in length and density. Thereafter, these rapid and reciprocal alterations of MT and MV underwent inversion. Thus MT structures began to reappear within 2 min, increasing progressively so that by 30 min their numbers were again substantial, although lengths remained diminished. During the same interval, the initial surge of luminal MV gradually subsided, to near-control appearance by 30 min. These coordinate, reciprocal, and biphasic responses are consistent with biochemical evidences of abrupt membrane perturbation associated with interception of estrogen at its cellular targets. The resultant modification of the intracellular environment may contribute to limited reorganization of cellular architecture and propagation of the hormonal signal.

rat; uterine epithelium; microtubule dynamics; surface membrane

SUBSTANTIAL EVIDENCE (reviewed in Refs. 13, 45, and 46) has revealed that estrogen elicits, within seconds, multiple signals attributable to surface perturbation of its cellular targets. Such signals include striking alterations in surface morphology of rat uterus, with significant increases in numbers of microvilli (MV) by 30 s, as detected by scanning electron microscopy (33). Within 1 min, MV density was further increased, with frequent clustering; the central cilium, so prominent in the control preparation, was no longer visible. These correlates, which were confined to physiologically active estrogens, were biphasic, with regression of MV density, length, and clustering and reappearance of the central cilium in many cells after 15 to 30 min, followed by recrudescence of the acute changes in surface activity at 1 h. These observations on early morphological effects of the active hormone (33) have recently been confirmed in cultured breast cancer cells (32).

Because of the likelihood that these and additional surface indicators of acute hormone action were associated with concomitant structural alterations of the plasmalemmal and cytoplasmic compartments (40, 45, 46), the present work was undertaken to examine by transmission electron microscopy (TEM) the influence of estrogen on the ultrastructural state of the target cell in situ, with special reference to the microtubular (MT) apparatus and the luminal surface. The data reveal, for the first time, that the cytoskeletal structures undergo striking and biphasic alterations in number and length that are correlated temporally with reciprocal changes in MV activity. Taken together with previous data on abrupt estrogen-induced activation of plasmalemmal adenylyl cyclase (44) as well as redistribution of surface membrane components (30), the present observations strongly indicate that extensive reorganization of cellular architecture is an early correlate of estrogen recognition by its target cell.

MATERIALS AND METHODS

Experimental procedures. With exceptions to be specified, the protocols were generally as outlined previously (33). Briefly, Sprague-Dawley rats were ovariectomized at 5–6 wk of age and maintained for 3 wk in the low-steroid environment, described elsewhere (40), which has been found to be critical to the success of these and other experiments conducted with physiological concentrations of active estrogens.

On the day of experiment, the animals were lightly anesthetized with subcutaneous sodium pentobarbital (Nembutal; 5 mg/100 g body wt). Fifteen minutes later, they were injected by saphenous vein with estradiol-17β ($E_2\beta$), freshly prepared in Dulbecco's phosphate-buffered saline (PBS; Gibco Laboratories, Grand Island, NY) from standardized alcoholic stock solution, as previously described (33), generally in a dose of 0.5 μg/0.25 ml per 100 g body wt. Control animals, likewise ovariectomized, received an equivalent volume of vehicle alone. After selected intervals, the right uterine horn was sectioned ~2 mm from the cephalic end to render it patent for

flushing with fixative in situ. It had earlier been established that the data obtained with either uterine horn were indistinguishable. The organ was immediately flushed with ~0.4 ml of a 2% solution of EM grade gluteraldehyde (Polysciences, Warrington, PA) in Tyrode's buffer, injected into the lumen with a 27-gauge needle that was inserted through the horn just above its junction with the corpus. The uterine horn was severed near the corpus, trimmed rapidly of mesometrial fat, and then, mounted on dental wax, immersed in a vial of 2% gluteraldehyde in Tyrode's buffer for 2 h at room temperature. Thereafter, specimens were postfixed in 1% OsO_4 in Tyrode's buffer for 2 h, dehydrated in the usual ethanol series, and Spurr-embedded. Sectioning systematically transverse to the long axis was carried out with a Sorvall MT 6000 apparatus. Thin sections (70–80 nm) were collected on Formvar-coated copper grids and stained successively with uranyl acetate and lead citrate. The specimens were examined in a JEOL JEM-100CX electron microscope operated at 80 kV. Micrographs were taken at random at magnifications ranging from 4.8 to 29 K. Photographic enlargement was generally ×3 or ×4.5 and very occasionally ×6.

For each time point, between two and eight samples were processed, with the exception of the control series, of which 15 were prepared over a range of time intervals. Placebo control and estrogen-treated samples within the same experiment were processed together through all stages of section preparation and staining. Primary emphasis was placed on observations at the acute time points, 2 min and earlier. It is to be noted that even a 30-s interval represents at least five circulation times in the rat (9), while the uptake of the hormone is virtually instantaneous (26).

Dimensions of Microtubules

Diameters. Multiple measurements of inner and outer diameters of longitudinal profiles of MT were made with a PEAK scale lupe ×7, averaged, corrected for magnification, and subjected to statistical analysis of variability. The data obtained by two observers independently varied by only ~2%. Because the MTs were most prominent in the preparations from ovariectomized animals without estrogen exposure, inner and outer diameters of these organelles were established from analysis of TEMs from placebo controls alone.

Lengths of microtubules. In a series of control samples as well as those treated with estrogen for a range of time points, all the longitudinal MT profiles appearing in randomly selected photomicrographic prints at a final magnification of ×87,000 were traced with a fine pen onto transparent celluloid sheets. These were then scanned blind, each on two separate occasions, on the digitizing tablet of a 4081 Tektronix Graphic System Analyzer. The resultant data were transferred to an IBM 3090 Main Frame Computer, transformed from arbitrary units to real values by comparison with a series of direct measurements of corresponding elements on representative TEM plates, and corrected for magnification. Relatively fewer cross-sectional profiles were seen.

The data were then subjected to statistical analysis for the maximum, minimum, and mean lengths of longitudinally oriented MT per plate. Also determined were the summated lengths per plate. The control values for these parameters did not vary significantly among the several observation time points. Comparison was made by Student's *t* test between a series of estrogen-treated specimens obtained between 35 and 120 s of exposure to hormone and a corresponding set of controls. Foreshortening errors in similar analyses are generally negligible (see Ref. 24). Although some MT are probably missed when tracings are made from the randomly selected micrographs, this minor source of error could not have altered appreciably the highly significant differences observed between experimental and control groups.

RESULTS

Dimensions of Microtubules in Luminal Endometrial Cells

Replicate measurements of outer and inner diameters of longitudinal MT profiles carried out on uterine TEM plates from a series of ovariectomized rats yielded a mean outside diameter of 21.0 ± 0.1 nm. This value is comparable with that of 25 nm, reported as an overall average in a variety of cells in situ (12, 38). The inside diameter ranged from 14.2 to 15.8 nm, compared with the literature mean of 15 nm, thus indicating wall thickness of 2.6 to 3.4 nm. MT lengths were variable and were markedly influenced by experimental conditions, as described below. However, basal values in the control preparations were within the normal limits in the literature (op. cit.).

Acute Effects of $E_2\beta$ on Microtubules

On even preliminary inspection of specimens from ovariectomized animals treated with the physiological level of 0.5 μg/100 g body wt of $E_2\beta$ for periods ranging from 35 to 80 s, it was immediately evident that the numbers of longitudinal as well as occasional cross-sectional MT profiles in representative micrographs were sharply diminished (Figs. 1–3). Figure 1, *A* and *B* depict representative placebo control specimens. The length of a single MT in each of these is in sharp contrast to that of the preparation treated in vivo for only 35 s (Fig. 2*A*), in which much shorter cytoplasmic MTs appear intact. In Figure 2*B*, which is a representative micrograph at 45 s of exposure to estrogen, there remains a single longitudinal MT profile of modest length. It should be noted also that the surface activity, as reflected in the occurrence of copious microvillar elements of considerable length within 35 to 45 s (Fig. 2, *A* and *B*), is in striking contrast to the quiescent plasmalemma typical of the controls (see Fig. 1*A*). By 80 s of estrogen action in vivo, the preparations were virtually devoid of MT (Fig. 3).

The lengths of MT profiles seen in TEMs from both control and estrogen-treated groups varied considerably. However, computer analysis of the data confirmed the fact that, whereas short lengths of MT occurred in both groups, the samples exposed to $E_2\beta$ in situ, even for as long as 30 min, were totally devoid of the lengths of 1 μm or greater that were characteristic of many of the placebo controls (not illustrated). Moreover, by at least

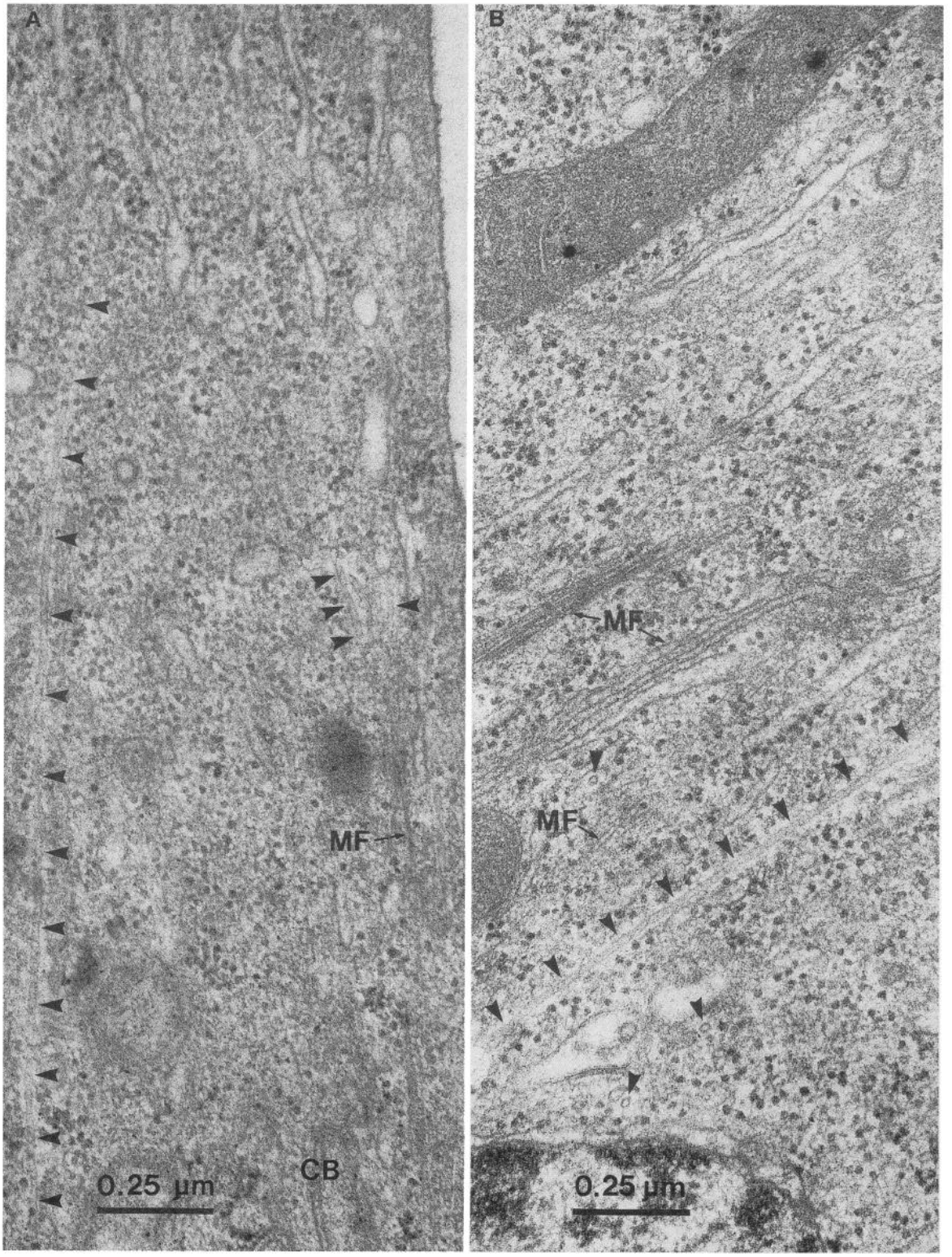

FIG. 1. Thin-section transmission electron micrographs (TEMs) of 2 representative uterine epithelial cells of individual, ovariectomized control rats injected intravenously with placebo vehicle. Serially disposed arrowheads identify characteristically long MT sectioned in profile in both *A* and *B*. Extensive subcortical array of MT in *A* is unlabeled. Also to be noted are quiescent luminal surface with limited MV activity in *A*, prominent array of microfilaments (MF), especially in *B*, and MT in cross section in latter (single arrowheads). CB, cell boundary. Other abbreviations are defined in text.

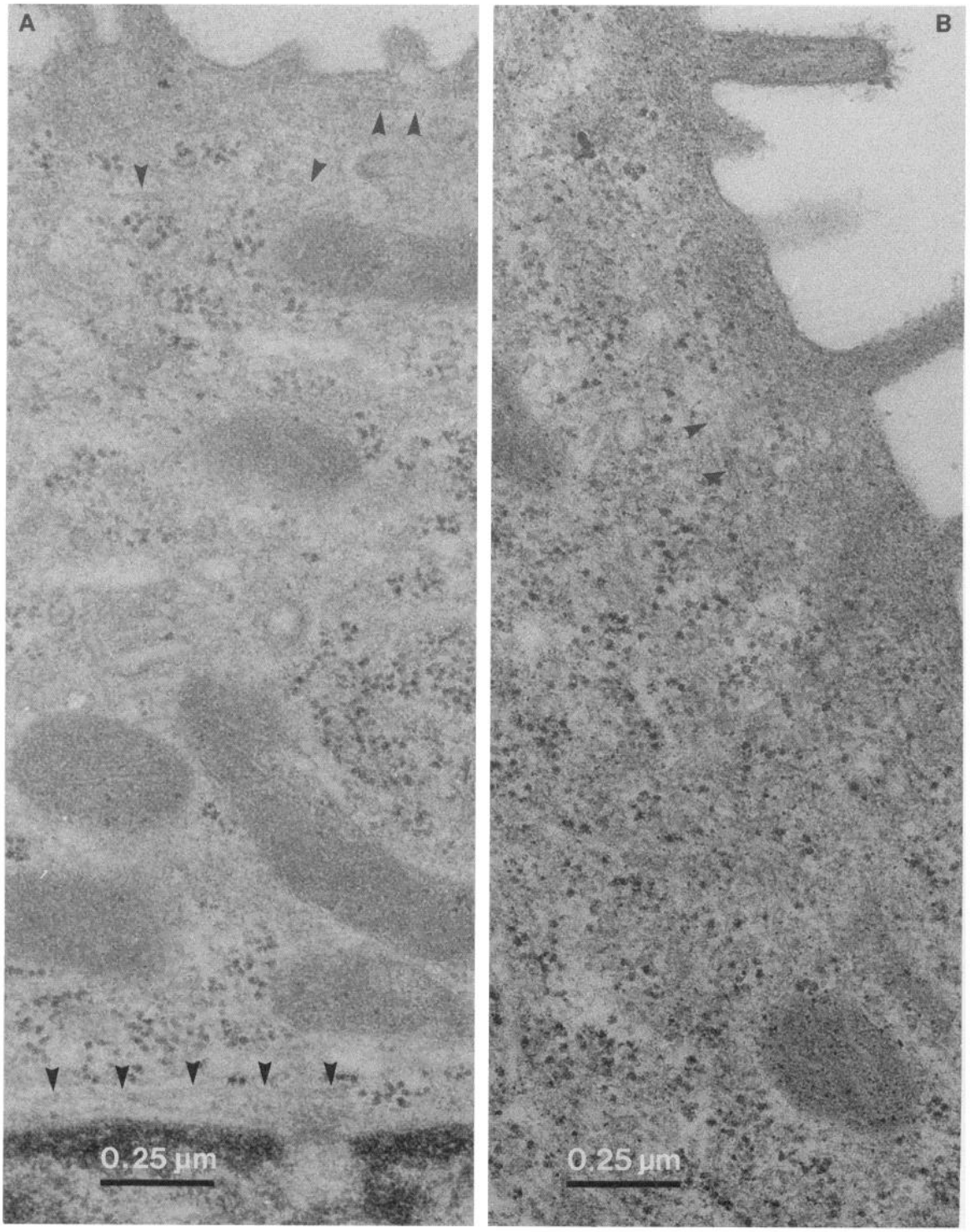

FIG. 2. Uterine epithelial cells at 35 (*A*) and 45 s (*B*) after $E_2\beta$, 0.5 µg/100 g body wt, administered iv to ovariectomized rats. MT (arrowheads) in *A* are appreciably shorter than those in representative control preparations (Fig. 1, *A* and *B*), while *B* is depleted of these organelles except for a short length in longitudinal profile (arrowhead). Also to be noted (*A* and *B*) are development of MV at luminal surfaces, even at these brief intervals after hormone. Abbreviations are defined in text.

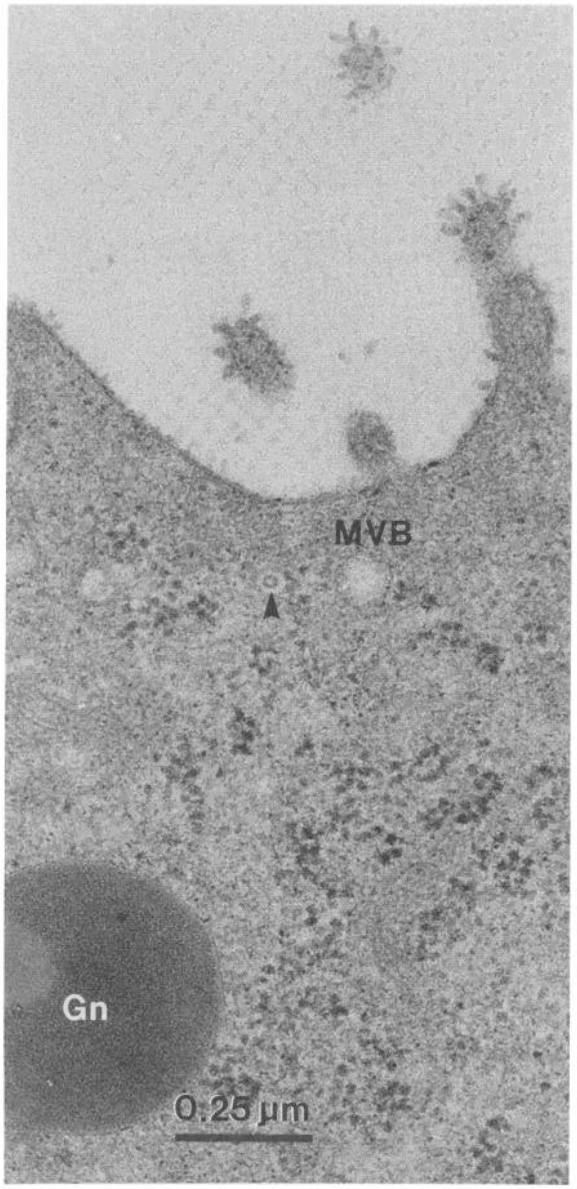

FIG. 3. Luminal epithelial cell 80 s after a single physiological dose of $E_2\beta$, iv, as in Fig. 2. Except for a single MT in cross section (arrowhead), specimen is essentially devoid of these organelles. Large granule (Gn) is typical of these cells, as is multivesicular body (MVB), latter at this early interval after hormone. Note increased lengths and complexity of MV.

35 s and up to 2 min of intravenous injection, estrogen elicited a striking diminution in mean lengths of those longitudinal profiles that were detectable in the randomly selected micrographs (Table 1; $P < 0.001$). These findings also had their counterpart in effects of $E_2\beta$ on the summated lengths of longitudinal MT profiles ($P = 0.01$; Table 1). Thus maximal, mean, and overall lengths of MT profiles per EM plate exhibited dramatic declines by as early as 35 s after administration of a physiological dose of estrogen. Notably, while the maximal MT lengths of estrogen-treated specimens were only about one-third

TABLE 1. *Acute effects of estrogen on lengths of endometrial microtubule profiles*

Group	Maximum/Minimum Lengths, µm	Mean Lengths/ Plate, µm	Mean Summated Lengths/Plate, µm
Control	2.40/0.05	0.42±0.04	8.84±2.65
Experimental	0.83/0.06	0.25±0.02	1.09±0.38
P	<0.001/>0.2	<0.001	0.01

Variations shown are means ± SE. For each group, ovariectomized rats were injected iv with placebo solution (control) or with 0.5 µg/100 g body wt estradiol-17β (experimental) at *time zero*, and their uteri were processed for transmission electron microscopy (TEM) at intervals from 35 s to 2 min thereafter. Lengths were calculated from computer analysis of tracings of longitudinal microtubule (MT) profiles in TEM plates, as described in the text. Length comparisons shown are between 5 representative control and an equal number of estrogen-treated specimens within indicated time frame. It should be noted that at times of estrogen action up to 2 min, many EM plates were entirely devoid of MT. Thus these values in experimental series represent residual and/or reconstituted MT, and are probably maximal.

those of the controls ($P < 0.001$), the minimum lengths were quite similar ($P > 0.2$; Table 1).

With only occasional exceptions, there was generally parallel orientation of the MT seen in longitudinal profile in both control and experimental preparations. These observations support the view that in luminal epithelial cells of uterus, as in many other cell types, the cytoarchitectural arrangement of MT is not a random network (see Refs. 12 and 38).

Transitory Nature of Abrupt MT Dismantling Under Estrogen Influence

Figure 4 depicts staging in the apparent restitution of the MT apparatus with increasing time after hormone administration. Within 2 min (Fig. 4*A*), the numbers of detectable longitudinal profiles of MT, although of relatively short lengths, were increased over their negligible concentration at the very early time periods (i.e., 35–80 s; Figs. 2, *A* and *B*, and 3). There was a progressive increase in the arrays of these organelles, begun by ~2 min, through the 30-min time point (Fig. 4, *A–D*). The transitory nature of these very early modifications of the cytoarchitecture under estrogen influence paralleled the time course of cell surface effects of the hormone, as noted with scanning electron microscopy (32, 33).

Biphasic Influence of Estrogen on Microvillar Apparatus

Figure 5 summarizes the very rapid effects of $E_2\beta$ on the development of microvilli at the luminal surfaces of uterine epithelial cells in situ. The relative quiescence of the plasmalemmal surface of control ovariectomized preparations (e.g., Figs. 1*A* and 5*A*) is in sharp contrast to the appreciable increase in numbers and lengths of MV by as little as 35 s after hormone (Fig. 5*B*). This influence of estrogen on both numbers and lengths of MV increased with time, as noted in Fig. 5, *C* (45 s) and *D* (80 s). By 2 min (Fig. 5*E*), the appearance of the

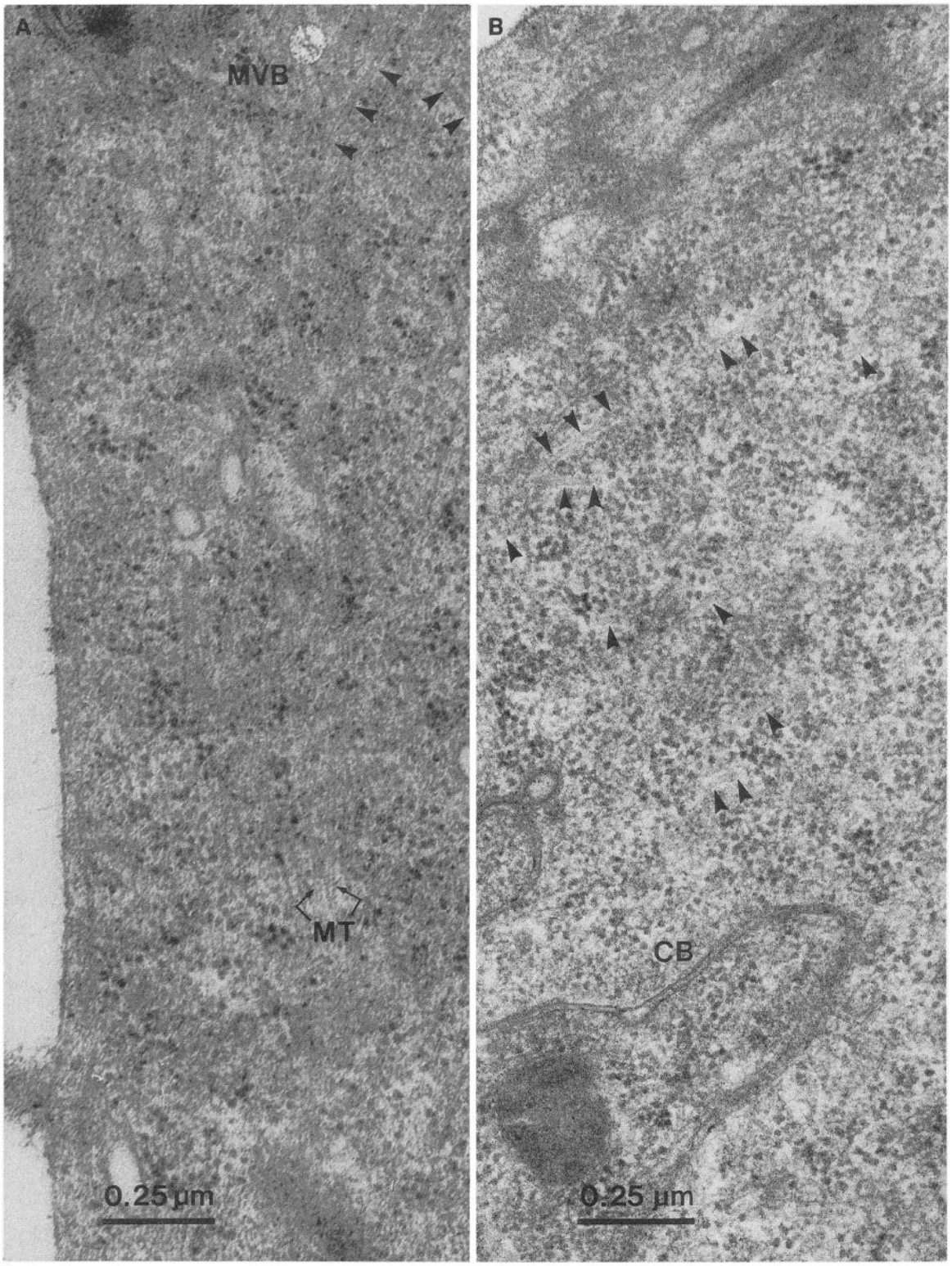

FIG. 4. Uterine luminal epithelial cells at further intervals after $E_2\beta$, 0.5 μg/100 g body wt, iv. By 2 min (*A*), considerable numbers of short MT in longitudinal profile (arrowheads) are once again evident. By 5 min (*B*) MTs, easily distinguished from cell boundary (CB), occur more frequently. Restoration of MT appears progressive through 15 (*C*) and 30 min (*D*; note clusters designated by symbol MT and arrows), while surface activity in form of MV gradually subsides (see Fig. 5). Abbreviations are defined in text. See facing page for *C* and *D*.

plasmalemma was quite complex, with a substantial increase in surface area (see also Fig. 6*B* for detail). These surface alterations underwent sharp involution, beginning perhaps by as early as 5 min (Fig. 5*F*) and progressing through 10 and 15 min (Fig. 5, *G* and *H*) up to 30 min (Fig. 5*I*). By the latter time, the surface was nearly indistinguishable from that of controls (Figs. 1*A* and 5*A*). Analysis of the second wave of MV activity evident by

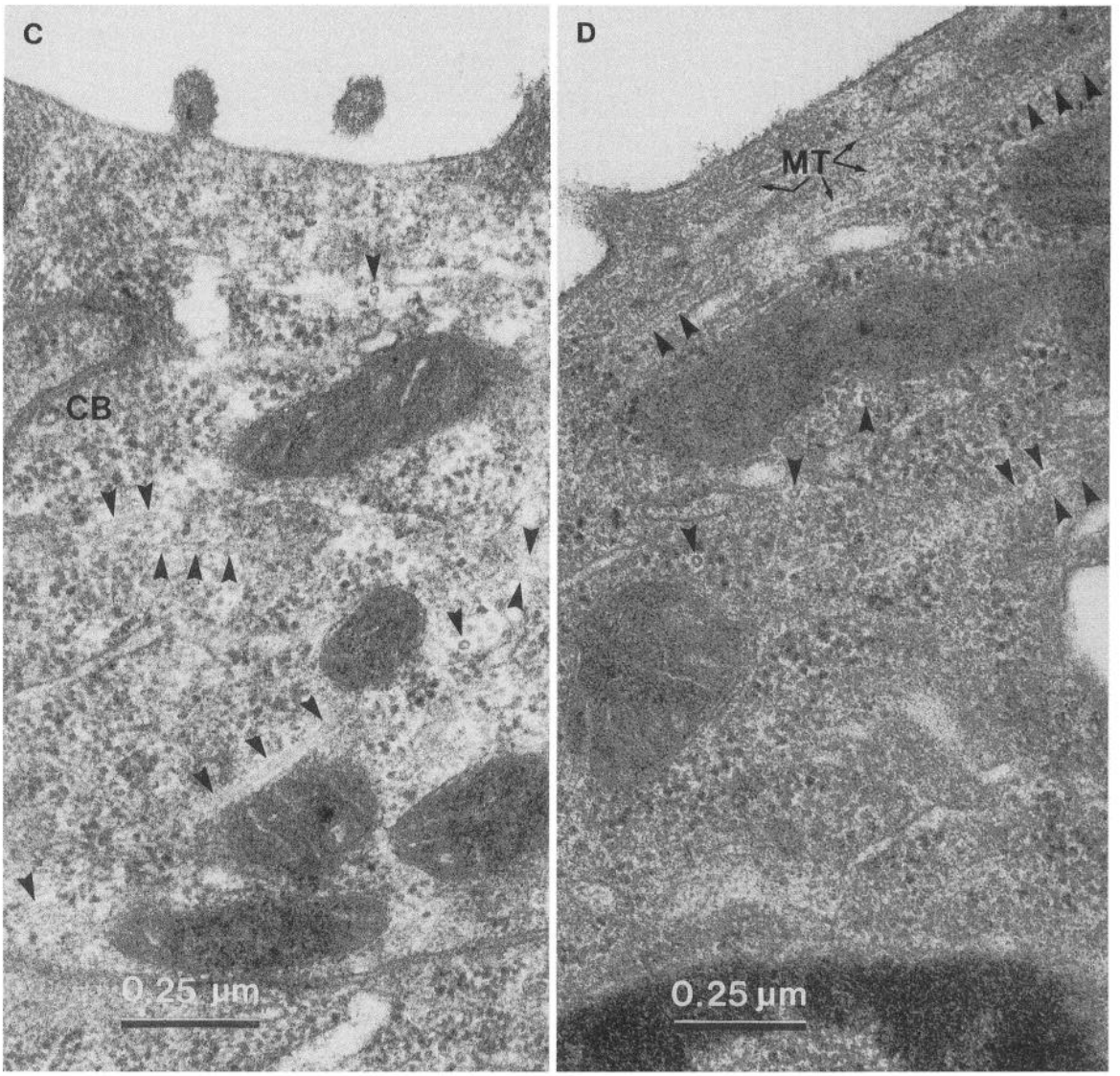

FIG. 4—*Continued.*

scanning electron microscopy after 1 h (33) was beyond the scope of the present investigation.

Figure 6 illustrates the remarkable MV activity elicited at 45 s (Fig. 6*A*) and at 2 min (Fig. 6*B*) by estrogen in situ and provides striking evidence of the burst of vigorous surface activity under these conditions. Indeed, the "hillocks" at the MV bases (Fig. 6*B*) may represent portions of clustering MV cut in tangential section. Figure 6*A* reveals an unusually large multivesicular body close to the luminal surface, a relationship that was not observed in the control series. Such organelles, also evident at 80 and 120 s after $E_2\beta$ (not illustrated) and which may be secondary to the enhanced endocytotic activity that is characteristic of early estrogen action (15, 31, 45, 46), could possibly serve as a means of conveyance of matériel to the expanding plasmalemmal area. Likewise, these organelles may be suggestive of incipient exocytotic function at the luminal surface (see Ref. 5).

DISCUSSION

Results of the present investigation provide new and further evidence that cytostructural changes are very early indicators of estrogen stimulation. The onset of these alterations, including profound depletion of extensive microtubular arrays concomitantly with development of copious luminal microvilli, occurred within the first 35 s of hormone action in vivo and thus appreciably earlier than would appear to be possible by transcriptional or translational regulation by the steroid. The time of onset of these cytostructural correlates (see Ref. 27) is comparable with that of other early evidences of the transduction and/or propagation of the hormonal signal, including cAMP elevation (44), ionic fluxes (28, 32, 45) leading to altered electrical activity (11), and accentuated delivery of closely limited amounts of certain enzymes and other components to the cell surface, to the cytoplasm, and to the nuclear compartment (40, 41, 45, 46). Indeed, these other rapid responses may themselves contribute to reorganization of target cell architecture (29, 30).

In common with the acute cytostructural changes in response to estrogen, the secondary restoration of MT assembly was accompanied by the subsidence of luminal

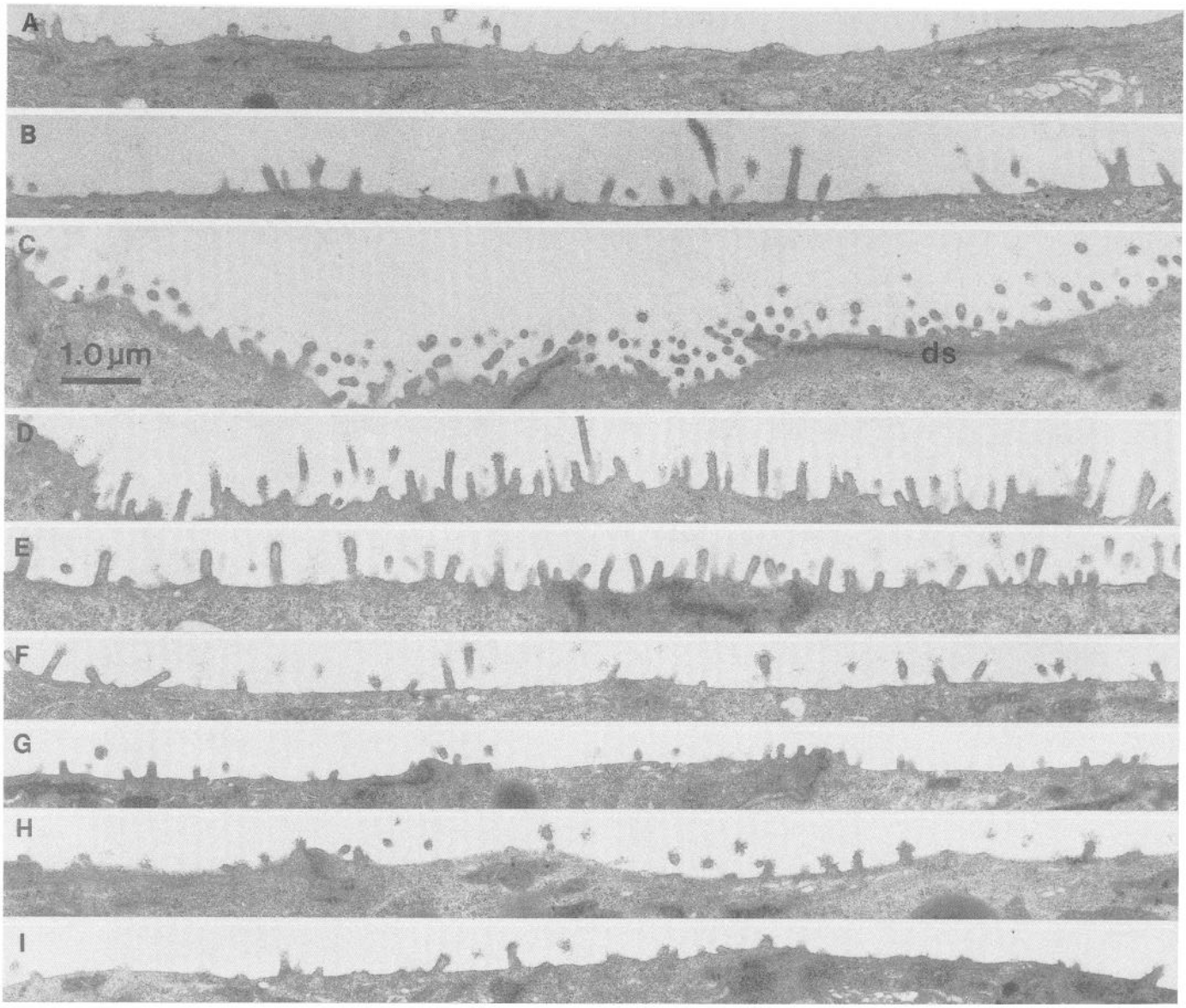

FIG. 5. Low magnification views of luminal surfaces of uterine epithelial cells of ovariectomized rats at brief intervals after iv administration of placebo control vehicle (*A*) or $E_2\beta$, 0.5 μg/100 g body wt (*B–I*). Relative paucity of MV in control preparation is in contrast to striking onset and progressive enhancement in this parameter at 35 (*B*), 45 (*C*), 80 (*D*), and 120 (*E*) s after exposure to hormone in situ. *F–I*: cell surfaces at 5, 10, 15, and 30 min, sequentially, reveal the remarkable subsidence of the above MV activity. Thus, by 30 min after estrogen (*I*), the degree of luminal surface investment with MV closely resembles relatively quiescent control state (*A*). ds, Desmosomes.

MV activity. These findings of the closely integrated timing and inversion of reciprocal MT dismantling and MV generation suggest that the cytoskeletal and microvillar changes may be attributable to some common mechanism(s) of physiological significance to the further propagation of the estrogenic response and subject to narrow limitation and/or rapid reversal.

The abrupt effects of estrogen on microtubular assembly did not depend on the state of MT preservation during the present experiments, for both control and $E_2\beta$-treated specimens were processed simultaneously through all stages of preparation. Similarly, geometric artifact in apparent MT length was improbable since the section angle transverse to the luminal long axis was systematically maintained constant throughout. Moreover, it is unlikely that restoration of the cytoskeletal assembly was necessarily dependent on new tubulin synthesis (see Refs. 4 and 20). In fact, on the basis of the time course of demolition/restoration of given fractions of the MT complement, it would appear that the products of disassembly of these structures survive in the cytoplasm in assembly-competent form, circumstances that conform to the dynamic instability model of MT turnover (see Refs. 19 and 34). Taken together, these findings suggest that shifts in the cytoplasmic environment (see also, Ref. 4) under estrogen influence, themselves subject to secondary limitations, provide the requisite trigger to the inverse and biphasic effects of the hormone on MT and MV dynamics.

A proximate mechanism that could account for such rapid (see Refs. 19 and 34), extensive, and apparently coordinated changes at the surface and in the cyto-

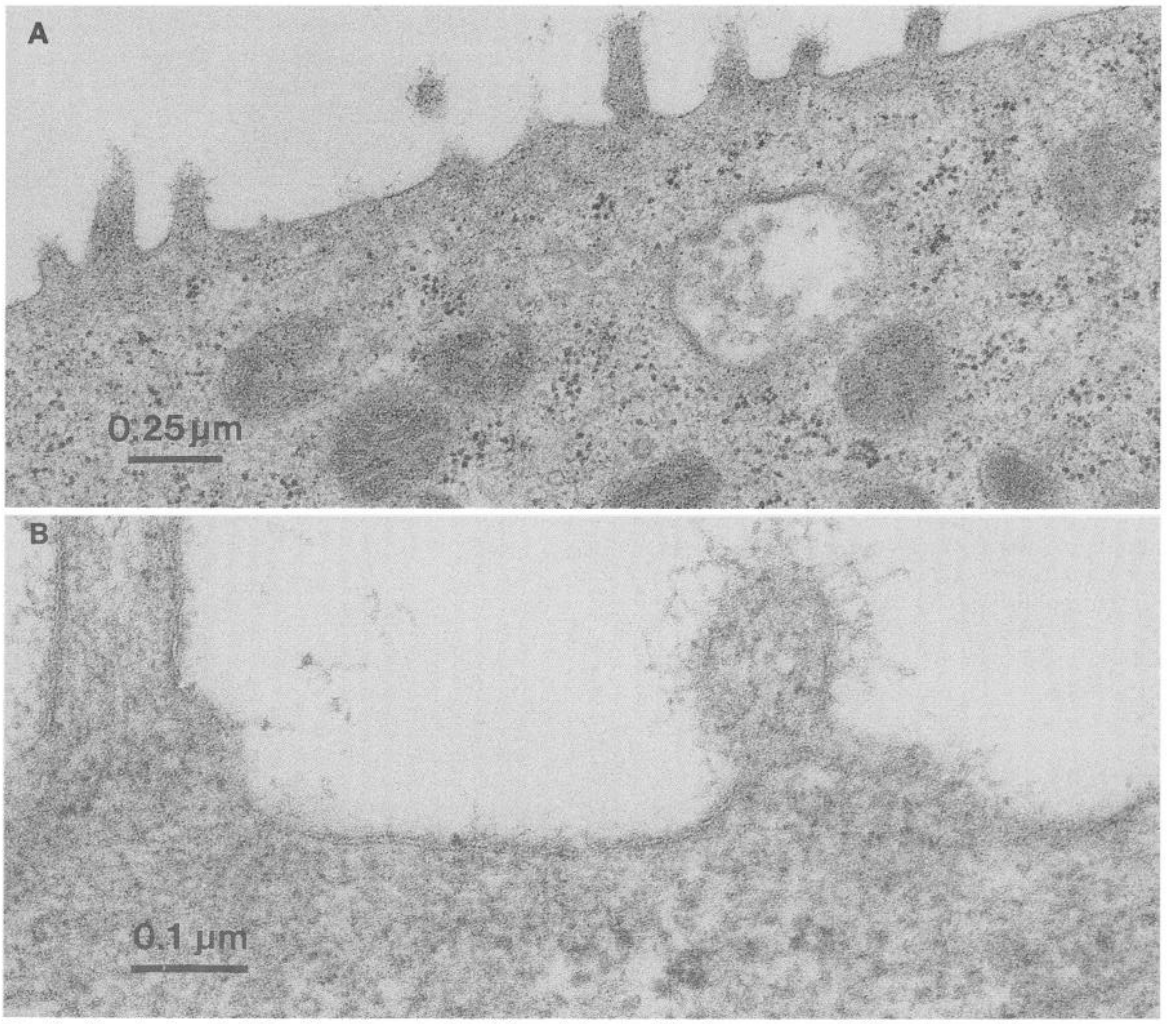

FIG. 6. Detail of luminal epithelial cell surface at 45 s (*A*) and 2 min (*B*) after estrogen. MV activity, with conspicuous glycoprotein decoration, characterizes both specimens. A large multivesicular body is shown in *A*.

plasmic compartment of the target cell in response to estrogen remains to be identified. The present observations would appear to require a poised, membrane-bounded system in which potential reactants critical for the alteration of the microenvironment are sequestered and from which they can be delivered in controlled amounts on signal by limited, dose-dependent labilization of the organellar bounding membrane. The lysosomal population represents just such a system, as has been documented for rapid, receptor-mediated responsiveness to estrogen as well as to an extensive array of other steroid and peptide hormones in a specific, dose-dependent, and target-selective manner (reviewed in Refs. 40–43, 45, and 46). Among the numerous hydrolytic and nonhydrolytic components of these highly conserved organelles that are liberated abruptly at strategic sites in the affected cell on capture of specific effectors are a Ca^{2+}- and calmodulin-dependent protein kinase (7), calmodulin (25), and Ca^{2+} (10, 23), modulators of the state of phosphorylation of tubulin and its associated proteins. Enhanced phosphorylation of tubulin (48), which can be extensive in seconds (5), or of microtubule-associated proteins (17), by calmodulin-dependent (5, 48) or cAMP-dependent (17, 39) protein kinases, respectively, leads to reversible inhibition of the capacity of tubulin to undergo self-assembly (17, 48; see also citations in Refs. 12 and 38). Similarly, phosphorylation suppresses the cross-linking of microtubule-associated proteins to actin filaments, an action that is counteracted by acid or alkaline phosphatase treatment (39). In contrast, the propensity of tubulin to associate with membranes, plasmalemmal as well as organellar (summarized in Ref. 1), is further accentuated by phosphorylation of residues in its highly anionic 4K COOH-terminal region and is likewise subject to reversal by phosphatase treatment (16). Specific phosphorylation of uterine α-tubulin under conditions of estrogen administration to ovariectomized rats (18) supports the possibility that protein phosphorylation mechanisms are involved in estrogen action on the cytoskeleton.

Dissipation by redistribution, recompartmentation, or degradation of the catalytic components liberated from membranous sites could underlie the counterregulation requisite to reversal of the MT depolymerization phenomenon. The increased availability of free calcium, which is evoked acutely by estrogen in isolated uterine

cells through enhanced uptake (28) as well as by mobilization from organellar sequestration, and which is known to inhibit MT assembly (14), is rapidly supplanted by a sharp efflux of Ca^{2+} (28), thus removing its inhibitory effect on MT polymerization. Moreover, the inward cAMP gradient established within 15 s in uterine cells by estrogenic molecules is also dispelled with time (44). Concurrent activity of lysosomal phosphatase represents still another means whereby the status quo of MT could be restored. Thus, in addition to tight control exerted by multiple factors on disassembly of these organelles under estrogen influence, reversibility of this complex process appears built into the regulatory cascade.

The remarkable accrual of MV within seconds of estrogen application (32, 33; present work) has its counterpart in the acute effects on plasmalemmal architecture of their respective targets by certain peptide hormones (2, 6, 8, 21), in some cases associated with early and reversible reorganization of actin filaments (22, 37). Moreover, rearrangement of cytoskeletal and adhesion-plaque structures of MCF-7 breast cancer cells, leading to numerous protrusions and ruffling, occurs as a dose-dependent response to estrogen in vitro (35). Systematic analysis of the effects of $E_2\beta$ on the several microfilament structures of uterine cells was beyond the scope of the present work. However, it was noted that conspicuous parallel arrays of filaments identified in certain control preparations (e.g., Fig. 1, *A* and *B*) were not observed among the earliest estrogenized samples. Thus the microfilamentous web in the cortical cytoplasm may share the temporary fate of the MT in being subject to limited and potentially reversible destabilization by the hormone. Such concerted effects may underlie the acute development of MV (see Ref. 36).

The specific outcome of a transitory loss of MT integrity is unknown. It has been postulated that such a step is obligatory in the progression of growth-arrested cultures, challenged by the appropriate specific peptidal growth-stimulatory agents, through the premitotic process leading to DNA synthesis and cell division (reviewed in Ref. 47). Results of the present investigation, taken together with earlier observations from this laboratory (40, 45, 46), indicate that estrogenic stimulation of proliferative activity in responsive cells is not only preceded by alterations in membrane-associated regulatory processes but also by a striking reorganization of cellular architecture that may contribute to propagation of the hormonal signal.

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REFERENCES

1. Andreu, J. M., and J. A. Muñoz. Interaction of tubulin with octyl glucoside and deoxycholate. 1. Binding and hydrodynamic studies. *Biochemistry* 25: 5220–5230, 1986.
2. Avivi, A. On the mechanism of TSH-induced formation of follicle-like structures in primary cultures of thyroid cells. *Cell Biol. Int. Rep.* 6: 1109–1118, 1982.
3. Bareither, M. K., and H. G. Verhage. Effect of estrogen and progesterone on secretory granule formation and release in the endometrium of the ovariectomized cat. *Biol. Reprod.* 22: 635–643, 1980.
4. Brinkley, B. R., S. M. Cox, D. A. Pepper, L. Wible, S. L. Brenner, and R. L. Pardue. Tubulin assembly sites and the organization of cytoplasmic microtubules in cultured mammalian cells. *J. Cell Biol.* 90: 554–562, 1981.
5. Burke, B. E., and R. J. DeLorenzo. Ca^{2+} and calmodulin-dependent phosphorylation of endogenous synaptic vesicle tubulin by vesicle-bound calmodulin kinase system. *J. Neurochem.* 38: 1205–1218, 1982.
6. Chinkers, M., J. A. McKanna, and S. Cohen. Rapid induction of morphological changes in human carcinoma cells A-431 by epidermal growth factor. *J. Cell Biol.* 83: 260–265, 1979.
7. Collins, C. A., and W. W. Wells. Characterization of endogenous protein phosphorylation in isolated rat liver lysosomes. *J. Biol. Chem.* 257: 827–831, 1982.
8. Connolly, J. L., S. A. Green, and L. A. Greene. Pit formation and rapid changes in surface morphology of sympathetic neurons in response to nerve growth factor. *J. Cell Biol.* 90: 176–180, 1981.
9. D'Amour, F. E., F. R. Blood, and D. A. Belden, Jr. *Manual for Laboratory Work in Mammalian Physiology* (3rd ed.). Chicago: Univ. of Chicago Press, 1965.
10. Davis, W. L., and R. G. Jones. Calcium lysosomes in rachitic and Vitamin D_3-replete chick duodenal absorptive cells. *Tissue Cell* 13: 381–391, 1981.
11. Dufy, B., J.-D. Vincent, H. Fleury, P. Du Pasquier, D. Gourdji, and A. Tixier-Vidal. Membrane effects of thyrotropin-releasing hormone and estrogen shown by intracellular recording from pituitary cells. *Science Wash. DC* 204: 509–511, 1979.
12. Dustin, P. *Microtubules* (2nd ed.). New York: Springer-Verlag, 1984, p. 1–482.
13. Duval, D., S. Durant, and F. Homo-Delarche. Non-genomic effects of steroids. Interactions of steroid molecules with membrane structures and functions. *Biochim. Biophys. Acta* 737: 409–442, 1983.
14. Fuller, G. M., and B. R. Brinkley. Structure and control of assembly of cytoplasmic microtubules in normal and transformed cells. *J. Supramolec. Struc.* 5: 497–514, 1976.
15. Garcia-Segura, L. M., G. Olmos, P. Tranque, and F. Naftolin. Rapid effects of gonadal steroids upon hypothalamic neuronal membrane ultrastructure. *J. Steroid Biochem.* 27: 615–623, 1987.
16. Hargreaves, A. J., F. Wandosell, and J. Avila. Phosphorylation of tubulin enhances its interaction with membranes. *Nature Lond.* 323: 827–828, 1986.
17. Jameson, L., T. Frey, B. Zeeberg, F. Dalldorf, and M. Caplow. Inhibition of microtubule assembly by phosphorylation of microtubule-associated proteins. *Biochemistry* 19: 2472–2479, 1980.
18. Joseph, M. K., M. A. Fernstrom, and M. S. Soloff. Switching of β- to α-tubulin phosphorylation in uterine smooth muscle of parturient rats. *J. Biol. Chem.* 257: 11728–11733, 1982.
19. Kirschner, M., and T. Mitchison. Beyond self-assembly: from microtubules to morphogenesis. *Cell* 45: 329–342, 1986.
20. Loizzi, R. F. Ovariectomy-induced tubulin polymerization in pregnant rat mammary gland. *Proc. Soc. Exp. Biol. Med.* 173: 252–255, 1983.
21. Mattson, P., and J. Kowal. Reversibility of the effects of ACTH and cytochalasin B on the ultrastructure and steroidogenic activity of adrenocortical tumor cells in vitro. *Tissue Cell* 15: 805–821, 1983.
22. Mellström, K., A.-S. Höglund, M. Nistér, C.-H. Heldin, B. Westermark, and U. Lindberg. The effect of platelet-derived growth factor on morphology and motility of human glial cells. *J. Muscle Res. Cell Motil.* 4: 589–609, 1983.
23. Nemere, I., V. Leathers, and A. W. Norman. 1,25-Dihydroxyvitamin D_3-mediated intestinal calcium transport. Biochemical identification of lysosomes containing calcium and calcium-binding protein (calbindin-D_{28K}). *J. Biol. Chem.* 261: 16106–16114, 1986.
24. Nicklas, R. B., and G. W. Gordon. The total length of spindle microtubules depends on the number of chromosomes present. *J. Cell Biol.* 100: 1–7, 1985.
25. Nielsen, T. B., J. B. Field, and J. R. Dedman. Association of

calmodulin with lysosomes. *J. Cell Sci.* 87: 327–336, 1987.
26. PECK, E. J., JR., J. BURGNER, AND J. H. CLARK. Estrophilic binding sites of the uterus. Relation to uptake and retention of estradiol in vitro. *Biochemistry* 12: 4596–4603, 1973.
27. PETERS, R. A. Hormones and the cytoskeleton. *Nature Lond.* 177: 426, 1956.
28. PIETRAS, R. J., AND C. M. SZEGO. Endometrial cell calcium and oestrogen action. *Nature Lond.* 253: 357–359, 1975.
29. PIETRAS, R. J., AND C. M. SZEGO. Surface modifications evoked by estradiol and diethylstilbestrol in isolated endometrial cells: evidence from lectin probes and extracellular release of lysosomal protease. *Endocrinology* 97: 1445–1454, 1975.
30. PIETRAS, R. J., AND C. M. SZEGO. Estrogen-induced membrane alterations and growth associated with proteinase activity in endometrial cells. *J. Cell Biol.* 81: 649–663, 1979.
31. PIETRAS, R. J., AND C. M. SZEGO. Specific internalization of estrogen and binding to nuclear matrix in isolated uterine cells. *Biochem. Biophys. Res. Commun.* 123: 84–91, 1984.
32. POURREAU-SCHNEIDER, N., Y. BERTHOIS, P. GANDILHON, P. CAU, P. M. MARTIN, AND M. PASSEREL. Early alterations at the plasma membrane of breast cancer cell lines in response to estradiol and hydroxytamoxifen. *Mol. Cell. Endocrinol.* 48: 77–88, 1986.
33. RAMBO, C. O., AND C. M. SZEGO. Estrogen action at endometrial membranes: alterations in luminal surface detectable within seconds. *J. Cell Biol.* 97: 679–685, 1983.
34. SAMMAK, P. J., G. J. GORBSKY, AND G. G. BORISY. Microtubule dynamics in vivo: a test of mechanisms of turnover. *J. Cell Biol.* 104: 395–405, 1987.
35. SAPINO, A., F. PIETRIBIASI, G. BUSSOLATI, AND P. C. MARCHISIO. Estrogen- and tamoxifen-induced rearrangement of cytoskeletal and adhesion structures in breast cancer MCF-7 cells. *Cancer Res.* 46: 2526–2531, 1986.
36. SAVION, N., AND Z. SELINGER. Inhibition of enzyme secretion and autophagy of secretory granules caused by action of high concentration of secretory hormones on rat pancreatic slices. *Methods Cell Biol.* 23: 359–378, 1981.
37. SCHLESSINGER, J., AND B. GEIGER. Epidermal growth factor induces redistribution of actin and α-actinin in human epidermal carcinoma cells. *Exp. Cell Res.* 134: 273–279, 1981.
38. SCHLIWA, M. *The Cytoskeleton. An Introductory Survey.* New York: Springer-Verlag, 1986, p. 1–326.
39. SELDEN, S. C., AND T. D. POLLARD. Phosphorylation of microtubule-associated proteins regulates their interaction with actin filaments. *J. Biol. Chem.* 258: 7064–7071, 1983.
40. SZEGO, C. M. The lysosome as a mediator of hormone action. *Recent Prog. Horm. Res.* 30: 171–233, 1974.
41. SZEGO, C. M. Lysosomal function in nucleocytoplasmic communication. In: *Lysosomes in Biology and Pathology*, edited by J. T. Dingle and R. T. Dean. Amsterdam: Elsevier/North Holland, 1975, vol. 4, p. 385–477.
42. SZEGO, C. M. Steroid-protein binding: from circulating blood to target cell nucleus. *Gynecol. Invest.* 7: 251–279, 1976.
43. SZEGO, C. M. Mechanisms of hormone action: parallels in receptor-mediated signal propagation for steroid and peptide effectors. *Life Sci.* 35: 2383–2396, 1984.
44. SZEGO, C. M., AND J. S. DAVIS. Adenosine 3′,5′-monophosphate in rat uterus: acute elevation by estrogen. *Proc. Natl. Acad. Sci. USA* 58: 1711–1718, 1967.
45. SZEGO, C. M., AND R. J. PIETRAS. Membrane recognition and effector sites in steroid hormone action. In: *Biochemical Actions of Hormones*, edited by G. Litwack. New York: Academic, 8: 307–463, 1981.
46. SZEGO, C. M., AND R. J. PIETRAS. Lysosomal functions in cellular activation: propagation of the actions of hormones and other effectors. *Int. Rev. Cytol.* 88: 1–302, 1984.
47. THYBERG, J. The microtubular cytoskeleton and the initiation of DNA synthesis. *Exp. Cell Res.* 155: 1–8, 1984.
48. WANDOSELL, F., L. SERRANO, M. A. HERNÁNDEZ, AND J. AVILA. Phosphorylation of tubulin by a calmodulin-dependent protein kinase. *J. Biol. Chem.* 261: 10332–10339, 1986.