

# CENTRIOLE MORPHOGENESIS IN DEVELOPING CILIATED EPITHELIUM OF THE MOUSE OVIDUCT

ELLEN ROTER DIRKSEN

From the Cancer Research Institute, University of California,  
San Francisco, California 94122

## ABSTRACT

The differentiating mouse oviduct has been used for the study of centriole morphogenesis because its epithelium is extensively ciliated and centriole formation occurs in a brief period after birth. Proliferative elements, consisting of an extensive fibrillar meshwork encrusted with 75 m $\mu$  granules, were encountered at all ages, but were the only centriole precursors present in younger animals (2–3 days). These large aggregates were found either physically associated with a mature centriole or alone, but never associated with procentrioles. It is likely, therefore, that although proliferative elements may be derived from preexisting centrioles, they do not directly produce new centrioles. An intermediate structure, the condensation form, found primarily in older animals (4–6 days), and produced by the packing of the proliferative element material, gives rise to daughter procentrioles. This association of procentriole and condensation form has been called a generative complex. Condensation forms undergo various stages of depletion, producing hollow spheres with thin walls or small osmiophilic aggregates as procentrioles grow in length and assemble their microtubules. From these observations it is concluded that synthesis of microtubular precursor protein is mediated by the mature centriole and that this protein is packaged into many condensation forms in order to allow the rapid assembly of a large number of centrioles in a brief period of time.

## INTRODUCTION

Evidence for the formation of centrioles from precursor structures which do not closely resemble the mature organelle has been recently established in a number of cell types (3, 8, 9, 10, 12, 13, 15, 16). Previous work in this laboratory demonstrated that in differentiating ciliated cells of rat tracheal epithelium, the greatest period of centriole morphogenesis occurred in the 19-day fetus (8). On the basis of these observations four main developmental stages of centriole formation were proposed: (a) The centrioles which participate in the last mitosis of the cell, or the recently matured centrioles, produce a fine fibrillar meshwork containing compact electron-opaque masses of

varying sizes (60–80 m $\mu$ ). These aggregates, called proliferative elements (*PE*), are not always found associated with a mature centriole, due probably to the plane of section. (b) The condensation forms (*CF*), considered to be the next stage in the formation of centrioles, are large, highly electron-opaque, often partially hollow masses (70–120 m $\mu$ ) which could be produced by the aggregation of the smaller, and morphologically similar masses in the proliferative elements. (c) Generative complexes (*GC*), the next morphological stage of centriole formation, consist of immature procentrioles which are physically connected, and in fact seem to arise from the condensation forms.

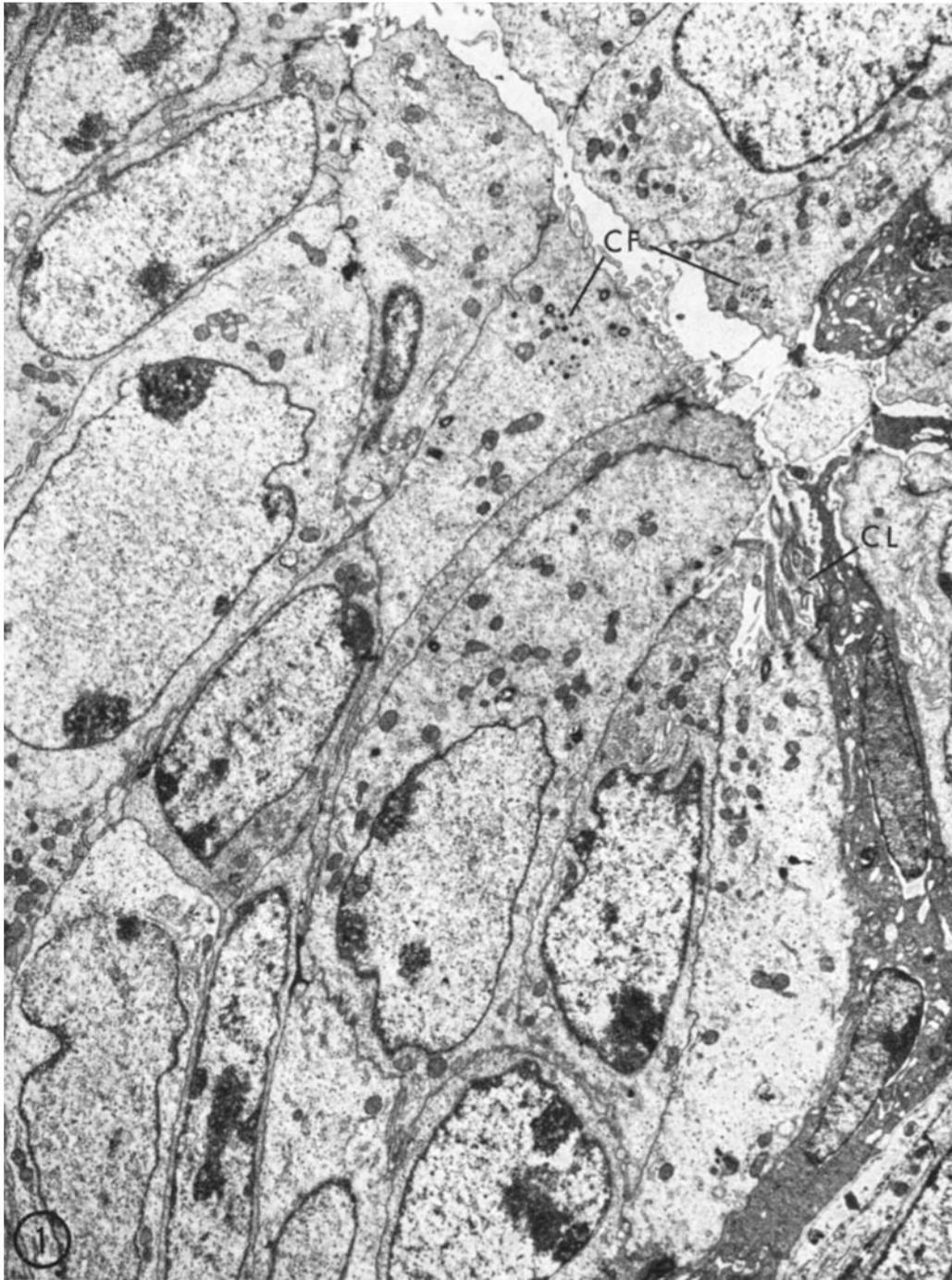


FIGURE 1 Low power electron micrograph of the oviduct epithelium of a suckling mouse. Condensation forms (*CF*) and basal bodies are found in two of the cells near the lumen, while one of the cells is already ciliated (*CL*).  $\times 6000$ .

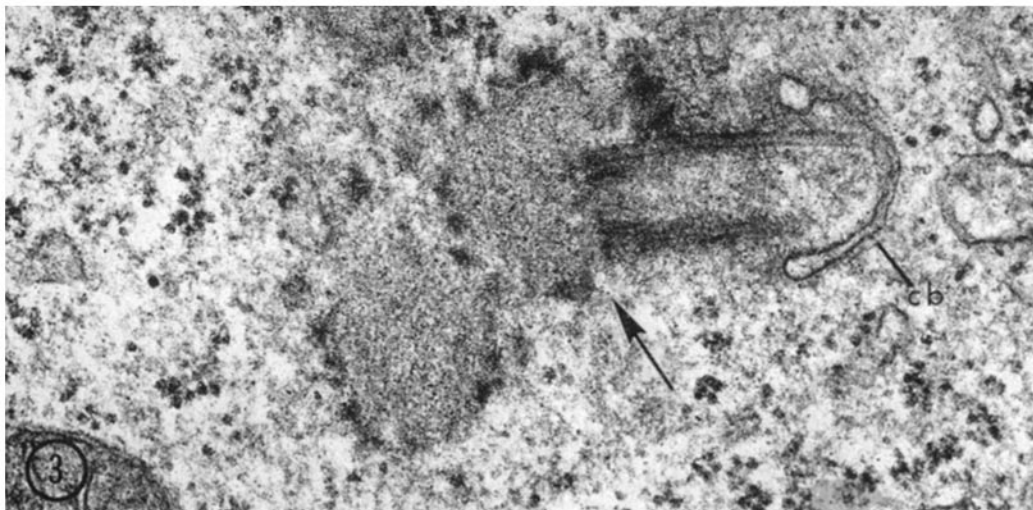
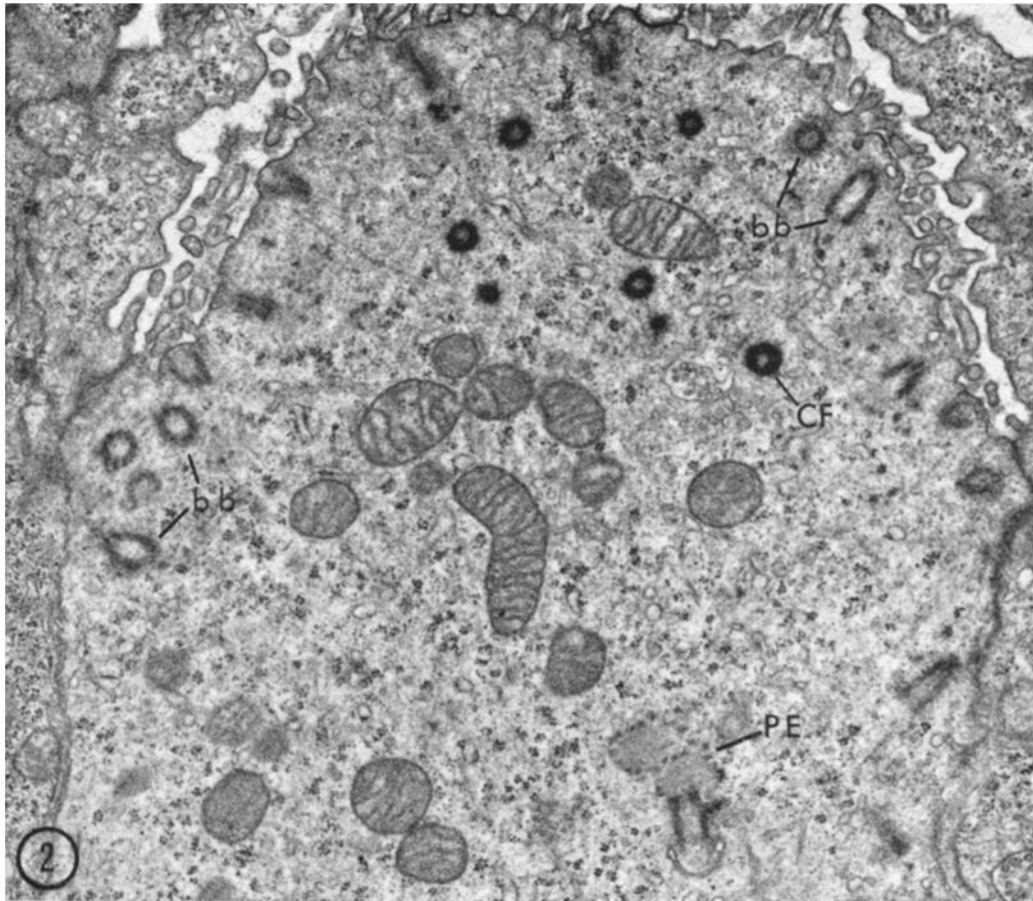


FIGURE 2 Apical portion of a cell in which basal bodies (*bb*) are oriented at the lumen. A proliferative element (*PE*) can be found deeper within the cell. A number of condensation forms (*CF*) are also found near the lumen.  $\times 20,000$ .

FIGURE 3 An enlargement of the proliferative element in Fig. 2. The fibrillar material is in direct continuity with the microtubules of the mature centriole (arrow). A ciliary bud (*cb*), usually found at that end of the centriole from which the ciliary shaft arises, is also present.  $\times 67,000$ .

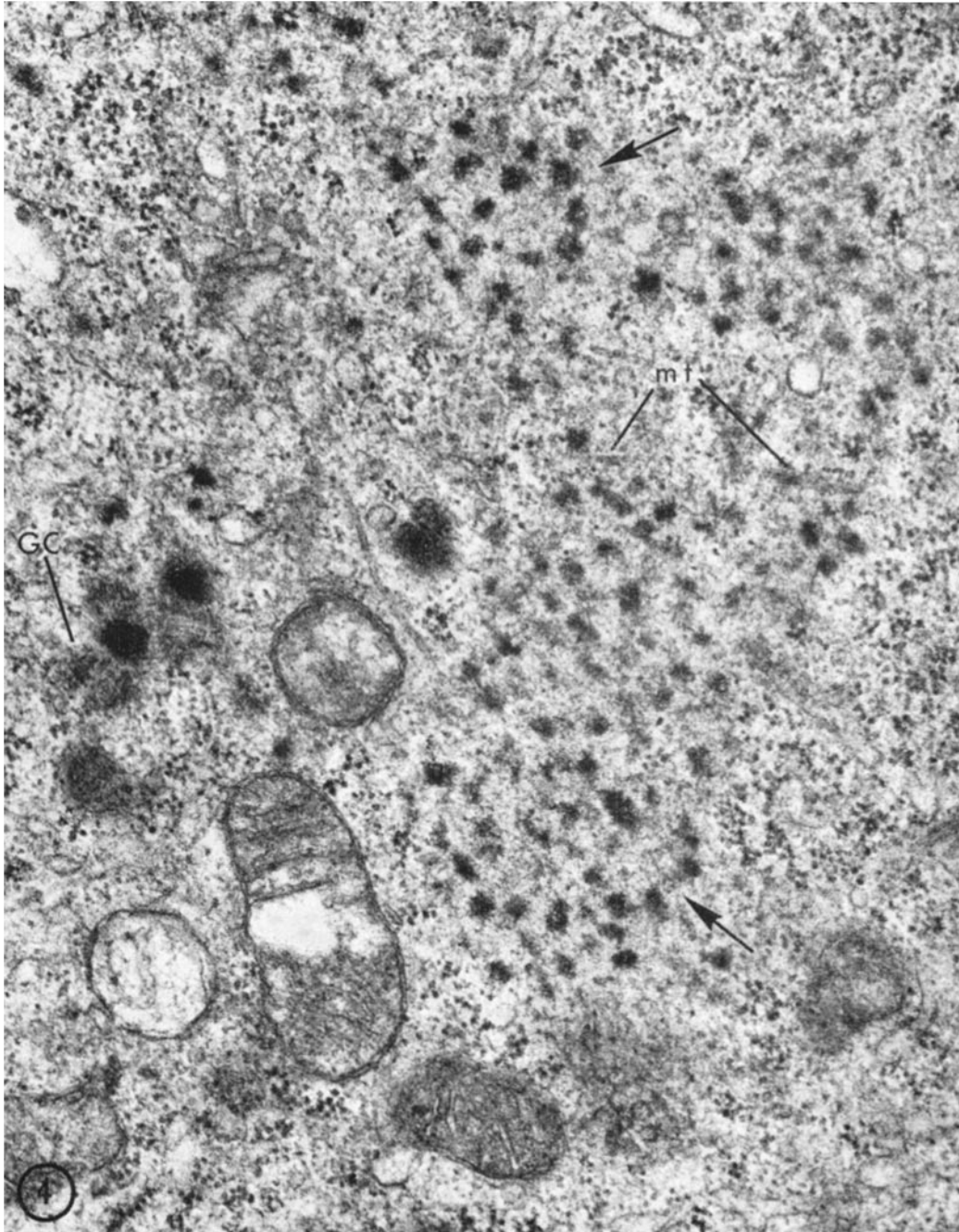


FIGURE 4 Section of a cell with large arrays of fibrillar material among which are interspersed dense granules (average 75–80  $m\mu$ ) in circular arrays (arrows). To the left, two generative complexes can be seen with condensation forms surrounded by pro-centrioles (*GC*). Microtubules (*mt*) can be seen coursing through the meshwork.  $\times 49,000$ .

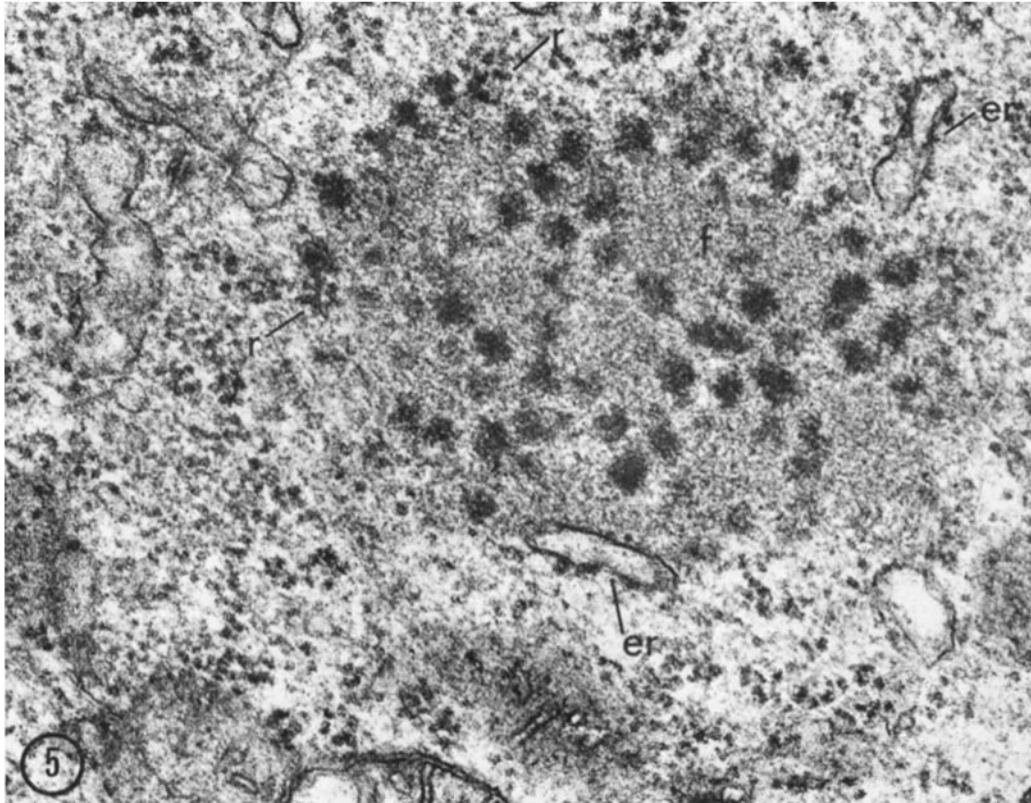


FIGURE 5 Elements of endoplasmic reticulum (*er*) are found near a large fibrillar array or proliferative element. In this plane of section no mature centriole is in evidence. A filamentous component (*f*) is surrounded by electron-opaque granules. Ribosomes are absent within this proliferative element, but are clustered near some of the dense granules (*r*).  $\times 69,000$ .

Procentrioles in these complexes occur in various stages of development. (*d*) As the procentrioles in the generative complexes mature, the condensation forms at the center seem to either disappear or become almost hollow.

In a preliminary report, observations indicated that in the presumptive ciliated cells of suckling mouse oviduct, similar events occurred during cell maturation (7). That is, in order to produce the number of centrioles needed to induce the full complement of cilia, the ciliated cells of oviducts from suckling mice undergo changes similar to those described for presumptive ciliated cells of embryonic rat tracheal epithelium. However, the fimbria of the mouse oviduct proved to be extremely suitable because its epithelium consists largely of ciliated cells, and cell maturation of oviduct epithelium, unlike that of respiratory

epithelium, occurs in a brief period shortly after birth.

The present work extends the observations on centriole formation to the ciliated oviduct epithelium of suckling mice, and includes a number of precursor forms not previously encountered either in respiratory epithelium or in other cell types in which this process has been studied.

#### MATERIALS AND METHODS

Female Swiss mice with their litters were obtained on the day on which their young had been born. At various ages (2-6 days old) the oviducts were removed from suckling females and fixed for 24 hr in 3.8% glutaraldehyde at pH 7.4 in sodium phosphate buffer at a final concentration of 0.025 M. The tissues were postfixated for 2 hr in 2% OsO<sub>4</sub> buffered at pH 7.5 with 0.1 M sodium phosphate. After rinsing with several changes of acetate-Veronal buffer, pH 6, the

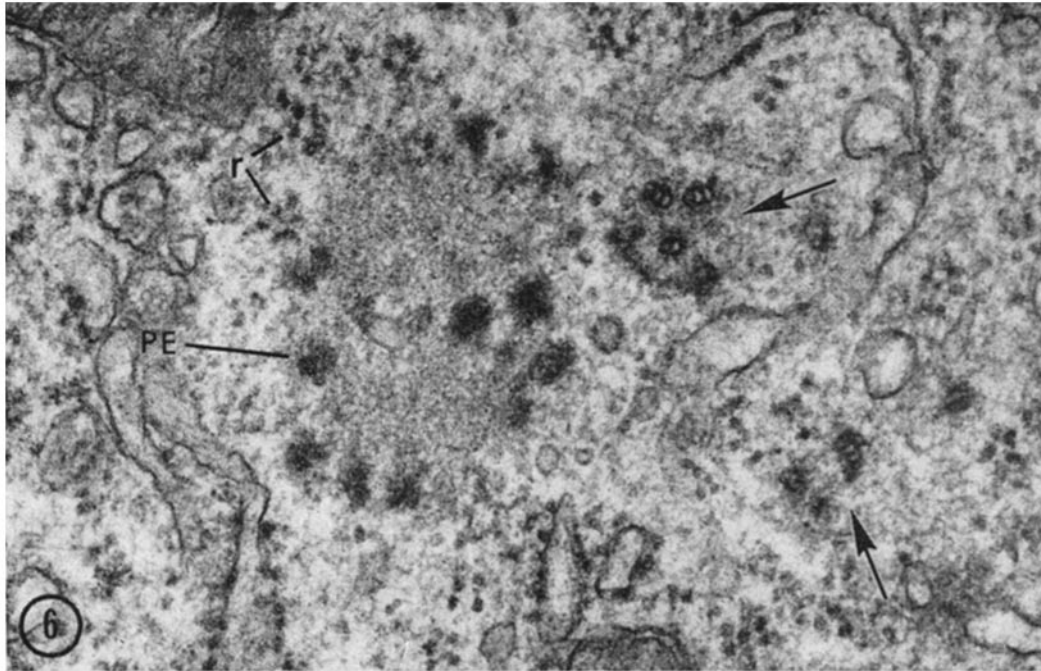


FIGURE 6 Doublet and triplet microtubular arrays (arrows) are found near a proliferative element (PE). Clusters of ribosomes (*r*) contact the filamentous material of the PE.  $\times 79,000$ .

tissues were stained with 0.5% uranyl acetate in the same buffer. Tissues were dehydrated in a graded series of acetone or alcohol followed by propylene oxide and embedded in Araldite. The sections, obtained on an MT-2 Porter-Blum ultramicrotome, were stained with 2% uranyl acetate for 30 min at room temperature followed by lead citrate for 20 min. Sections were examined and micrographed in an RCA-EMU 3G electron microscope operated at 100 kv, with 150  $m\mu$  condenser and 35  $m\mu$  objective apertures.

## OBSERVATIONS

### *The Proliferative Event*

The epithelium of the fimbria and ampulla of the mature mouse oviduct contains many ciliated cells, but those parts of the oviduct past the isthmus have few cells of this type. Although cilium formation begins to occur shortly after birth, it is not until after the first week that most of the cells have developed cilia. The epithelium of suckling mice will have a number of cells which are already ciliated, while at the same time many cells will be in the process of maturation and will thus contain many centriole precursors (Fig. 1). It is this latter

type of cell which has been of interest in this study of centriole formation in newborn animals. Observations of the fimbriated portion of the oviduct of 1 day-olds have, except in rare instances, failed to demonstrate centriole precursors of almost any type. At 2 and 3 days the cells are usually without cilia but with clusters of electron-opaque masses of varying sizes. Such structures have been previously called proliferative elements (8). Often, in a single section of the developing ciliated border, about 20% of the cells will contain these clusters.

When present in the same plane of section, only mature centrioles have been found associated with the large fibrillar masses (Figs. 2, 3, 14; arrows). This has led to the assumption that the previously existing centriole gives rise to or, in some manner, influences the formation of the fibrillar material (8). In those cases where pro-centrioles are found in the vicinity of proliferative elements, they are primarily related to the generative complexes with which they are associated (Fig. 7). However, the association of mature centriole and fibrillar meshwork (Figs. 2, 3) is not found as frequently as proliferative elements without centrioles (Figs. 4, 5, 6). This might be

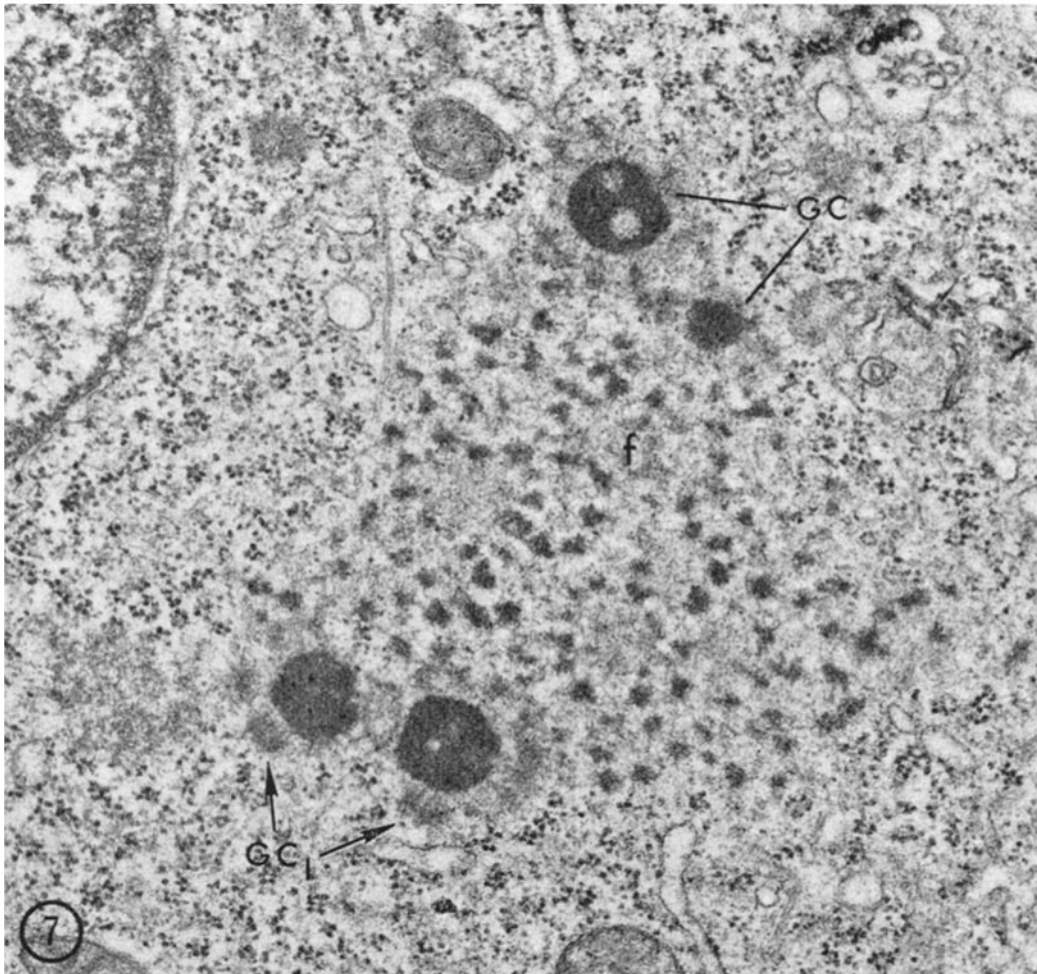


FIGURE 7 Four generative complexes at GC, GC<sub>1</sub>, with very immature procentrioles are found at the periphery of a very large proliferative element. This consists of filamentous material (f) delineated into circular patterns by the usual electron-opaque granules. At GC<sub>1</sub> the walls of short microtubules can be discerned (arrows). Ribosomes, although in close association with the proliferative element at its edges, are excluded from the inner area.  $\times 41,000$ .

interpreted to mean that this association is a rapid event which, because of the sampling problem, does not occur frequently.

Occasionally, doublet and triplet microtubular elements are encountered free in the cytoplasm (Fig. 6, arrows) which represent early stages in centriole assembly.

In the cells of 4-day-olds, various stages of centriole formation are present, but the most striking feature at this age is the number of proliferative elements which can be found. Cells occur whose cytoplasm, from apex to base, contains arrays of

proliferative elements (Fig. 4). At the same time a considerable amount of structural orientation begins to appear in the fibrillar masses. Circles, consisting of electron-opaque granules, surround a fine meshwork of amorphous material (Fig. 4, arrows; Figs. 5, 6). It is of interest to note that ribosomes and polysomes are excluded from such areas, while smooth-surfaced vesicles are often encountered within the proliferative elements.

Endoplasmic reticulum has been found associated with proliferative elements (Fig. 5), and it is of interest to speculate on its role in relation to

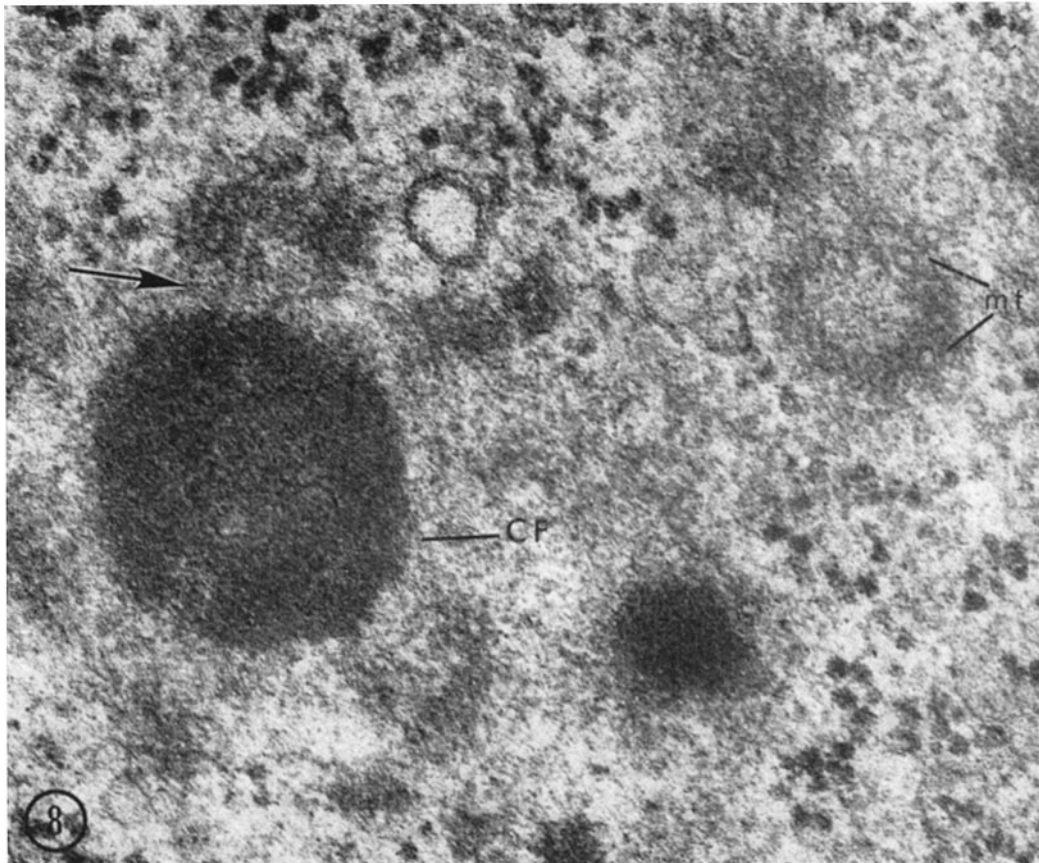


FIGURE 8 A generative complex whose central condensation form (*CF*) is  $380\text{ m}\mu$  and is surrounded by several pro-centrioles. Evidence for continuity between pro-centriole microtubules and condensation form can be seen in the rodlike extension (arrow). To the right, a pro-centriole in cross-section is seen to contain several partially assembled microtubular singlets (*mt*). These are found within the amorphous annular matrix.  $\times 134,000$ .

the synthesis of the microtubular protein which will, subsequently, be involved in the assembly of centrioles.

#### *The Growth Phase*

In the fimbriated portion of the oviduct of 5- and particularly 6-day-old mice, cells with massive arrays of proliferative elements and generative complexes are very frequent. These latter structures often take bizarre and complex forms, of which only a few examples can, of necessity, be shown.

The most predominant of the intermediate configurations in centriole formation is the generative complex. It consists of a central electron-opaque

core, varying in size from  $100\text{ m}\mu$  to  $700\text{ m}\mu$ , surrounded by immature centrioles in various stages of growth (Figs. 7, 8, 9, 10, 11). The central mass either is almost completely dense (Figs. 7, 8) or has varying degrees of hollowness (Figs. 9, 10, 11, 12). Immature pro-centrioles, which consist of discs of dense material with little internal organization, will contain individual microtubules in their matrix. In Fig. 8 such an annulus contains two distinctly delineated microtubules (*mt*), while in other discs varying numbers of singlets have been seen.

The detailed structure of the condensation form has remained elusive. Internal structure of some complexity is often suggested (Figs. 8, 10, 13) but



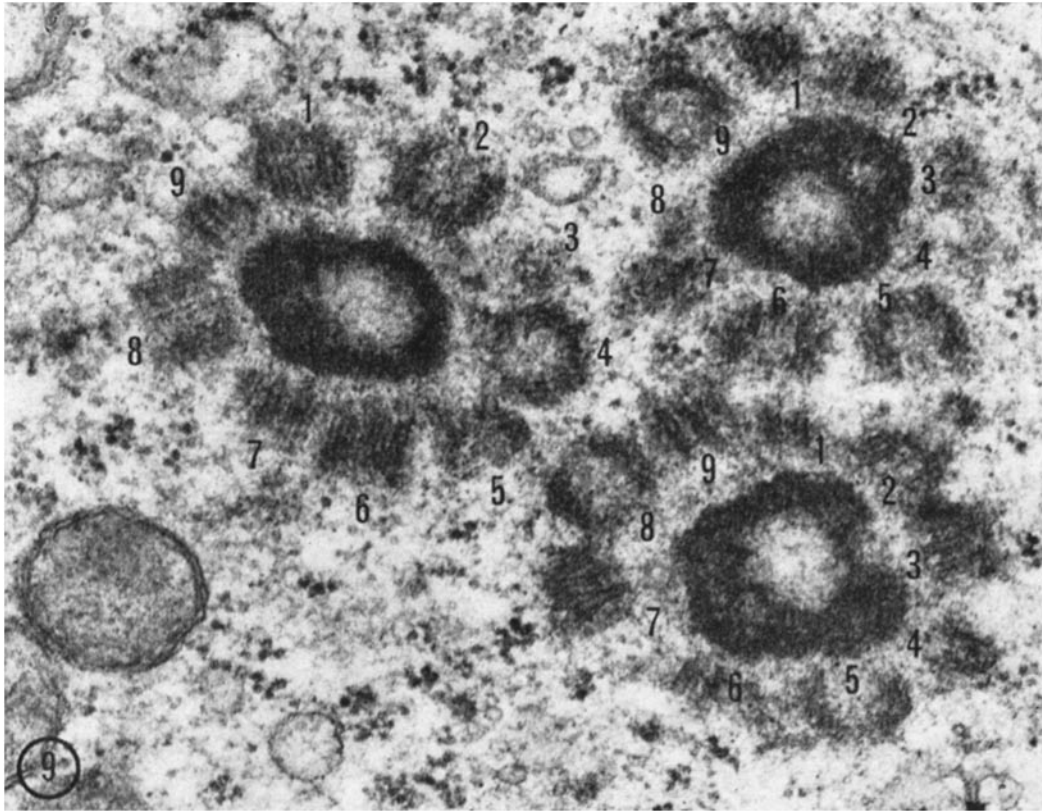


FIGURE 9 Three generative complexes with hollow condensation forms, each surrounded by nine procentrioles. The generative complex at the upper right-hand corner appears to have fewer procentrioles because at positions 4 and 8 the plane of section has missed them. At this stage the microtubular nature of the procentrioles is evident.  $\times 63,000$ .

the density is so great that visualization is difficult even in the thinnest sections. On occasion, helices have been observed within the condensation forms (Fig. 13, arrow). Because of the extreme density of these organelle precursors it can be assumed that they consist of very concentrated deposits of protein.

Several lines of evidence implicate the condensation form as the source of microtubular protein for the assembly of the mature centriole. (a) The condensation form is very large and dense when in association with very immature procentrioles (Figs. 7, 8). (b) As procentrioles mature, the condensation form may decrease in size or become a hollow sphere (Figs. 9, 10, 11, 12, 13). (c) The wall of the condensation form is thinnest where the microtubular elements of the maturing centrioles are most distinct (Figs. 11, 12). Once

centrioles have developed to their mature length, only remnants of condensation forms remain (Fig. 14). (d) In favorable sections there appears to be a continuity between condensation forms and procentrioles by way of a fibrous bridge (Figs. 8, 11; arrows).

In order to ascertain whether the osmiophilic material in condensation forms decreased during centriole growth, volume calculations were determined at early (e.g. Figs. 7 and 8) and late (e.g. Fig. 11) stages of procentriole maturation. These were based on the assumptions that the condensation form is a sphere and that the sections from which the measurements were taken passed through the maximum diameter of that particular condensation form. The amount of osmiophilic material in condensation forms at early and late stages of procentriole growth was found to be

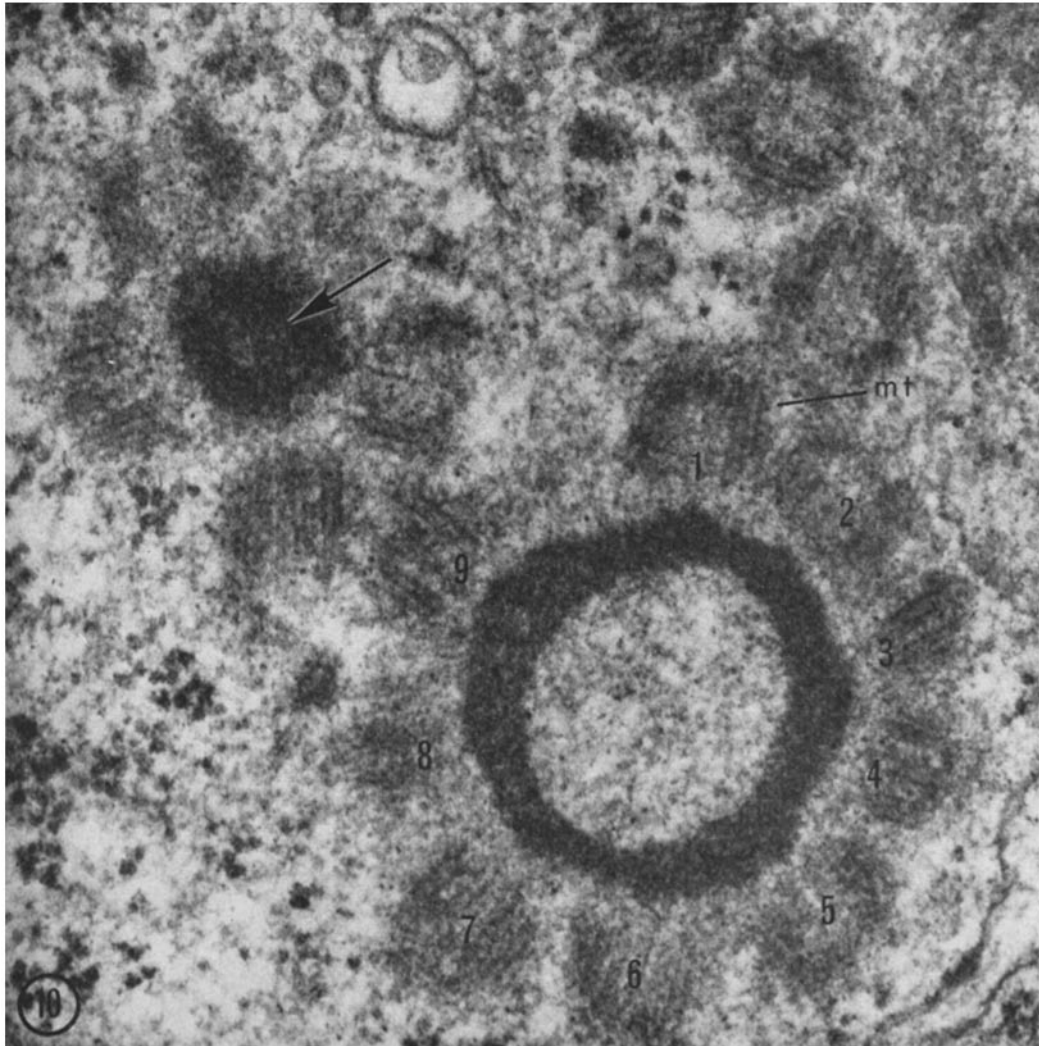


FIGURE 10 A generative complex with the condensation form ( $550\text{ m}\mu$ ) having a wall  $100\text{ m}\mu$  thick. Nine procentrioles are also seen to surround this hollow structure. The microtubules (*mt*) of the procentrioles are very distinct. A generative complex at the upper left demonstrates the globule-like appearance of this dense mass (arrow).  $\times 96,000$ .

$3.5 \times 10^{-14}$  cc and  $2.8 \times 10^{-14}$  cc, respectively. These represent averages of measurements obtained from seven representative electron micrographs for each stage. Although these observations must be interpreted with caution because of the uncertainties about whether a particular section passes through the maximum diameter of a condensation form, the amount of osmiophilic material does appear to decrease as procentriole maturation progresses.

There seems to be no set number of centrioles which can arise from a single condensation form, but as many as nine procentrioles have been found in one plane of section (Figs. 9 and 10). Although immature centrioles are found primarily related to condensation forms, occasionally procentrioles will also be found surrounding a mature centriole (Fig. 12,  $GC_2$ ). A few instances of this have been seen, and in one case the contact between mature and nascent centrioles was much closer than that

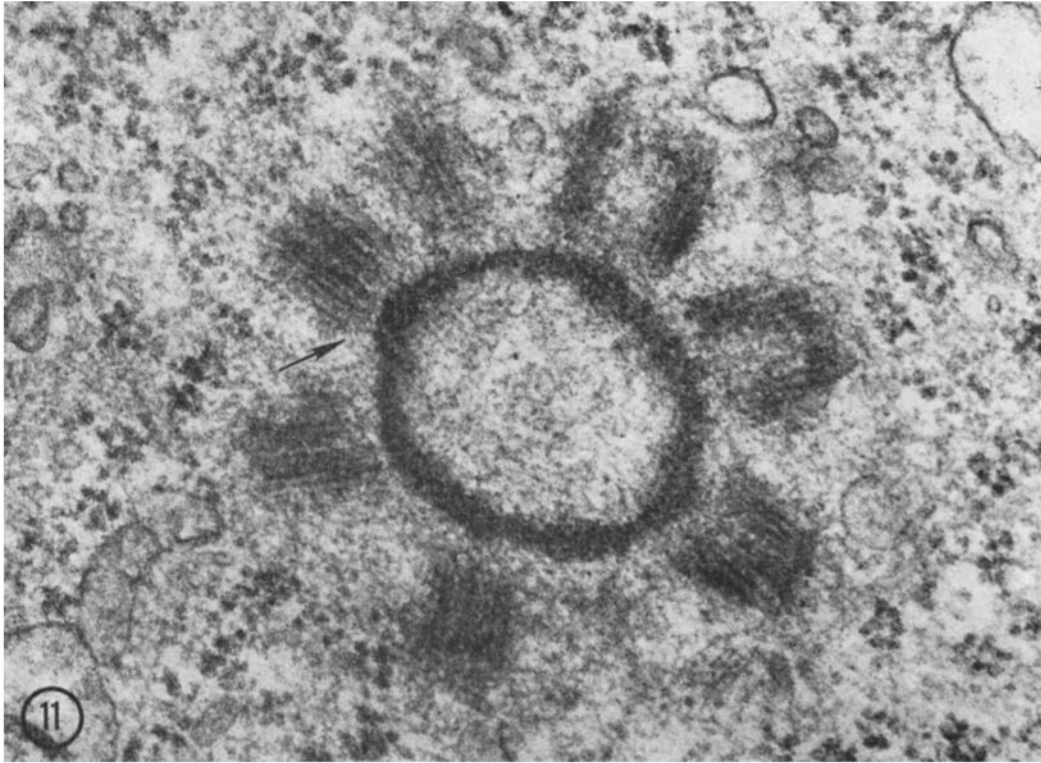


FIGURE 11 The wall of this generative complex is extremely thin ( $55\text{ m}\mu$ ), while the total diameter of the hollow sphere is  $640\text{ m}\mu$  by  $510\text{ m}\mu$ . Microtubules of the procentrioles are often connected by fine strands to the sphere (arrow).  $\times 77,000$ .

seen in Fig. 12. It is also of interest that in this same section there is a more typical condensation form-procentriole relationship.

It is clear from the foregoing that centriole formation in the oviduct ciliated epithelium is not synchronized, even within a single cell. Some cells might have cilia, while others are still forming new centrioles (Fig. 1). More important, within a single cell there are basal bodies at the cell surface, while lower down in the cell an early proliferative element is present (Fig. 2). Often, a cell has not formed cilia but is filled with mature centrioles and "depleted" condensation forms as well as proliferative elements, possibly on a fresh round of replication (Fig. 14, arrows).

Even as cilia have appeared, condensation forms of various sizes remain behind (Figs. 15, 16). The fate of these condensation forms is still not clear, but they must be reabsorbed since mature ciliated cells do not contain them.

#### DISCUSSION

A considerable body of work has accumulated which suggests that centriole morphogenesis is a highly complex process whereby precursor material, probably consisting of microtubular protein, is assembled into the nine triplets of the centriole after being synthesized in the cytoplasm. This process of self-assembly is possible since microtubules have been recently shown to consist of two major proteins similar in general composition and molecular weight (18, 20). Further, radioautographic evidence (7) has also shown that, at least in oviduct differentiating ciliated cells, a considerable amount of the proteins synthesized are found in centrioles and centriole precursors. It is also possible to separate microtubule protein synthesis from microtubule assembly, as has been demonstrated in colchicine-blocked ciliogenesis in *Xenopus* ectoderm (17) and in *Chlamydomonas*

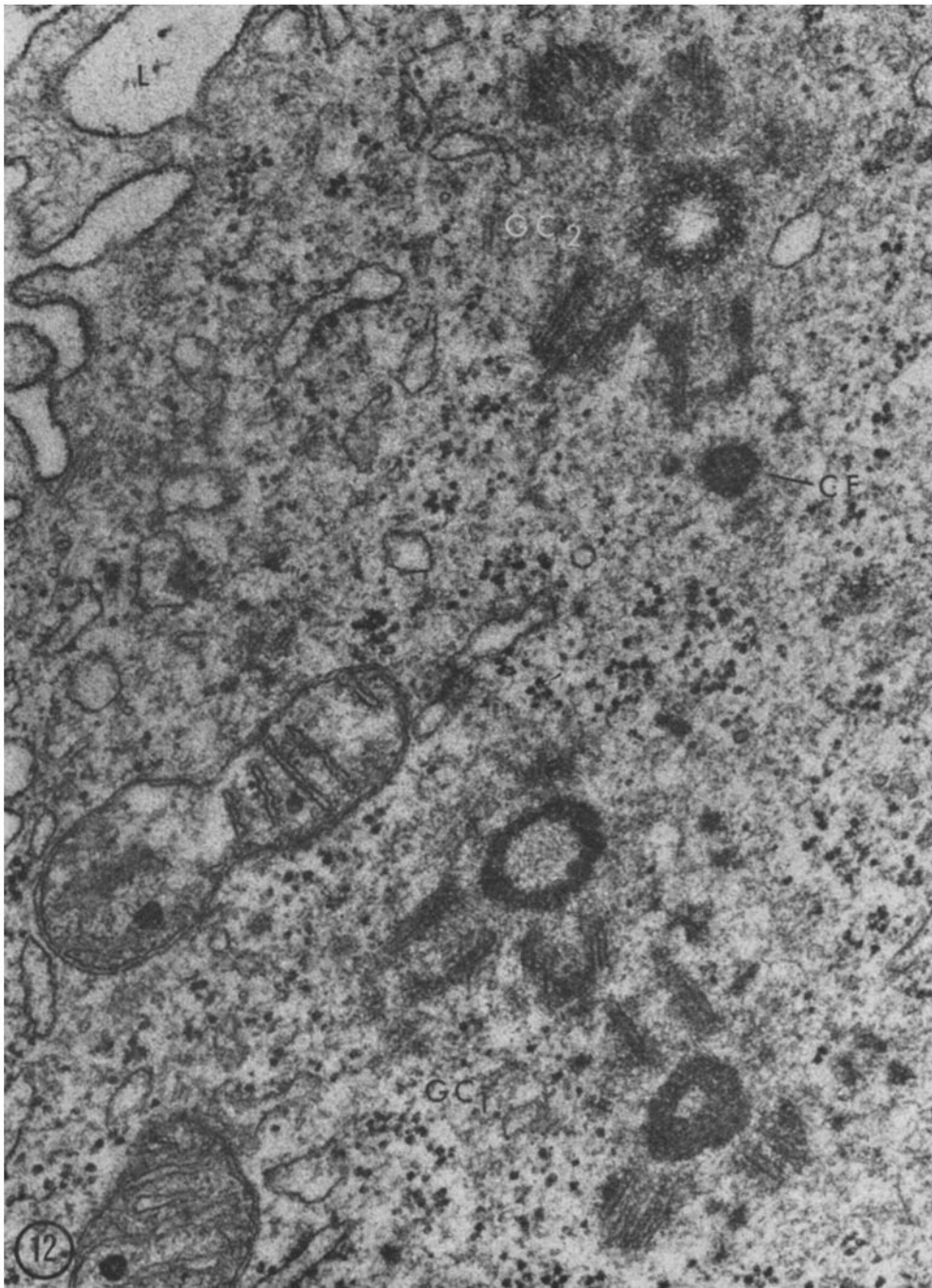


FIGURE 12 Three generative complexes are present in this section near the lumen (L). At  $GC_1$  two hollowed spheres, surrounded by procentrioles, have the typically amorphous appearance of this precursor form, with the wall of one being thicker than the other. The center of a third generative complex at  $GC_2$  consists of a mature centriole, a situation which is encountered less frequently. Note that the length of the procentrioles has increased. *CF*, condensation form.  $\times 60,000$ .

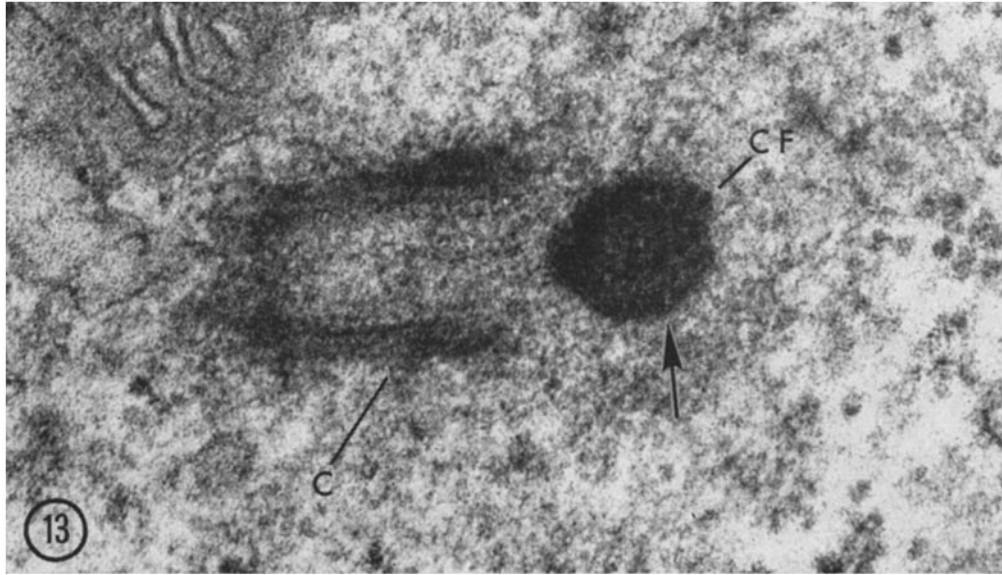


FIGURE 13 A nearly completed centriole (C) with a condensation form (CF, 170 m $\mu$ ). Substructure is discernible, consisting of helical arrays within the matrix (arrow).  $\times 127,000$ .

flagellar elongation (4). These data lead to the inference that once microtubular protein is synthesized, no other mediator is necessary in order to generate centriolar structure.

That microtubular protein can exist in a precursor form in the cytoplasm is demonstrated by the studies of Behnke (2) on the breakdown of microtubules in blood platelets due to cold exposure. These microtubules reassembled without any other mediator once the platelets were placed at room temperature. The small amorphous electron-opaque masses found near the partially reassembled microtubules resemble the structures near centrioles in parthenogenetically activated sea urchin eggs (6), the electron-opaque clusters enmeshed in the fibrillar material of proliferative elements (8), the granules of the fibrogranular aggregates (15), and the procentriole-precursor bodies (16), all structures present in cells producing centrioles.

#### *The Function of the Mature Centriole*

Most authors have not been able to resolve the relationship between the mature centriole and the amorphous material (PE) which now appears to be the earliest candidate for centriole precursor. This material has been found associated with mature centrioles in tracheal epithelium (8) and in this present study.

Stockinger and Cireli (19) reported that although procentriole precursor material gives rise to centrioles, this material arises *de novo*. Steinman (16) has suggested that the first event in centriole formation is the conversion of the dense "procentriole precursor bodies" into procentrioles. Sorokin (15) believes that the fibrogranular aggregates of material are the initial centriole precursors and that these aggregates accumulate near, but not necessarily in direct continuity with, one of the preexisting centrioles in the immature cell. Sorokin (15) and Frasca et al. (9) have further described a close association between these granular aggregates and annulate lamellae. Kalnins and Porter (12) suggest that one of the mature diplosomal centrioles is involved in generating the material from which procentrioles are assembled. Brenner (3) has not been able to find a physical relationship between a mature centriole and the procentriole precursor material in monkey oviduct. In fetal rat trachea, however, Dirksen and Crocker (8) published evidence for a physical continuity between mature centrioles and the amorphous fibrillar meshwork. Evidence for such association has also been found in presumptive ciliated cells of the mouse oviduct. At no time, however, has there been evidence of an association between immature procentrioles and these large amorphous masses of material.

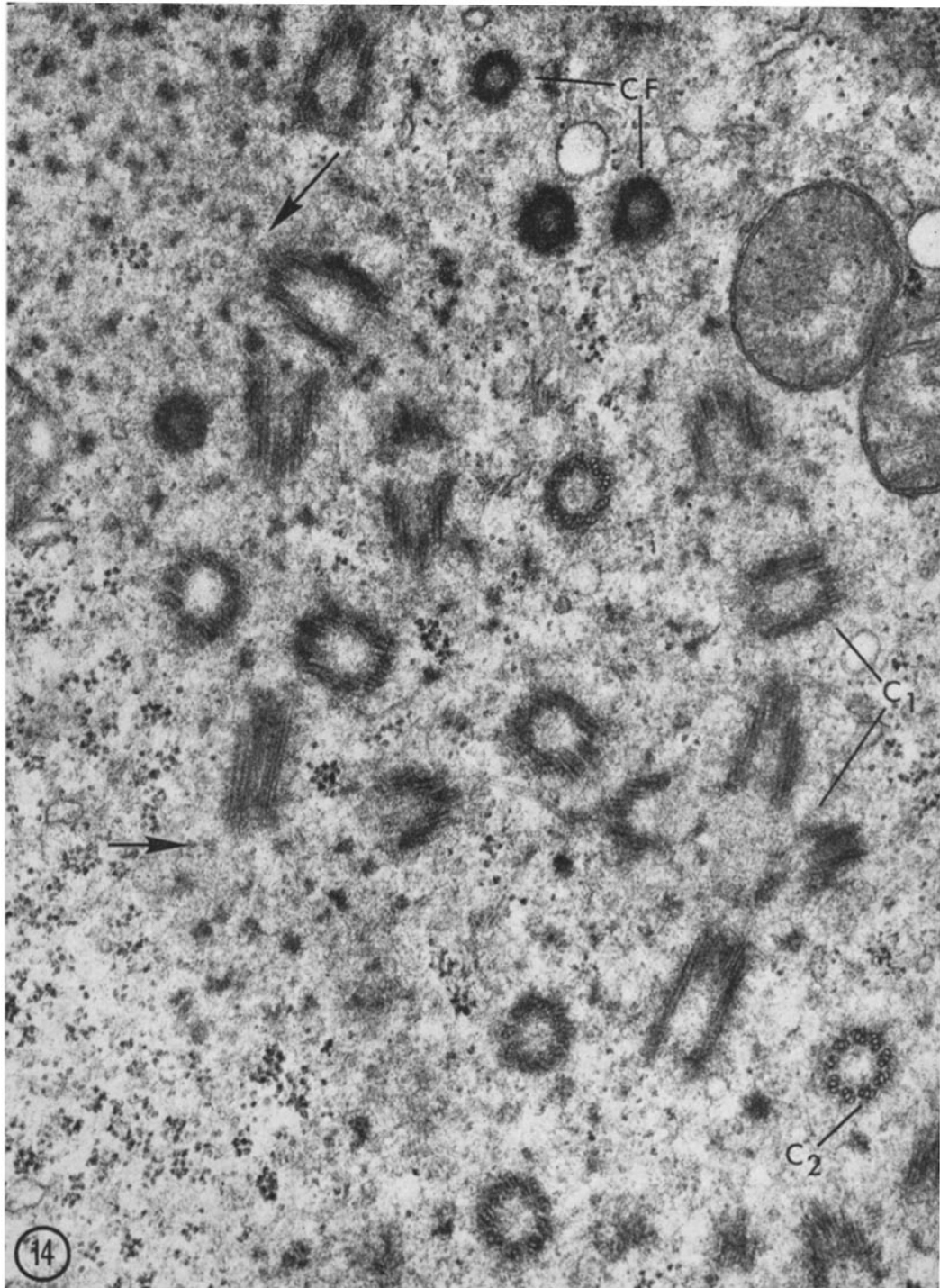


FIGURE 14 Section of a cell showing a large number of centrioles in longitudinal and in cross-section ( $C_1$ ,  $C_2$ ). Condensation forms ( $CF$ ,  $180\text{ m}\mu$ ) without surrounding procentrioles are seen, while two mature centrioles are associated with masses of fibrillar material (arrows), and could be identified as proliferative elements.  $\times 48,000$ .

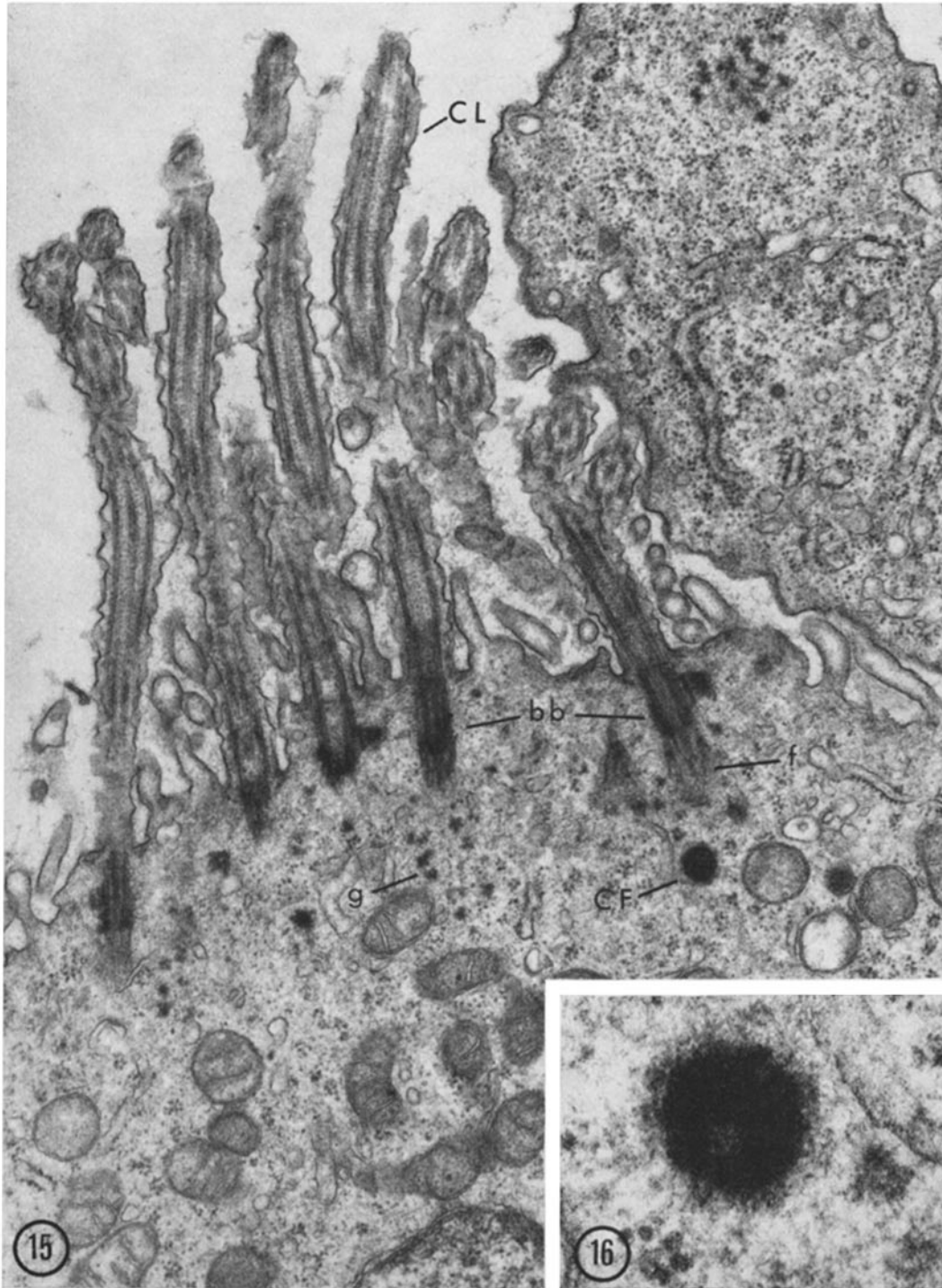


FIGURE 15 A cell with many cilia (*CL*) and a condensation form (*CF*) below a basal body (*bb*). Filamentous material (*f*) can be seen associated with basal bodies as well as electron-opaque granules (*g*) similar to those present in proliferative elements. At upper right, portion of another cell containing a small cluster of granules encrusted in fibrillar material typical of a proliferative element.  $\times 29,000$ .

FIGURE 16 Higher magnification of the condensation form in Fig. 15 ( $180 \mu$ ). There is a slightly less electron-opaque, eccentrically located core, and a fine raspberry-like matrix substance.  $\times 116,000$ .

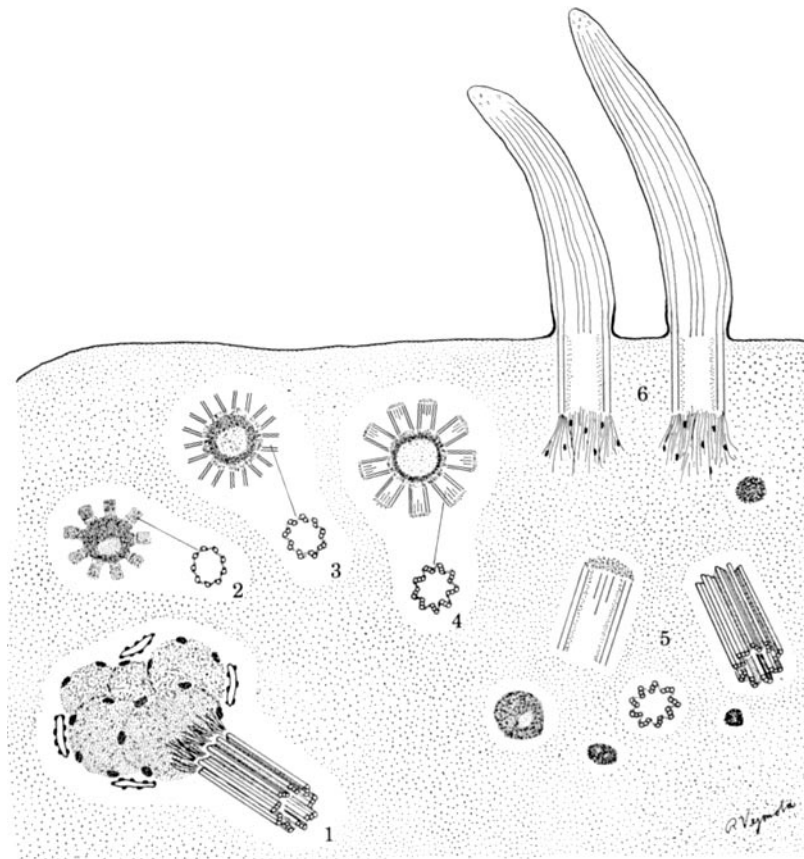


FIGURE 17 A diagrammatic representation summarizing the sequence of events which occurs during centriole and cilium development in the epithelium of mouse oviduct. 1, The beginning of centriole morphogenesis is marked by the synthesis of large amounts of microtubular precursor protein by the endoplasmic reticulum, mediated in some manner by the mature centriole. This precursor material is filamentous or fibrillar in nature and is encrusted with  $75\text{ m}\mu$  granules. 2, The condensation form of this generative complex consists of densely packed material generated earlier by the proliferative element. It is likely that many condensation forms are derived from the material produced by one proliferative element. Nine immature procentrioles, whose walls at this time consists of single microtubules, surround this condensation form. 3, As the wall of the condensation form becomes thinner, due to the utilization of its material in the assembly of microtubules, the procentrioles grow longer as well as adding another set of microtubules. 4, The depleted condensation form, now a hollow sphere, is still surrounded by maturing centrioles, whose walls are composed at this time of the full complement of triplets. 5, The mature centrioles migrate to the apex of the cell while remnants of condensation forms remain behind in the cytoplasm. 6, These centrioles will now function as basal bodies and proceed to generate cilia.

### *The Origin and Fate of the Generative Complex*

Once microtubular protein has been synthesized under the influence of the mature centriole, it must be packaged in some manner into the electron-opaque condensation forms. This is the least understood event in centriole morphogenesis

of vertebrate ciliated epithelia. This structure has, in turn, been called a "deuterosome" by Sorokin (15) and "procentriole organizer" by Steinman (16). Kalnins and Porter (12) found clusters of immature procentrioles around central cylinders. These cylindrical cores, however, are morphologically different from the condensation forms or deuterosomes.



The variety of generative complexes which have been observed in the differentiating ciliated epithelium of the suckling mouse oviduct allows the interpretation that young centrioles actually grow by virtue of the utilization of material from the central electron-opaque condensation form.

As the microtubular triplets of the centrioles are assembled, the material of the condensation forms disappears. This was evident in the differentiating ciliated cells of rat tracheal epithelium (8) and is extensively demonstrated in the present material. The progression of assembly from singlets to doublets to triplets observed in *Paramecium* (5), in *Tetrahymena* (1), and in *Chlamydomonas* (11) has also been partially demonstrated in *Xenopus* (16, 17) as well as in the present study.

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