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PROGRAM OF F-ACTIN IN THE FOLLICULAR EPITHELIUM DURING OOGENESIS OF THE GERMAN COCKROACH, *BLATTELLA GERMANICA*

Keywords: Panoistic ovariole, vitellogenesis, chorionation, follicle cell, ovulation

ABSTRACT. Extensive programmed structural and functional changes of insect follicular epithelium during oogenesis provide a model to study modulation of cytoskeletal organization during morphogenesis in a non-dividing cell population. Rhodamine-phalloidin staining of whole mounted and cryosectioned oogenic follicles reveal changing F-actin filament organization from pre- to post-vitellogenic stages consistent with the presumptive dorsal-ventral orientation of the future embryo. Filaments are not abundant in pre-vitellogenic follicle cells up to day 2. Differences between dorsal and ventral follicle cells appear first on day 3. Obviously patent follicle cells are seen only on the ventral follicle surface which exhibits stronger F-actin fluorescence than the dorsal non-patent epithelium. On the presumptive ventral side of mid-vitellogenic follicles morphologically distinct bundles of actin filaments orient peripherally into projections connecting adjacent follicle cells and from the center of follicle cells apically into macrovillar projections extending toward the oocyte surface. The mid-vitellogenic dorsal follicle cell layer also possesses macrovillar extensions containing F-actin which reach and appear to penetrate the oolema. During chorion deposition major reorganization of actin of follicle cells takes place. After chorion deposition all F-actin filaments within a given follicle cell are arranged into large parallel bundles with semi-regular cross-striations which exclude fluorescent label. The parallel orientation of actin striated filament bundles within each follicle cell appears to be random with respect to the orientation of bundles in neighboring follicle cells over much of the mid-latitude of the follicle epithelium. At anterior and posterior follicle poles a more axial orientation of striated bundles is evident. This muscle-like tissue arrangement is appropriate for cooperation in ovulating the chorionated oocyte from the follicle into the oviduct.

Introduction

The *Blattella germanica* follicular epithelium undergoes substantial morphological alterations during oogenesis. Cell shape and cell to cell contacts have been reported to vary from densely packed columnar, closely apposed cells during pre-vitellogenesis to somewhat spherical shape and reduced cell-to-cell contact during vitellogenesis (Tanaka, 1973). The morphological transformation from a closely packed epithelium to one with wide extracellular spaces provides the pathway for extraovarian yolk precursors to reach the oolema and is known as 'patency' (Davey and Huebner, 1974; Davey, 1981; Telfer *et al.*, 1982). Subsequently the follicle cell patency disappears as the cell layer performs its well-studied role in chorion deposition (Regier and Kafatos, 1985; Margaritis, 1985).

Cytoskeletal elements play important roles in morphogenesis (Cohen, 1979a,b). Microtubules and microfilaments are present in *Rhodnius prolixus* follicle cells and a role has been suggested for this cytoskeleton in mediating follicle cell shape changes associated with 'patency' (Huebner, 1976; Abu-Hakima and Davey 1977a). An analysis of cytoskeleton developmental changes during follicle cell morphogenesis and differentiation is available for the polytrophic meroistic ovariole of *Drosophila* (Gutzeit, 1990)

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and for the telotrophic meroistic ovariole of *R. prolixus* (Watson and Huebner, 1986), but studies for the ancestral (King and Büning, 1985) panoistic ovary are lacking. Dipteran and lepidopteran follicles have distinctly different follicle cells covering the oocyte and nurse cell regions (Regier and Kafatos, 1985; Margaritis, 1985). However, the general impression of the cellular organization of the panoistic ovariole's follicle cell layer is that it is very simple, requiring little diagrammatic elaboration. We hope to change that impression.

The F-actin filamentous patterns in follicle cells of *B. germanica* were studied using rhodamine labelled phalloidin (RhP). The relative abundance, organization and dynamics of the follicle cell F-actin microfilaments cytoskeleton during the principal developmental stages of follicle cells were characterized. Differences in F-actin filament organization of ventral vs dorsal follicle cells during vitellogenesis were discovered. A muscle-like organization and cross striation of the actin bundles in follicle cells at the time of ovulation is described for the first time.

Materials and Methods

Animals

Cohorts of *B. germanica* were synchronously cultured at 30°C (Kunkel, 1966). Oocytes from first parturition females only were used for these experiments. The stage of the terminal follicle is measured in days after feeding first parturition adults at 30°C (Kunkel, 1973). In the text 'follicle' is used to indicate the oocyte plus the surrounding follicle cell layer, and 'follicle cells' for the follicular epithelium.

Insect saline

The physiological insect saline solution (ISS), pH 6·8, had the following composition (mM/l): NaCl, 145; KCl, 14; $CaCl_2$, 4; $MgSO_4$, 5; H_3PO_3, 11; sucrose, 110. Penicillin (125 mg/l) and streptomycin (50 mg/l) were added as preservatives (Bowdan and Kunkel, 1990).

Fluorescent probe

Rhodamine-phalloidin (RPH) (Sigma Chemical Company, St Louis, MO, USA) was used to label filamentous actin (Wulf *et al.*, 1979).

Preparation of whole mounted oocytes

Ovaries were dissected from the body cavity and cleaned of deposits of fat body in ISS. Individual ovarioles were fixed with 4% paraformaldehyde and 0·1% glutaraldehyde (in ISS) for 2 hr at room temperature. Fixation was followed by three washes with ISS. Follicles were then permeablized with 0·15% Triton X-100 in ISS for 5 min at room temperature, then washed three times with ISS. Fluorescent labeling was done in RPH solution (300 ng RPH/ml ISS) for 20 min at room temperature. After three washes in ISS the follicles were mounted on microscope glass slides in mounting buffer (50% glycerol: 50% ISS containing 2·5% n-propyl galate).

Preparation of cryosectioned oocytes

After fixation (4% paraformaldehyde and 0·1% glutaraldehyde in ISS) and permeablization (0·15% Triton X-100 in ISS) follicles were infiltrated with successive concentrations (10%, 20%, 30%) of sucrose solution for at least 2 hr before sectioning. Follicles were embedded in HISTO PREP frozen tissue embedding media, sectioned on an International Cryostat, Model CT1, and stored in a freezer. Follicles were sectioned longitudinally in the dorso-ventral plane. The oocyte nucleus marks the presumptive ventral surface (presumptive germ band). Before staining sections were dried in air for 2 hr, and washed three times (10 min each) with phosphate buffered saline (PBS), pH 7·2. Fluorescent staining was done in 0·3 ug RPH/ml ISS for 20 min at room temperature and after three additional washes in PBS, sections were mounted in mounting buffer.

Fluorescent microscopy and photography

Fluorescence microscopy was performed on a Nikon Optiphot microscope using ×100 objectives. Photographs were taken using Kodak TMAX film, ASA 400, and developed using Kodak TMAX developer (Eastman Kodak Company, Rochester, NY).

Transmission electron microscopy

The follicles were fixed in 2·5% glutaraldehyde buffered to pH 7·4 with 0·1 M piperazine-N,N′-bis [2 ethanesulfonic acid] (PIPES) (Sigma, USA). After fixation for

2 hr at 4°C, the follicles were washed three times (15 min each) at room temperature with PIPES buffer, and were post-fixed for 3 hr at room temperature in a 1% OsO_4 solution buffered to pH 7·4 with 0·1 M PIPES. The follicles were quickly rinsed with distilled water and were dehydrated through a graded ethanol series (25%, 50%, 70%, 95%, 100%, 100%). The follicles were then embedded in an epon resin mixture with the aid of the intermediate solvent, propylene oxide. The specimens were sectioned with a Sorvall MT-2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined on a JEOL 100 transmission electron microscope.

Results

General morphology of follicle cells

Follicle cells form a single-layered epithelium surrounding the developing oocyte. At late pre-vitellogenic stage the follicle cells rapidly increase in number, becoming cuboidal and tightly packed. Their cuboidal nature is maintained until the onset of vitellogenesis when they become columnar as described in *Periplaneta* (Anderson, 1964). Figures 13 and 15 are examples of columnar follicle cells of a day 3 follicle. With the onset of vitellogenesis patency of the follicle epithelium develops; i.e. intercellular spaces between follicle cells appear (Fig. 1) and increase gradually throughout vitellogenesis (Figs 2, 5). Although the follicle cell/oocyte interspace widens with the onset of rapid vitellogenesis, macrovillar extensions from the apical border of follicle cells still interdigitate (Figs 4, 5) and maintain contact with oocyte microvilli (Fig. 6). During the progress of vitellogenesis the shape of ventral follicle cells become somewhat spherical as they separate from one another to become patent (Figs 17, 19, 21) while dorsal follicle cells remain tightly attached to one another and change from columnar to squamous as they are stretched. The follicle cells at this stage are binucleate (Fig. 2) and have stopped dividing. To cope with the increase of oocyte size, coinciding with the most rapid deposition of yolk protein, the follicle cells gradually stretch and flatten. At the end of vitellogenesis and the beginning of chorion deposition, both dorsal and ventral follicle cells again closely associate in a hexagonal pavement block fashion and the patency of fully stretched and flattened ventral follicle cells disappear (Fig. 3).

The follicle cells of *B. germanica* display a typical characteristic of insect follicle cells, a huge, binucleate, polynucleolar nucleus (Fig. 1). The binucleate character appears by day 2 and applies to all vitellogenic follicle

Figs. 1–3. Differential interference contrast (DIC) micrographs of selected stages of ventral follicle cell layer showing growth and shape change. Labels: *N* nucleus; *Ne* nucleolus; *Cy* follicle cell cytosol; *S* intercellular space between adjacent follicle cells.

Fig. 1. Onset of vitellogenesis (day 3) showing rounded binucleate cells with small intercellular spaces. ×400.

Fig. 2. Mid-vitellogenic (day 4) follicle is highly patent with large intercellular spaces evident. The large binucleate cells have prominent nucleoli which are testimony to the follicle cell layer's active synthetic role. Cells have grown substantially relative to day 3 cells (Fig. 1). ×400.

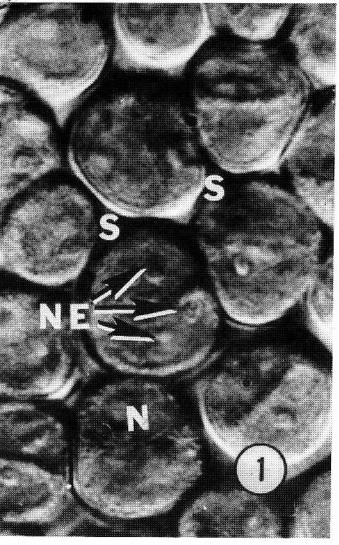
Fig. 3. Just prior to ovulation (day 6) the follicle cells lose their patency, becoming involved with chorion production and preparation for ovulation. ×400.

Figs. 4–6. Follicle cell/oocyte interface of vitellogenic follicles.

Fig. 4. Early vitellogenic (day 3) showing non-patent dorsal follicle epithelium with macrovilar extensions of the follicle cell apex toward the microvillate oocyte surface. ×7500.

Fig. 5. Mid-vitellogenic (day 4) showing patent follicle cell layer with extensions from apical side of follicle cells interleaved with oocyte microvilli. ×5000.

Fig. 6. Interdigitation between macrovilli from follicle cell and microvilli of oocyte surface. Arrows indicate contacts between macrovilli from follicle cells and microvilli of oocyte surface. ×33,000.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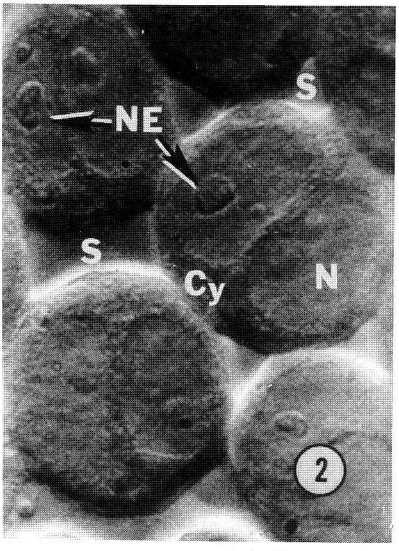
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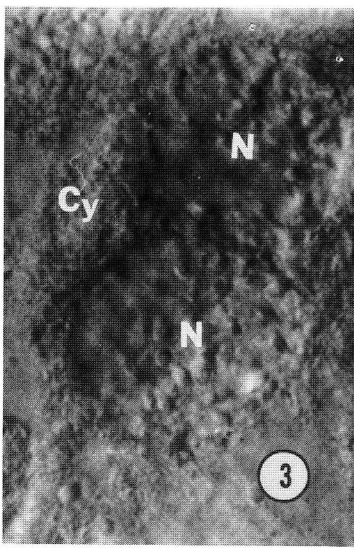
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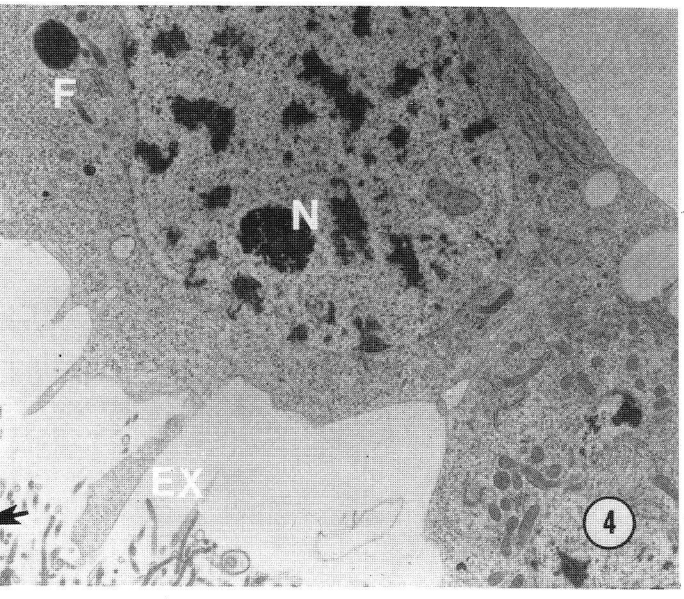
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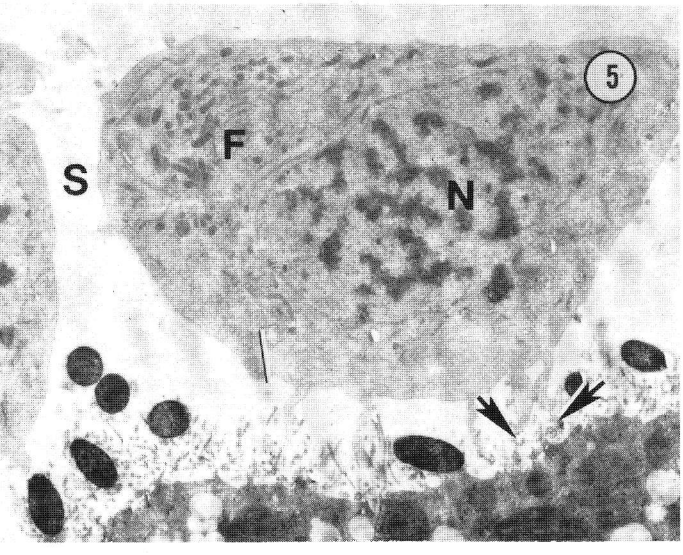
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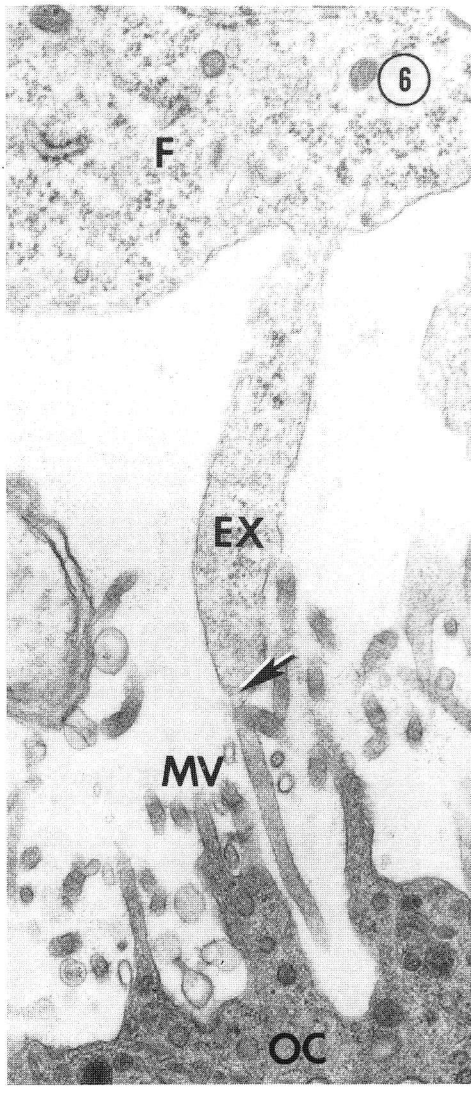
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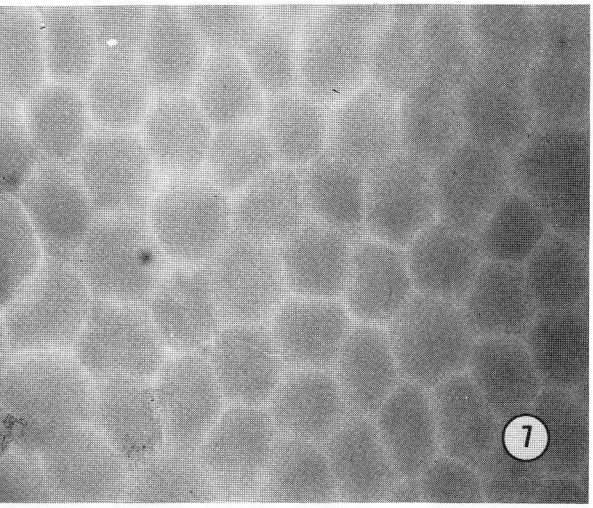
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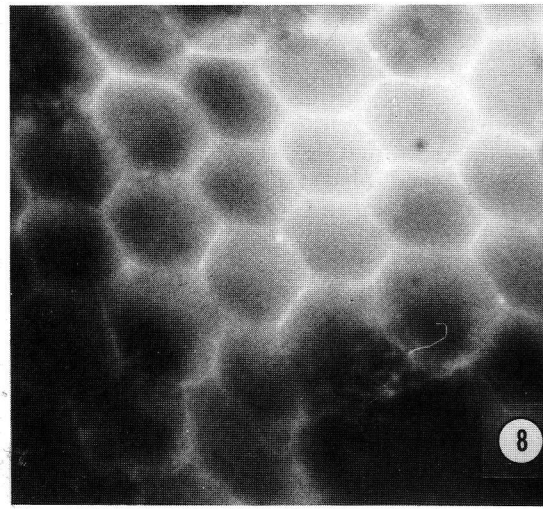
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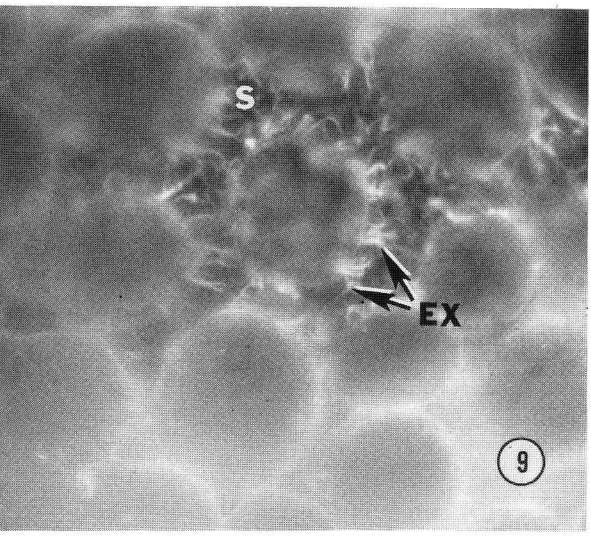
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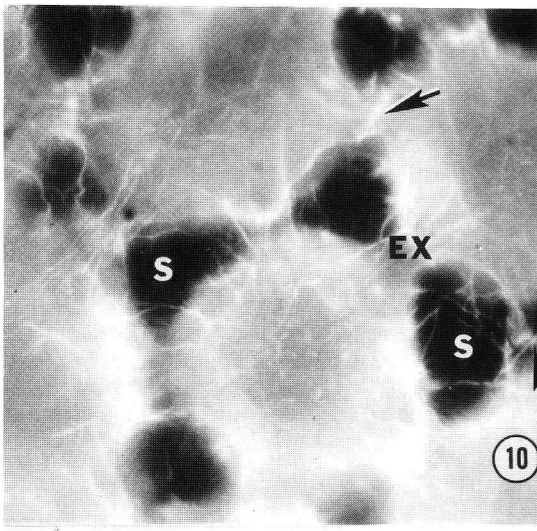
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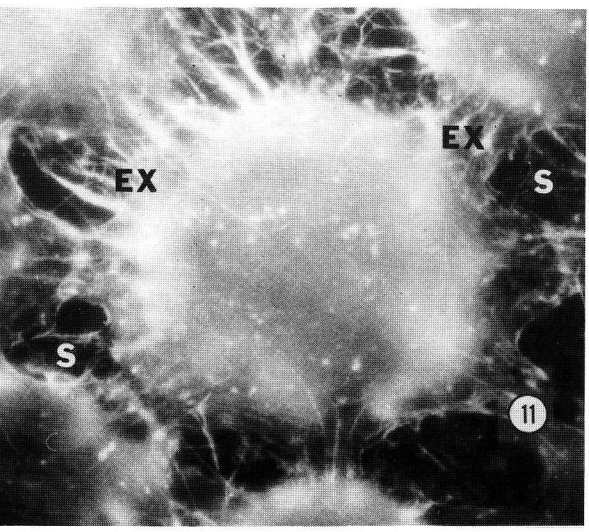
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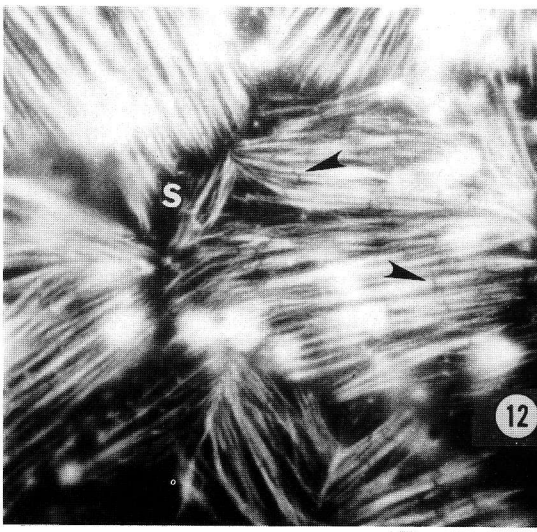
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Fig. 10). A lower fluorescence in the center of the follicle cell is due to the presence of huge double nuclei excluding F-actin. During mid-vitellogenesis (day 4 and day 5) the strong fluorescence of F-actin filaments and prominent actin filament bundles are localized at the periphery of the cytoplasm, and also concentrated within the lateral extentions connecting adjacent patent follicle cells (Figs 10, 11).

Following vitellogenesis but before ovulation all follicle cells, both dorsal and ventral, form a squamous epithelium. All F-actin filaments within a given follicle cell become arranged into parallel bundles. These bundles stretch from edge to edge of each flattened follicle cell and lie roughly parallel to the plain of the follicle cell epithelium and adjacent oocyte surface. The labeling with rhodamin-phalloidin is homogeneous and uniform along the bundles but is interspersed at points by non-fluorescent cross striations (Fig. 12).

F-actin patterns of follicle cells in cryosectioned follicles

The rhodamine-phalloidin fluorescence o early-vitellogenic follicles is displayed as a bright sheet of fluorescence along the latera contacts of follicle cells (similar to tha observed within the pre-vitellogenic follicle cells, data not shown). These sheets are

present within the columnar, apposed, nonpatent early follicle cells (arrowheads in Figs 14, 16). The apical border of follicle cells and cortex of the oocyte display a much stronger fluorescence compared to the central cytoplasm of follicle cells and ooplasm. This feature was obscured in whole mounts by looking face-on through the thinnest dimension, but enhanced in the cryosections viewed on edge.

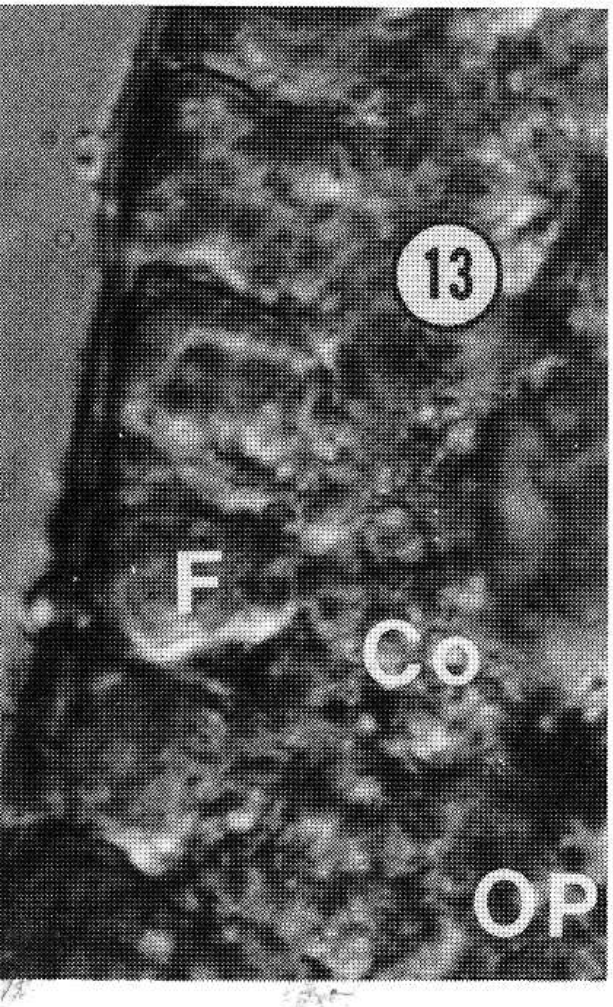
The patent mid-vitellogenic follicle cells (day 4 and day 5) contain extensive F-actin filament fluorescence which is concentrated on the apical surface (Figs 19, 21). The dense fluorescence reveals during optical sectioning (not shown) detail that can be described: F-actin bundles extend from the center of the cytoplasm to the apical surface and extend into macrovilli reaching toward the oocyte surface and occasionally seeming to penetrate the oocyte surface (Fig. 22). These bundles are parallel and regularly arranged as depicted schematically Fig. 23. Relatively strong fluorescence is also present within the lateral cell extensions connecting adjacent follicle cells (arrowheads in Figs 19, 20). However, the ooplasm is largely free of F-actin filament bundles.

A most interesting observation on this developmental series is that the ventral follicle cells have different morphology and different F-actin patterns from the dorsal follicle cells. As shown in Figures 13–22, and schematically in Figure 23, these differences appear in early vitellogenic follicle cells. When extracellular spaces between follicle cells start to appear on the ventral side (Figs 9, 13, 14) the follicle cells on the dorsal side are still tightly apposed to each other (Figs 15, 16). The differences are also reflected in the distribution of F-actin filament fluorescence. The fluorescence at the peripheral edges of dorsal follicle cells is much clearer and more discrete than that of ventral cells (arrowheads in Figs 14, 16). During mid-vitellogenesis the longitudinal cross- sectional view of lateral connecting arms between ventral follicle cells become much narrower than that of dorsal follicle cells leading to a beaded appearance of the ventral epithelium (Figs 19, 21 vs 20, 22, and 17 vs 18). The dense fluorescence of baso-apically oriented parallel F-actin filament bundles which are present on the apical side of ventral follicle cells (Figs 19, 21) is absent in dorsal follicle cells (Figs 20, 22). During the progression of vitellogenesis the F-actin pattern of ventral follicle cells remains the same (Fig. 21), but dorsal follicle cells lose distinguishable F-actin associated with boundaries between follicle cells (Fig. 22), which they previously showed (Fig. 16). Brightly stained F-actin bundles in follicle cell macrovillar extensions become deeply embedded in ooplasm (arrows in Fig. 22).

Discussion

The microtubules and microfilaments, present in *R. prolixus* (Huebner and Anderson, 1970, 1972; Abu-Hakima and Davey, 1977a) and *Drosophila* follicle cells (Gutzeit, 1986a,b), have been interpreted as having a cytoskeletal role in the patency of intercellular spaces (Huebner, 1976; Abu-Hakima and Davey, 1977b; Huebner and Injeyan, 1980). No previous study has followed in detail the changes in arrangement and abundance of F-actin filaments in the insect panoistic follicle cell during differentiation associated with oogenesis. We utilized rhodamine-phalloidin fluorescence to provide a view of these developmental changes. Rhodamine-phalloidin binds specifically to filamentous actin (Wieland, 1977) and does not cross react with other cytoskeletal proteins or components associated with the cytoskeleton (Faulstich *et al.*, 1988). This compound has proven to be a reliable and powerful method to visualize actin microfilament patterns (Taylor and Wang, 1980; Wieland *et al.*, 1983; Warn *et al.*, 1984, 1985).

Rhodamine-phalloidin fluorescence provides additional evidence that the initiation of patency is correlated with an increase in and reorganization of actin microfilaments in follicle cells. During the development of patency of the vitellogenic follicles of the blood-sucking bug, *Rhodnius*, follicle cells round up (Davey, 1981). This has been shown to be an hormonally regulated event involving both ionic flux and actin rearrangement (Davey, 1981). In the cockroach we find less dense F-actin fluorescence within the pre-vitellogenic follicle cells and a progressively increased overall intensity of fluorescence at the onset of vitellogenesis. In addition, the F-actin forms into morphologically discrete bundles which are visible at the light microscope level. This pattern of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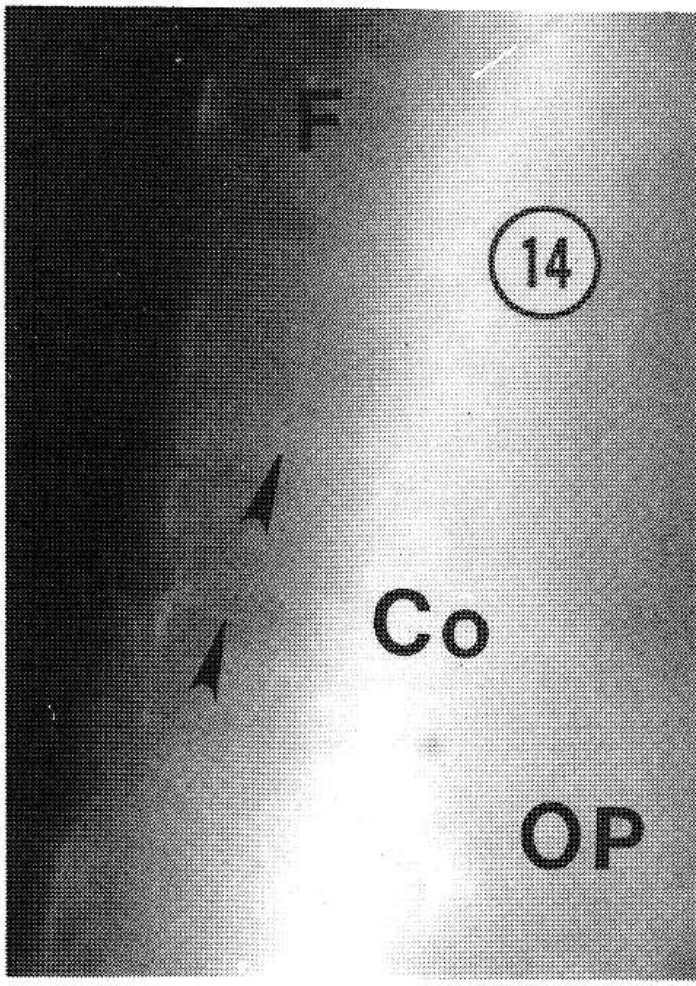
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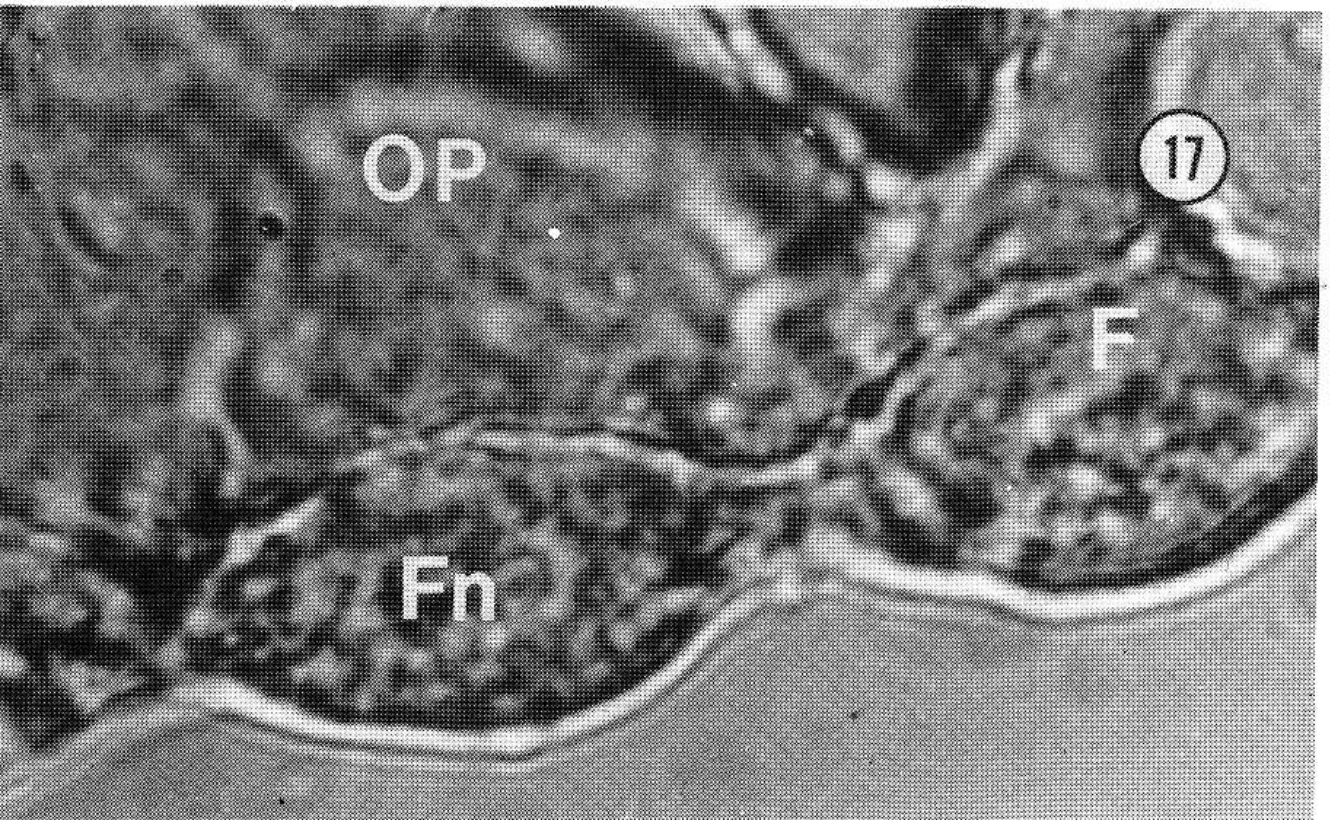
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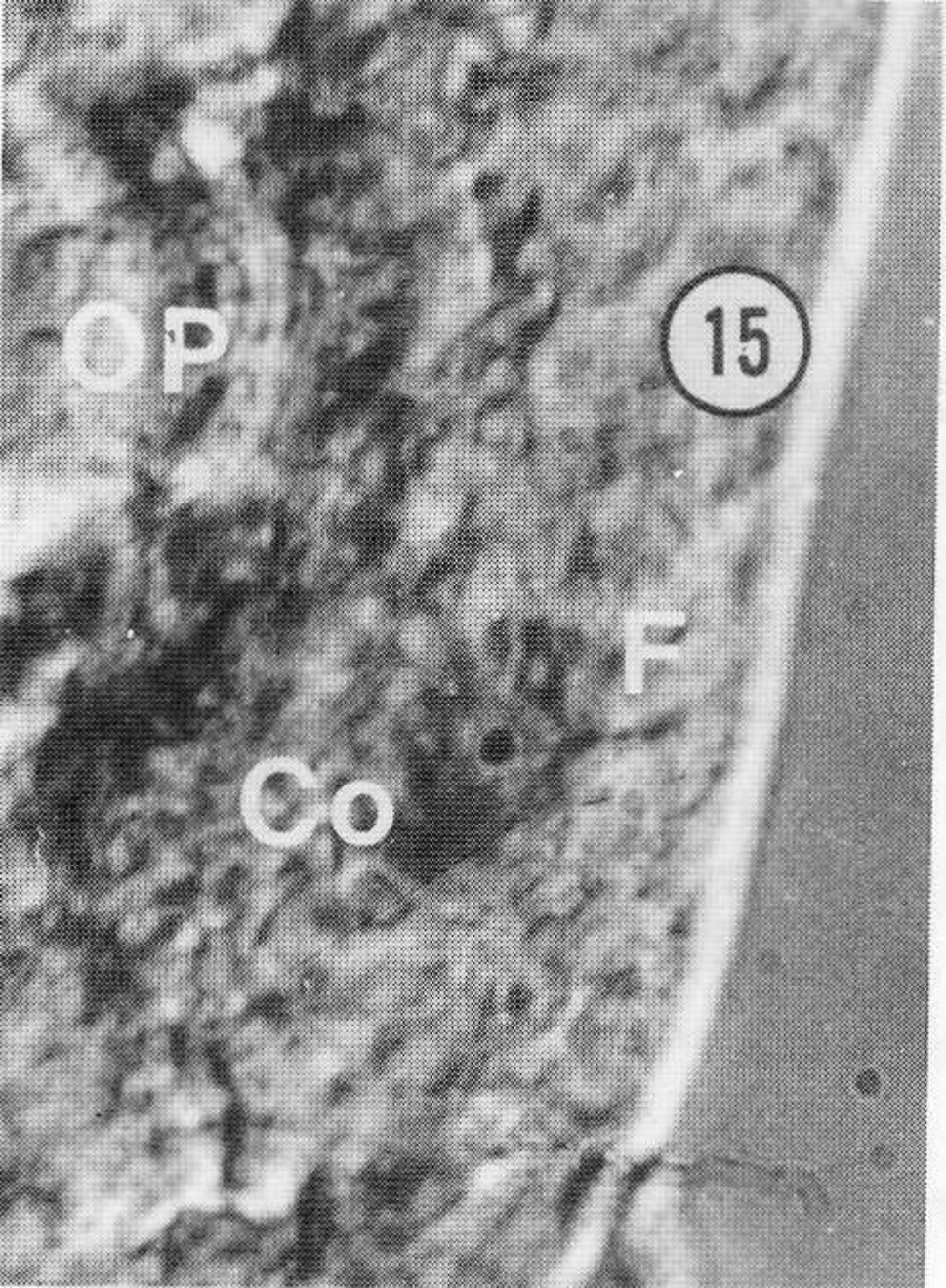
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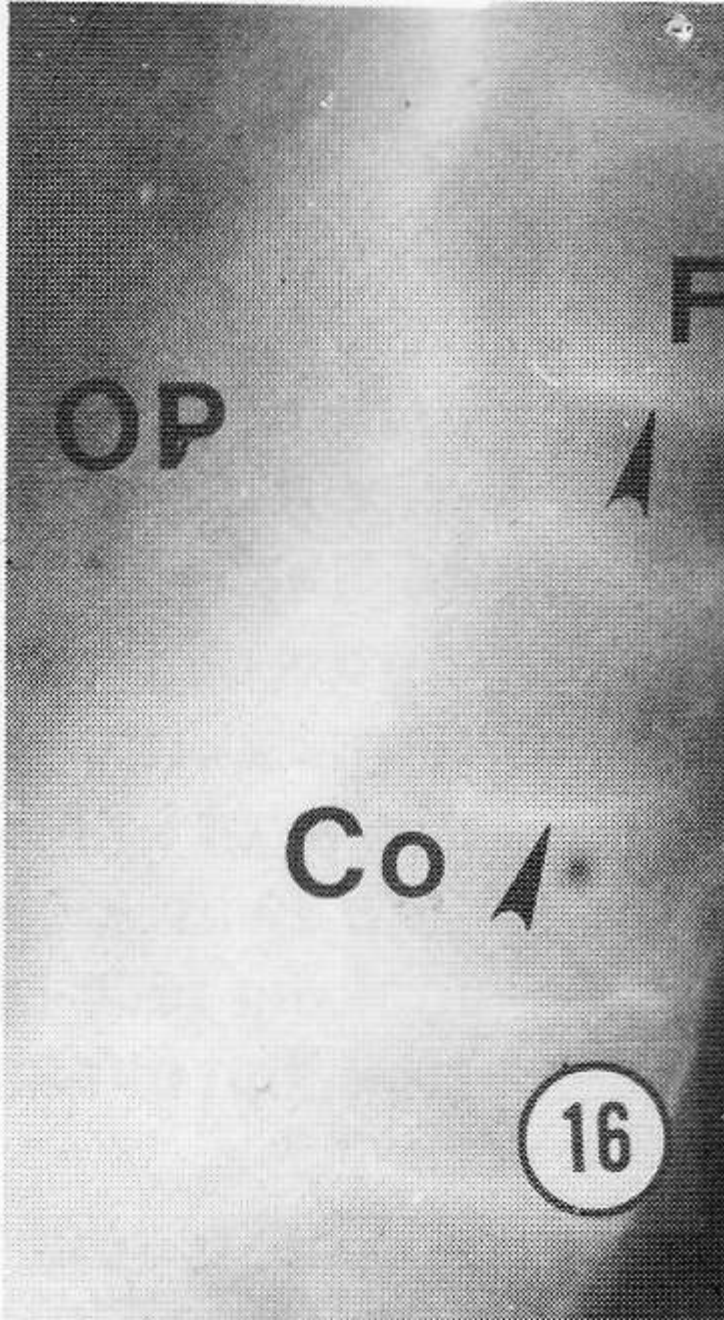
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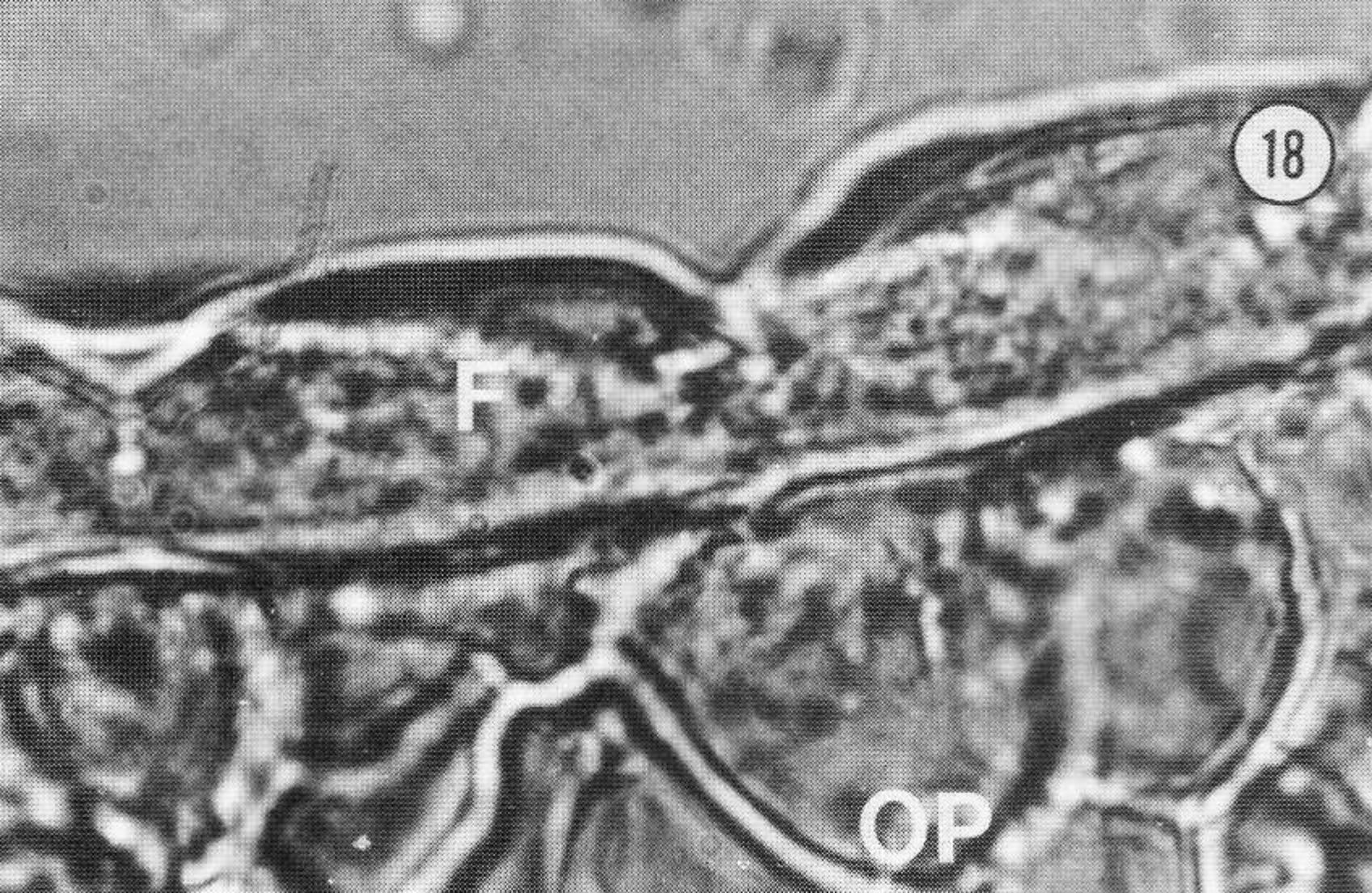
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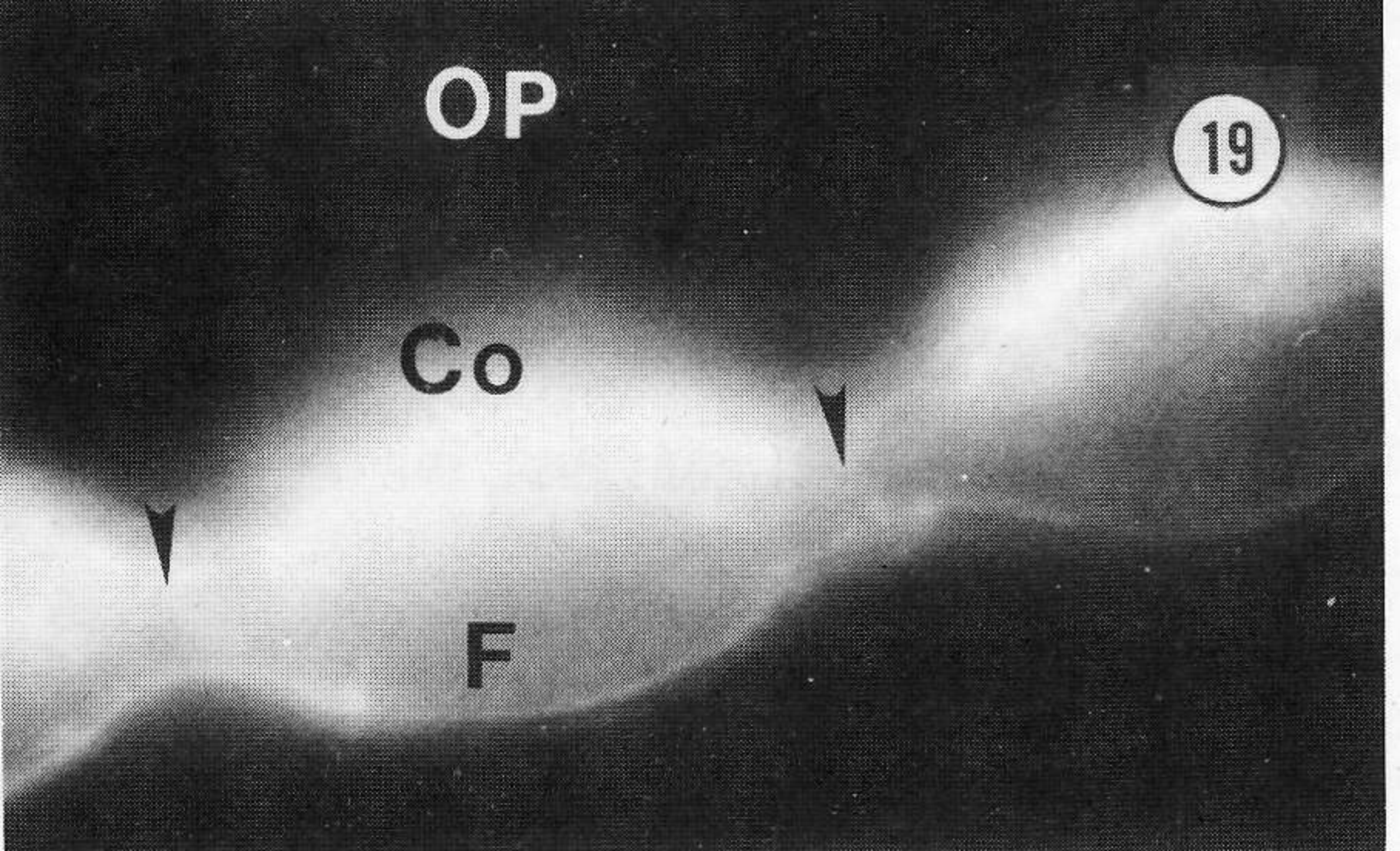
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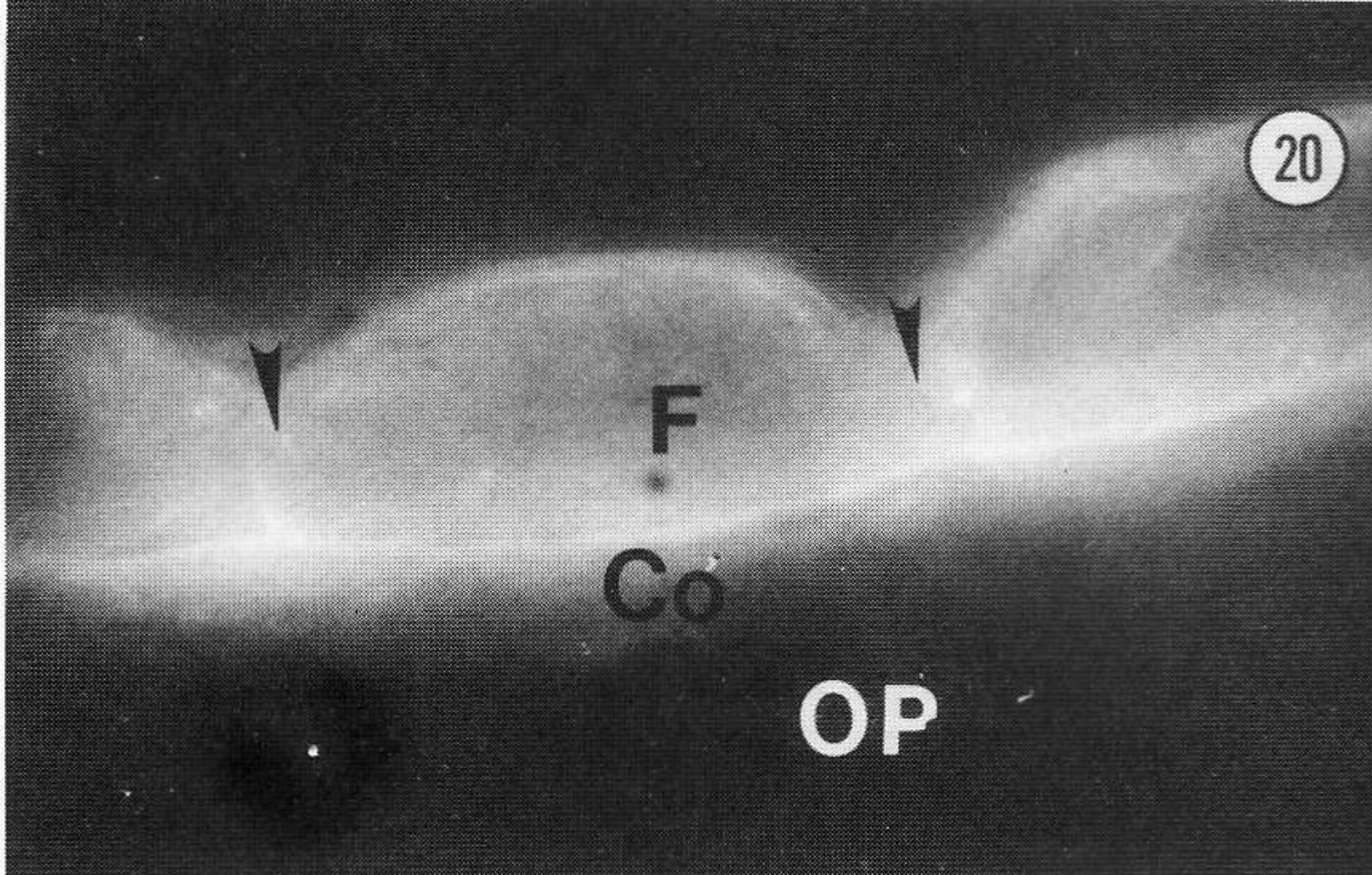
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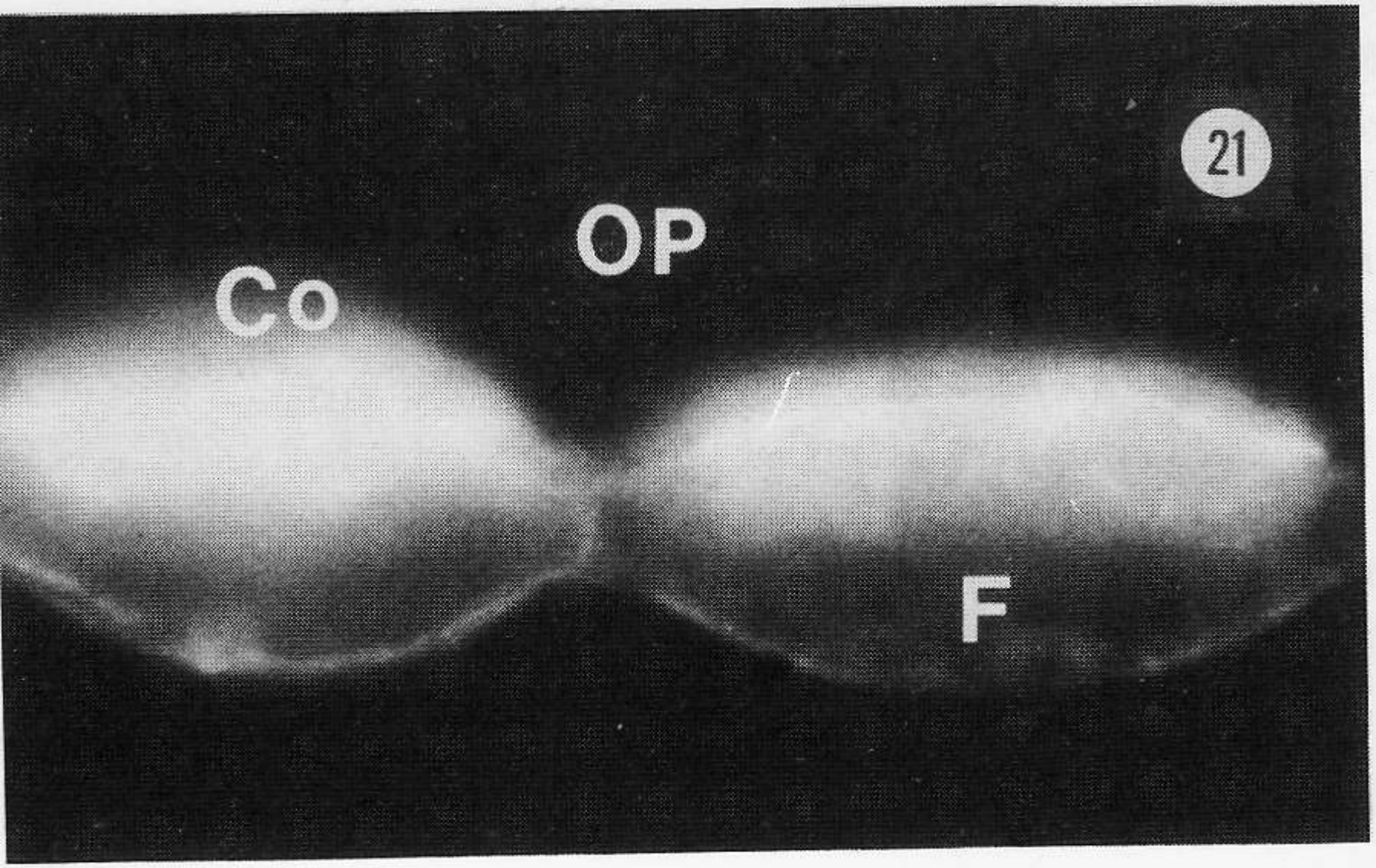
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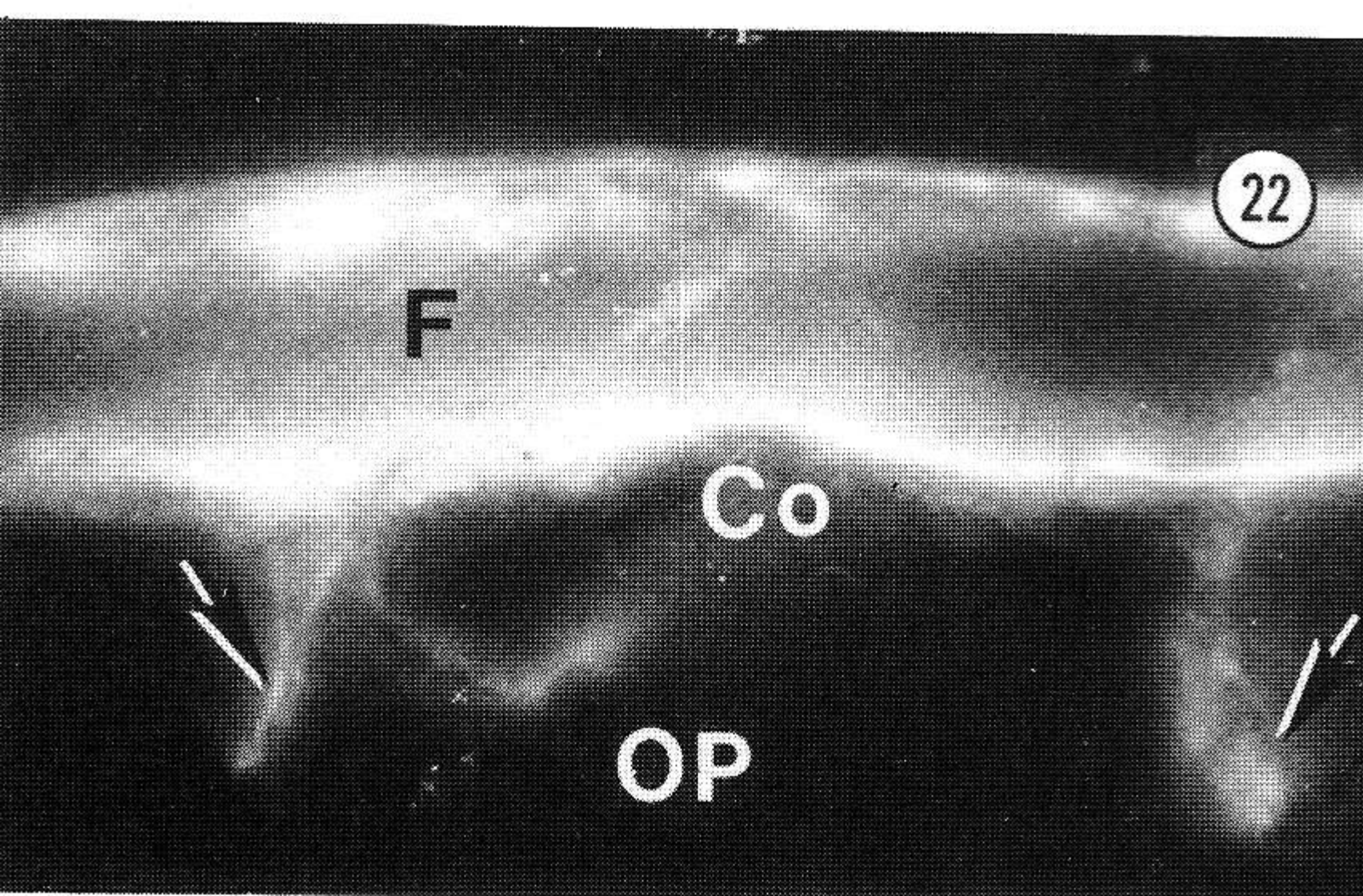
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actin filament increase and distribution is one of the particular characteristics of patent follicle cells (Figs 7–11).

F-actin filaments can form the main cytoskeletal constituents of very stable cell structures such as the intestinal brush border (Mooseker, 1976; Hirokawa *et al.*, 1982) or the sterocilia of the cochlear hair cells (Tilney and Saunders, 1983; Tilney *et al.*, 1983). The formation of prominent F-actin bundles within particular structures of the patent follicle cells indicates their association with the modulation of cell shape at those locations. In particular, F-actin is associated with the formation of the lateral connections between adjacent follicle cells (Figs 9, 10, 11) and the macrovillar extensions toward the oocyte surface (Figs 19, 22).

Microfilaments are often transitory cell structures as exemplified by the formation of the contractile ring or cleavage furrow of mitotic cells (Fujiwara *et al.*, 1978; Schroeder, 1981). In some instances of transitory function the use of stabilizing agents during fixation and staining are necessary to improve the visualization of the actin bundles. This is not the case with the follicle cell layer F-actin bundles; they seem to be highly stable without extra effort. In non-muscle cells together with myosin they can form various contractile systems. The thick F-actin parallel bundles with cross striations present in post-vitellogenic follicle cells (Fig. 12) may aid the transition of the follicular epithelium into a squamous sheet and also assist the ovulation of mature oocytes into the oviduct by filamentous contraction. The random orientation of adjacent cell bundle axes in the mid-latitude of the follicle is consistent with an isomorphic contraction which would contract the follicle cell layer and overlying tunica propria to expel the chorionated oocyte singly into the oviduct. A contraction of the more axialy orientated striated bundles in the posterior pole latitudes would cooperate with opening the follicle cell sphincter which would facilitate the ovulation process. This late ovulatory function of follicle cells has received little attention but represents a distinct temporal and substantial spatially differentiated morphological stage compared to the previtellogenic, vitellogenic and choriogenic stages. The panoistic insect follicle may be an ideal system in which to study this ovulatory role which may be obscured in more complex insect follicle types and in

Figs. 13–22. Sagital cryosections of follicles viewed by DIC and F-actin staining at different stages of development. General labels: *F* follicle cell; *Fn* follicle cell nucleus; *Co* cortex of oocyte; *Op* cytoplasm of oocyte.

Fig. 13. Ventral day 3 follicle cell epithelium. ×380.

Fig. 14. The apical cytoplasm of Figure 13 follicle cells are highly fluorescent. Follicle cell borders with other follicle cells are barely distinct with the RPH stain. ×380.

Fig. 15. Dorsal day 3 follicle cell epithelium. ×380.

Fig. 16. The apical cytoplasm of Figure 15 follicle cells continues to be highly fluorescent. Follicle cell borders with other follicle cells are highly distinct with the RPH stain. ×380.

Fig. 17. Ventral day 4 follicle cell layer showing beaded look of patent epithelium. ×380.

Fig. 18. Dorsal day 4 follicle cell layer showing ribbon-like non-patent epithelium. ×380.

Fig. 19. The apical cytoplasm of Figure 17 is highly fluorescent and a disc like structure (arrow) is seen in the extensions joining adjacent cells. ×380.

Fig. 20. The apical cytoplasm of Figure 18 is not fluorescent and the mild strictures between cells have prominent actin bundles. ×380.

Fig. 21. Ventral (day 5) follicle cells continue to have bright apical staining and mildly bright discs of actin in epithelial borders. ×380.

Fig. 22. Dorsal (day 5) follicle cells lose some of the prominence of actin bundles associated with cell–cell borders. ×380.

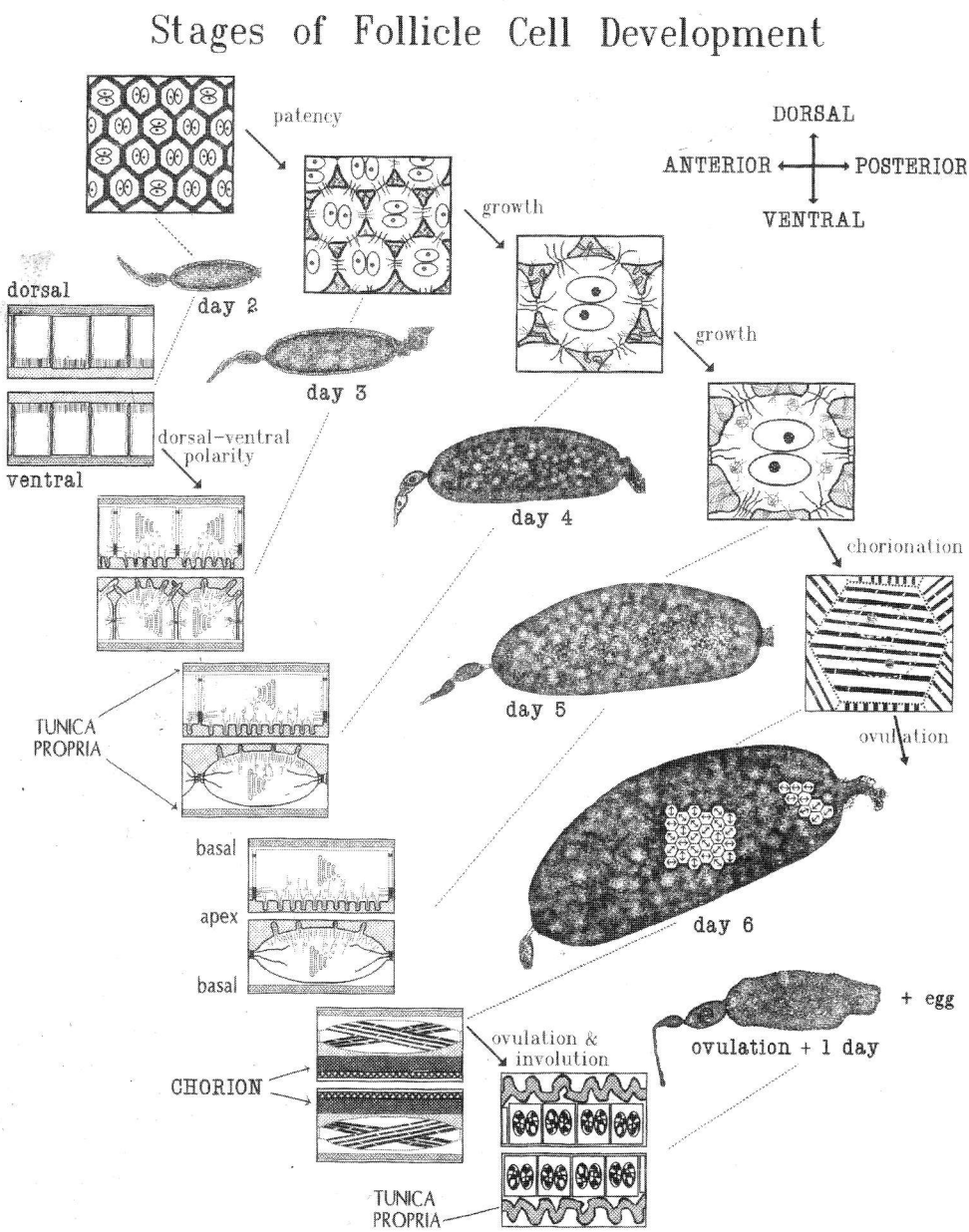

Fig. 23. Changes of F-actin distribution and organization in the follicular epithelium during oogenesis of *B. germanica*. The changes of size and shape of whole follicles at different stages of oogenesis are depicted as a 7 day cycle of a follicle. The orientation of the presumptive embryo relative to ovarian follicles is indicated by a compass rose (upper right). Upper inset drawings are ventral surface views of stages of whole mounted follicle cells showing the development of patency and the growth which occurs in the absence of cell division. Lower inset drawings are sagittal sectioned views of dorsal and ventral follicle cells showing the differences in dorsal vs ventral morphology and transition to the contractile tissue on day 6. The ovulation of the oocyte is the last major function before involution depicted as ovulation + 1 day. ×32; insets ×100.

the more solid organ structure of vertebrate ovaries.

During early vitellogenesis the apical border of follicle cells and the cortical zone of the oocyte intensely stain with rhodamine-phalloidin (Figs 14, 16), suggesting that both of them contain a cytoskeleton of actin microfilaments at that time, like the microvilli of vertebrate intestinal brush border (Hirokawa *et al.*, 1982). The abundant microvilli from the oocyte and the less abundant, but prominent, macrovilli from the follicle cells extend from their respective cell types and interlock at pre- and early vitellogenic stages (Mazzini and Giorgi, 1985; Anderson and Kunkel, 1990; Figs 4–6). The actin filaments of the oocyte become much less prominent at later stages. This may be consistent with the filamentous distribution in the meroistic oocytes of *Drosophila*. In these follicles F-actin plays a major role in both nurse and follicle cell physiology, but not in oocyte physiology (Gutzeit, 1986a; Gutzeit and Huebner, 1986). Passage of material to the appropriate surface of the follicle epithelium and structural support for the macrovillar extensions, which may be conduits of follicular products to the oocyte, are possible roles for the observed F-actin centripetal bundles.

Besides the passive permissive role via patency during vitellogenesis, follicle cells also contribute directly to the yolk, the cytoplasm (Telfer *et al.*, 1982) and the chorion (Margaritas, 1985) of the growing oocyte. Secretory activity is characteristic of follicle cells in general (Raven, 1961). In our observations the apical side of mid-vitellogenic follicle cells shows intensive fluorescence which is composed of parallel F-actin bundles extended from the center of follicle cells centripetally to the apical surface (Figs 19, 21). Because one of the key roles that actin plays is cytoplasmic transport, the parallel F-actin bundles localized on the apical side of follicle cells may function to facilitate the transport of secretory vesicles and materials, and also to polarize cytoplasmic transport toward the extracellular space between follicle cells and oocyte.

The observations of different cell shapes and different F-actin filament organization between ventral and dorsal follicle cells are correlated with the dorsal-ventral polarity of vitellogenic follicles (Figs 17–23). *B. germanica* has a short germ band type embryonic development. The germ band develops on the presumptive ventral surface of the oocyte. The patent follicle cell layer thus overlays precisely the surface of the oocyte on which the early embryo is going to develop (Kunkel, 1991). It is possible that the origin of dorsal-ventral polarity of the oocyte is related and partially determined by the prior local differentiation of dorsal and ventral follicle cells. It is likely, due to their different morphological organization, that the dorsal and ventral follicle cells make different contributions to the developing oocyte. It has been shown that the polarity of the dorsoventral axis in the *Drosophila* embryo is defined by a protein (Tl ligand) which is only produced by a ventral population of follicle cells during oogenesis (Stein *et al.*, 1991). Examining the relation of follicle cell organization to polarity in this short germband cockroach oocyte may lend insight into the general question of follicle cell-oocyte communication since the germ plasm is more localized relative to the follicle cell layer in this type organism (Kunkel, 1991).

As summarized in Figure 23 our study reveals changes of F-actin filament pattern during the terminal differentiation of the follicular epithelium in a panoistic ovary. F-actin bundles participate in shape related structures such as macrovillar extensions from follicle cell apices and the hexagonal spokes connecting follicle cells which appear during patency. F-actin bundles course laterally in such cells to seemingly anchor in the actin plates associated with cell-to-cell adhesions in radial extensions. The F-actin also participates in a regionally differentiated way during day 3 through 5 of vitellogenesis when the lateral plasmalemmal borders of dorsal and terminal follicle cells tend to stain strongly for F-actin while the ventral follicle cells have minimum contacts laterally with one another and lack the actin matte of cell cell adhesions. The F-actin filaments may also be involved in developmentally timed transport and contractile processes of the follicular epithelium. Ultimately, shortly after ovulation, the F-actin and other cytological structures of the emptied sheath of follicle cells must involute in a programmed manner and make way for the new terminal oocyte in the ovariole which begins to grow within one day after ovulation, and whose own follicle layer needs to attach to the

oviduct (Fig. 23, ovulation + 1 day). The series of F-actin transitions described here adds to the already impressive programmed sequence of gene expressions carried out in a patterned way by the insect follicle cell layer.

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