Vinblastine and Griseofulvin Reversibly Disrupt the Living Mitotic Spindle

Abstract. *Using polarized light we have studied the effects of various mitotic poisons on mitotic spindles of living* Pectinaria *oocytes; we have studied fixed specimens with phase and electron microscopy. Vinblastine caused attrition and eventual disappearance of spindle structure as rapidly as did colcemid, and subsequent recovery from this treatment was at least as fast as that from colcemid. Griseofulvin, however, was easily the best agent for rapid, reversible, and repeated dissolution of the spindle. Agents that arrest metaphase may act on nondividing cells by interfering with the organization of other gelated structures.*

Particular stages of mitosis have been difficult to study because most dividing cells pass so rapidly through the mitotic cycle. For identification of an arrester of metaphase, the test agent has generally been applied before the cell begins to divide, and the accumulated metaphase patterns have then been counted in fixed specimens. However, Inoué was able to study the effect of colchicine on the living mitotic spindle by picking a specimen with a prolonged metaphase and by making the spindle visible with polarization microscopy (*1*). We have combined rectified polarization microscopy with phase and electron microscopy to study the effects of various metaphase-arresting agents on spindle dissolution and recovery.

Freshly spawned, unfertilized oocytes of the marine annelid *Pectinaria gouldi* (the "ice-cream-cone worm") persist for several hours at the first meiotic metaphase. The mitotic spindles can be seen in polarized light, and the amount of oriented spindle material can be estimated as measured retardation, induced by spindle birefringence. When we perfused (80 to 150 μm/min) such specimens with the chemotherapeutic, periwinkle (*Vinca*) alkaloid vinblastine (as the sulfate, 1×10^{-5} mole/liter), there was a decrease in size (see cover, bottom) and in retardation (Fig. 1, bottom) of the spindle and, within 6 to 12 minutes at 24° to 25°C, complete dissolution. When the oocytes were subsequently perfused with artificial seawater, recovery of spindles began after about 20 minutes and was complete by 40 to 60 minutes (Fig. 1). This reversible effect was repeatable in the same preparation, and was at least as efficient as that of *N*-deacetyl-*N*-methylcolchicine (colcemid, 1×10^{-5} mole/liter) (*2*).

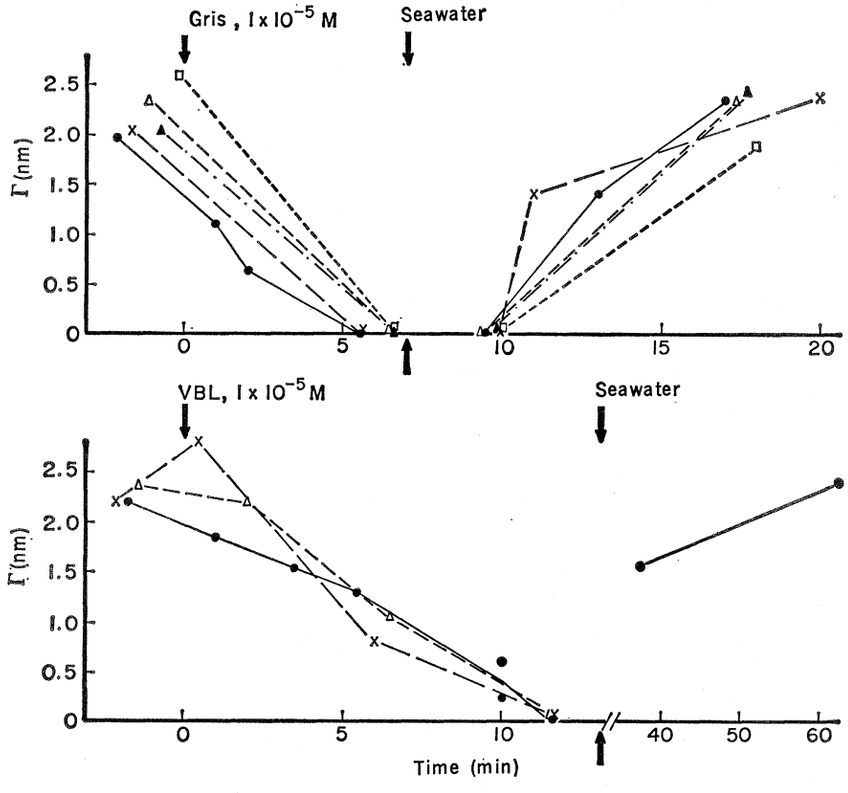

Fig. 1. Birefringence of mitotic spindles of individual *Pectinaria* oocytes is estimated as retardation (Γ) measured in polarized light. Observations were made at intervals after vinblastine (VBL) or griseofulvin (Gris) were added to the artificial seawater perfusate. After total disappearance of the spindle birefringence, the drugs were removed from the perfusate and recovery of retardation was followed. Note that the abscissa is broken in the lower figure: in the time required for one cycle of dissolution and recovery when vinblastine was used, three cycles were possible with griseofulvin. *N*,*N*-dimethylformamide (0.5 percent), which was required to keep the griseofulvin in solution, had no effect on spindle retardation.

Certain effects of vinblastine, relating to the mitotic cycle as a whole, have been thought to be reversed or impeded by glutamate (*3*). However, working with cells at metaphase, we found no effect of glutamate (1×10^{-3} mole/liter) on the initial spindle retardation, no impedence of dissolution by vinblastine, and no facilitation of subsequent recovery. In 40 percent D_2O-seawater, which increases the amount of oriented spindle material, the results were similar to those seen with colcemid (*2*); dissolution by vinblastine was retarded, but recovery was not hastened. This last finding is consistent with Inoué's view of a dynamic equilibrium between organized and disorganized spindle components. Vinblastine, like colcemid, would keep the components disorganized.

At 1×10^{-5} mole/liter, vincristine (as the sulfate), a chemotherapeutic *Vinca* alkaloid with a structure similar to that of vinblastine, did not abolish spindle birefringence. At 1×10^{-4} mole/liter, dissolution required 24½ minutes, and recovery was incomplete, with the frequent induction of small tri- and tetrapolar spindles. In this respect, vincristine is like colchicine; in *Pectinaria*, concentrations of colchicine required for dissolution are higher than those of colcemid, and recovery after treatment with colchicine is often incomplete. The molecular difference within each set of analogs is very small: vincristine differs from vinblastine only by having an *N*-formyl instead of an *N*-methyl group; colchicine differs from colcemid only by having an *N*-acetyl instead of an *N*-methyl group. The higher concentrations of vincristine and colchicine required for spindle dissolution relative to those of vinblastine and colcemid, and the inconsistent reversi-

bility of their effects, could be caused by problems of cell entry and then by strong binding to spindle precursors, but other alterations are also possible. At very low concentration (1×10^{-7} mole/liter), podophyllotoxin caused spindle dissolution in about 20 minutes, but recovery was incomplete.

When we perfused oocytes with the fungistatic antibiotic griseofulvin (1×10^{-5} mole/liter), there was a decrease in spindle size and birefringence (Fig. 1, top), disappearance of the spindle in 3½ to 6½ minutes, and complete recovery in 5½ to 11 minutes. We could carry out this reversible dissolution repeatedly in a single oocyte (Fig. 2), and recovery was complete even with a tenfold increase in the concentration of griseofulvin.

By fixing our specimens for phase and electron microscopy at various intervals after treatment with vinblastine, griseofulvin, or colcemid (all at 1×10^{-5} mole/liter) (*4*), we were able to confirm and extend the observations made on living oocytes (see cover): (i) Ultrastructural effects of vinblastine and griseofulvin on the mitotic apparatus were essentially the same as those of colcemid. (ii) In fixed specimens we confirmed the diminished spindle size noted in polarized light in the treated living material. (iii) We found that the diminished birefringence was the result of there being fewer microtubules. (iv) We did not find randomly oriented microtubules in the region of miniature spindles, suggesting that the decreased birefringence represents a loss of microtubules rather than their disorientation. However, a paucity of organelles persisted in the region previously occupied by the untreated spindle (see cover, middle). We believe that this clear zone is occupied by spindle material. (v) During shortening of the treated spindle, the centriole anchored in the cortex persisted in that region, and the remainder of the mitotic apparatus had thus moved toward the cortex (*1*, *2*).

These agents, then, are useful additions to the colchicine analogs for studying the molecular architecture of the mitotic spindle. Moreover, this system may in turn serve as a model for the action of the various spindle disrupters on nondividing cells (*5*). For instance, the effect of colchicine on the mitotic spindle has been thought to represent a special case of its effects on various organized, labile, fibrillar systems concerned with structure and movement (*6*). Since the advent of glutaraldehyde fixation (*7*), the now ubiquitously seen microtubules have been thought in many cases to be at least markers for this kind of activity, and they may be important in the development of protoplasmic gel strength (*8*). The dissolution of microtubules by colchicine has been demonstrated in nondividing cells (*9*), including the mature, human polymorphonuclear leukocyte; the functional effects of colchicine on this cell type may partially account for the control of acute gouty arthritis by this alkaloid (*10*). The cell proteins to which colchicine binds, and their possible relationship to microtubules or other structural elements (or both), are of considerable current interest (*11*).

Disrupters of the mitotic spindle other than colchicine may also affect gelated structures in nondividing cells. Indeed, vinblastine has some of the same functional and structural effects on (nondividing) human polymorphonuclear leukocytes as colchicine (*12*). Interference with gelated structures other than spindles may be important in the action of spindle-disrupting chemotherapeutic agents on, for instance, neurons, or of the fungistatic antibiotic griseofulvin on certain superficial dermatophytes.

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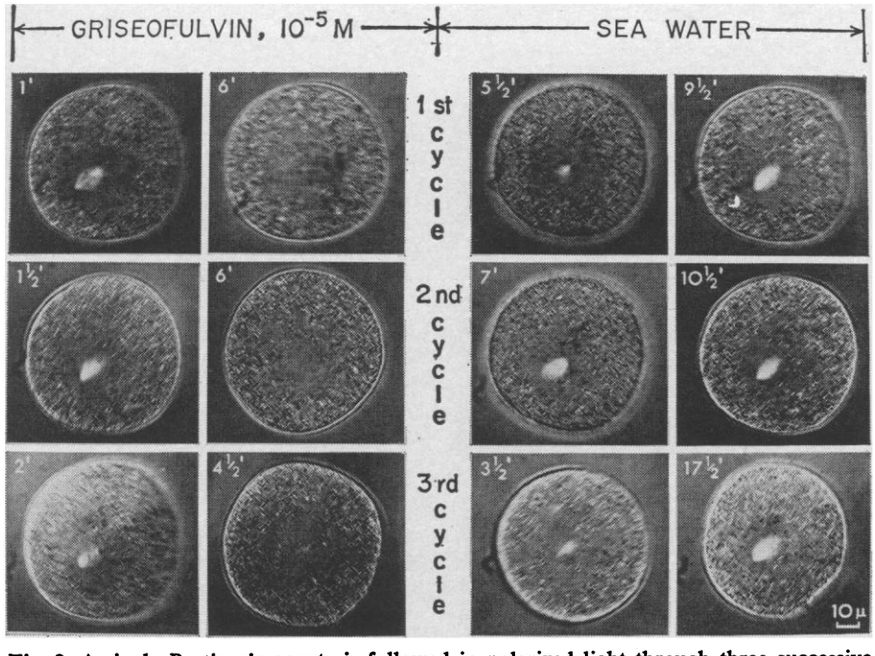

Fig. 2. A single *Pectinaria* oocyte is followed in polarized light through three successive cycles (horizontal rows) of spindle dissolution by griseofulvin and subsequent recovery in artificial seawater. The numbers refer to the time in minutes that the photomicrographs were taken after perfusion was begun with griseofulvin (first two columns) or with seawater (last two columns). The actual time for dissolution in each cycle was 3½ to 6½ minutes; for recovery, 5½ to 11 minutes. The spindles are slightly tilted from the horizontal.

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13. This work was done in part at the Marine Biological Laboratory, Woods Hole, Mass.; it was supported by grants from the John A. Hartford Foundation, PHS (AM-10493, AI-271, CA-10171, A-5514, GM-14834), and NSF (GB-5120). S.E.M. is a senior investigator of the Arthritis Foundation. We thank Miss S. Lesnieski and Mrs. S. Branson for technical assistance. We thank Ayerst Laboratories, New York, N.Y., for supplying the griseofulvin (microsize).

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Cupulae in Shark Neuromasts: Composition, Origin, Generation

Abstract. *Cupulae on the surface of the sensory epithelium of canal neuromasts of the shark lateralis system have been demonstrated. They are mounds of mucoid material secreted primarily by the supporting cells of the neuromast epithelium. Individual columns of mucus in fixed, stained sections produce striations perpendicular to the neuromast surface. The hair of the sensory cell is embedded in mucus at the bases of coalescing columns produced by a group of surrounding supporting cells. There is evidence that mucus production is continuous and is accompanied by regeneration of secretory cells and elimination of necrotic cells at the distal surface. It follows that cupular material is being discarded either intermittantly or continually, along with trapped cell debris and other detritus.*

We have found cupulae (Figs. 1–4) on the surface of neuromasts of the head and lateral canals of juvenile *Sphyrna lewini* (60 cm, total length), near-term pups of *Carcharhinus melanopterus* (39 cm), and adult *C. menisorrah* (133 to 152 cm). These delicate gelatinous structures have been demonstrated in several orders of teleost fish (*1*) but not with certainty in elasmobranch canal organs. In free neuromasts (pit organs) of sharks, we found only one of many preparations which showed a cupula (*2*), and this lacked the typical striations usually associated with cupulae and may have been an anomalous mucus secretion.

The cupular material of shark canal neuromasts, apparently of viscous rather than jelly-like consistency, is lost in freehand sections of fresh material but is occasionally retained in frozen preparations. It is often retained in permanent preparations of carefully handled, rapidly fixed (Bouin's, Susa's, 10 percent formalin) material, embedded and sectioned in paraffin (7 to 10 μm) and stained in various dyes (Mallory's, Giemsa, toluidine blue, periodic acid-Schiff, hematoxylintriosin, and so forth), but it is shrunken by dehydration and frequently is torn from the surface of the epithelium in sectioning.

The cupula is an elongated mound of mucoid material covering the surface of the neuromast epithelium and extending into the lumen of the canal. Its base is an elongate oval in shape and plane, convex or concave in contour, conforming to the shape and contour of the epithelial surface. In permanent sections its maximum height is about two to three times that of the sensory epithelium, and it occupies only about 20 percent of the area of the canal lumen. In fresh-frozen sections the cupula is much higher and of much greater cross-sectional area; it nearly fills the canal lumen. In permanent sections, the height decreases abruptly at the ends of the sensory epithelium, but not necessarily to zero. In some longitudinal sections, the cupulae of adjacent neuromasts are continuous across the peripheral layers of inwardly curved supporting cells (mantle cells) which separate the two neuromasts at their ends (Fig. 1).

In the permanent sections, the cupula has a free, irregular distal surface without any detectable limiting membrane (Figs. 1–3). The cupular material reacts positively with periodic acid-Schiff reagent, showing the presence of polysaccharides; it stains metachromatically in Giemsa and toluidine blue, showing the presence of mucopolysaccharides. It consists of numerous columns of dark-staining mucus separated by more lightly staining granular mucus, producing the striated appearance. When the cupula is cut at an angle, the cut ends of the columns present an areolar appearance. Trapped in and among the columns are nuclei and other cellular debris, which apparently are being discarded from the sensory epithelium, hairs torn from the sensory cells, occasionally blood corpuscles from hemorrhages, and sometimes sand grains which must have entered through the canal tubules.

The cupular material originates in the supporting cells (including the mantle cells) of the neuromast epithelium. The supporting cells extend from basal membrane to distal surface and, within the peripheral layer of mantle cells, surround the shallower sensory cells. Metachromatic staining shows the presence of mucopolysaccharides within the supporting cells, extending from their distal ends to about the level of the nuclei of sensory cells where the supporting cells are greatly constricted (Fig. 6). In some sections, slender threads of mucus project from their necks and merge with the cupular material torn from the surface (Fig. 4). Some of the cells which are discharging mucus appear senescent, with partially vacuolated interiors and pycnotic nuclei lying close to the distal surface of the epithelium (Fig. 5). Necrotic cells are seen frequently among the sensory cells. Mitotic figures are seen occasionally. These observations give evidence that the secretory supporting cells are being continually formed by cell division, and are being discarded through the surface of the epithelium, contributing to the cell debris in the cupula. Whether a cell discharges mucus just once or several times before becoming necrotic is uncertain. Cross striations, occasionally seen in the mucus column, could represent periodic discharge of one cell or successive discharge of several.

Facial sections of both free and canal neuromasts show one sensory cell neck surrounded by four or five supporting cell necks, with the distal cell membranes joined by intercellular cement. The arrangement is such that each short sensory hair (about 6 μm long, Fig. 5) is completely surrounded by the bases of coalescing columns of mucus.

Continual production of cupular material proximally implies continual or intermittant loss distally. Erosion of the material, and, incidentally, removal of accumulated detritis, may be accomplished by a slow head-to-tail flow of water through the canals, which we have demonstrated in a juvenile *S. lewini* (*3*).

The cupula of the shark neuromast, described here as a mound of mucus, may differ from that of teleosts in viscoscity (degree of polymerization of the mucopolysaccharides) or in composition. In living *Lota vulgaris*, the cupula can be displaced as a unit for a short distance (10 μm) over the surface of the neuromast, and toward its tip it is plastic, bending when probed (*4*). In living *Rhyncocymba nystromi*, it