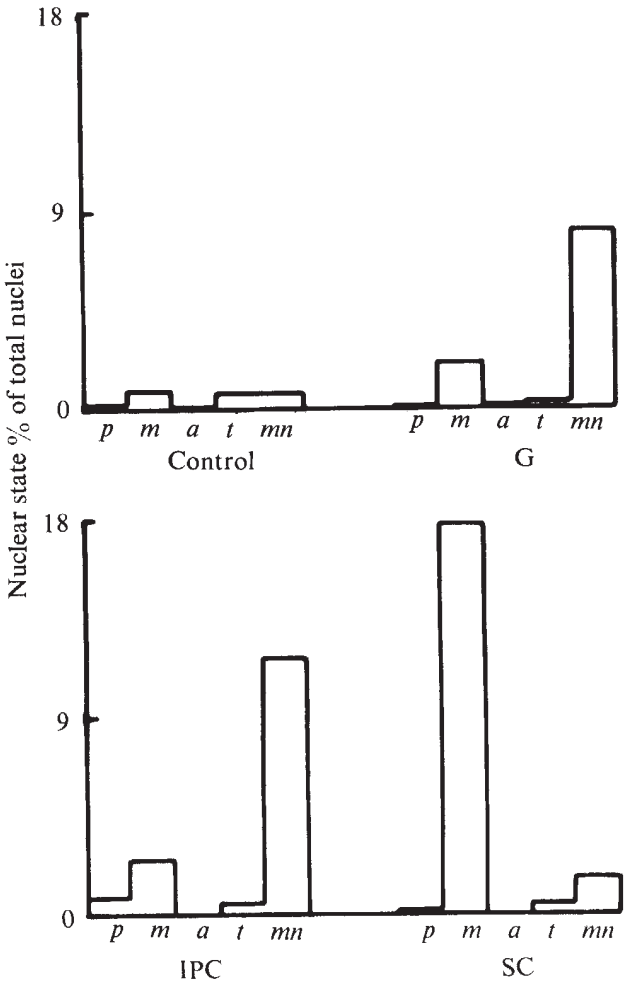


**Fig. 5** Phase accumulation data for control colonies and colonies growing on mitotic inhibitors, *p*, prophase, *m*, metaphase, *a*, anaphase, *t*, telophase, and *mn*, multinucleate. The pattern for sodium cacodylate shows the block of mitosis at metaphase. The patterns for griseofulvin (G) and isopropyl-*n*-phenylcarbamate (IPC) are similar but differ from that of sodium cacodylate (SC). Griseofulvin and IPC increase the number of metaphase nuclei but they also produce large numbers of multinucleate hyphal compartments.

nuclear division in a classical manner. We have used a further term, multinucleate, to indicate a cell containing two or more nuclei each with an interphase appearance. The phase accumulation data, for all the mitosis inhibitors tested, fell into two groups, typified by sodium cacodylate and IPC (Fig. 5). Sodium cacodylate showed a colchicine-like effect in blocking large numbers of cells at metaphase and few multinucleate cells were seen. IPC, however, showed a significant peak at metaphase and very large numbers of multinucleate cells. Figure 5 shows how the phase accumulation data for griseofulvin closely resemble those of IPC but are very different from those of sodium cacodylate.

The nuclei of griseofulvin induced multinucleate compartments were always observed in close proximity (Fig. 3). In such cells the daughter nuclei appeared to move only a very short distance apart after metaphase and then reverted to the early interphase state by re-enclosure in the nuclear envelope. In this sort of situation griseofulvin may act by inhibiting the postulated sliding of microtubules[3].

IPC is known to produce disorganization of spindle microtubules in dividing plant cells[4]. Our ultrastructural observations on *Basidiobolus* have shown that griseofulvin produces very similar effects (K. G. and A. P. J. T., unpublished). There is an increasing amount of evidence suggesting that griseofulvin affects microtubules in systems other than fungal hyphae[5–8]. The ability of griseofulvin to block mitosis, in a manner differing from that of colchicine, should lead to its use as a valuable research antibiotic.

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# Antimitotic Action of Griseofulvin does not Involve Disruption of Microtubules

THE antimitotic action of griseofulvin in plant and mammalian cells has been attributed to a colchicine-like disruption of microtubules. Treatment of *Vicia faba, Allium* root tips or HeLa cells with griseofulvin, for example, resulted in the accumulation of cells at metaphase[1–3]. The griseofulvin-inhibited cells contained abnormal arrays of chromosomes, identical to those produced by treatment of dividing cells with colchicine. Using polarization microscopy, Malawista *et al.*[4] found that the addition of $10^{-5}$ M griseofulvin to dividing *Pectinaria* oocytes reduced the size of the meiotic spindles, as measured by loss of birefringence. This action was considerably more rapid than with either podophyllotoxin or vinblastine sulphate; moreover, on removal of the griseofulvin, recovery of the spindle also occurred much more rapidly[4]. Recent evidence indicates that several of the drugs which arrest metaphase interfere with microtubule function through different mechanisms. Colchicine and the vinca alkaloids, for example, bind to tubulin (microtubule protein) at different molecular sites[5,6]. Griseofulvin does not prevent the binding of colchicine to tubulin, nor does it affect the ability of the vinca alkaloids to stabilize the colchicine binding activity of tubulin[5]. This suggests that griseofulvin does not bind at either the colchicine or vinca alkaloid binding sites.

It is now possible to polymerize microtubules *in vitro*, in crude supernatant fractions of brain containing tubulin, by removal of calcium ions by chelation with ethylene bis(oxyethylene-nitrite) tetraacetate (EGTA)[7,8]. We studied the effect of griseofulvin on this, and found that microtubule polymerization *in vitro* was not prevented. Examination of griseofulvin-inhibited HeLa cells by electron microscopy showed morphologically normal but disoriented microtubules.

Incubation of the crude 30,000*g* supernatant brain extract containing EGTA at 37° C resulted in the formation of 250 Å diameter microtubules. Addition of $5\times10^{-5}$ M colchicine, $2\times10^{-5}$ M podophyllotoxin, and $2\times10^{-5}$ M vinblastine sulphate or vincristine sulphate immediately before initiation of the polymerization reaction completely prevented microtubule formation. Polymerization *in vitro*, however, was not affected by the presence of griseofulvin (Fig. 1). At the highest griseofulvin concentration tested, $6\times10^{-5}$ M, the microtubules formed were indistinguishable

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from those found in untreated samples, and the degree of microtubule formation did not seem to differ significantly.

Inhibition of multiplication of HeLa cells by griseofulvin was concentration-dependent, 50% inhibition being obtained at $4\times10^{-5}$ M griseofulvin, and this concentration producing a mitotic index (c-mitotic cells) of 48% after 30 h of growth (data not shown). Examination of the cells, inhibited in metaphase, showed normal microtubules (Fig. 2). There seemed to be both pole-to-pole and chromosomal microtubules; the latter inserted in the usual manner into the kinetochore, and their numbers were estimated to fall into the normal range. The mitotic centres were of normal appearance, except that there was an occasional increase in the number of small vesicles in the vicinity of the centrioles. The centrioles also showed the usual morphological appearance, and were confined to the mitotic centres. An increase in mitotic centres, however, each containing two centrioles, was observed in many of the drug-treated cells, a finding which may explain the multipolar mitotic patterns observed with the light microscope[2,3]. The numerous cells found in incomplete mitosis showed mitotic arrest in or after metaphase; subhaploid numbers of chromosomes were often found in close apposition to a mitotic centre (Fig. 2). The number of chromosomes associated with any one of the multipolar mitotic centres seemed to be random. HeLa cells, treated with $2\times10^{-6}$ M colchicine or $2\times10^{-5}$ M podophyllotoxin, were arrested at metaphase, and, in contrast to the findings with griseofulvin-inhibited cells, they did not contain microtubules.

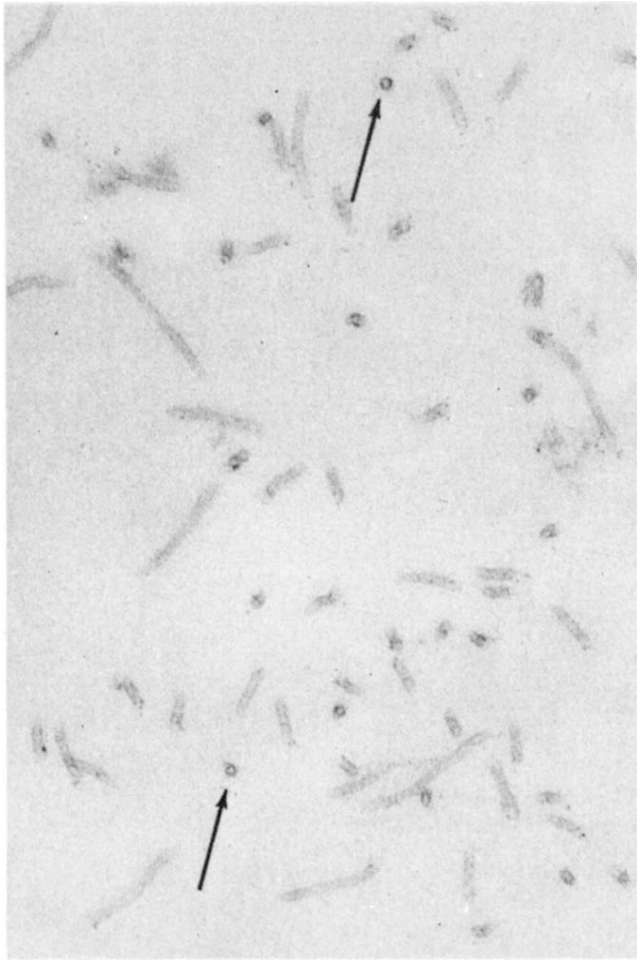

**Fig. 1** Microtubules polymerized *in vitro* in the presence of griseofulvin ($6\times10^{-5}$ M). The arrows point at cross-sectioned microtubules or 250 Å diameter. *In vitro* polymerization was carried out with 30,000*g* supernatant extracts of sonicated 13 to 17-d-old chick embryo brains in 20 mM sodium phosphate, 100 mM sodium glutamate, *p*H 6.75. Extracts contained 0.5 mg ml$^{-1}$ of tubulin, 1 mM $MgCl_2$, 1 mM GTP, and 10 mM EGTA. Polymerization was initiated by raising the temperature from 0° to 37° C. The reaction was carried out for 30–60 min. Polymerized microtubules were fixed as a suspension with prewarmed (37° C) buffered 2% glutaraldehyde[11]. Microtubules were pelleted by centrifugation (10,000*g*, 30 min), then post-fixed in 1% $OsO_4$, and embedded in 'Maraglas' by standard procedures[12,13]. Ultrathin sections were stained with uranyl and lead salts[14,15]. Magnification: ×70,000.

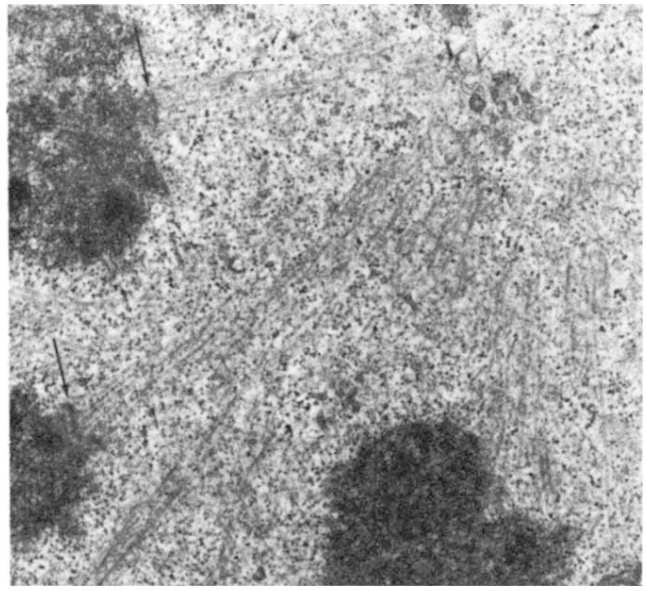

**Fig. 2** Section of a HeLa cell blocked in mitosis by exposure to $2\times10^{-5}$ M griseofulvin for 24 h. Normal microtubules radiate from a mitotic centre which occupies the right upper quadrant of the illustration to and past chromosomes. Note the normal attachment of the microtubules to kinetochores (arrows). HeLa monolayer cultures were maintained as described previously[16]. The griseofulvin was dissolved in dimethylsulphoxide (DMSO) so that all cultures (control, griseofulvin, podophyllotoxin, and colchicine-treated) contained final DMSO concentration of 0.1%. Cell growth was not affected by the DMSO. After treatment with the drugs for 6, 12, 24, or 30 h, cells were collected by scraping them off the glass in calcium- and magnesium-free balanced salt solution containing 0.02% EDTA, and mitotic indices and cell numbers were determined. Control and treated cells utilized for electron microscopy were fixed and processed as in Fig. 1. Magnification: ×27,500.

Thus, although the action of griseofulvin on mitosis resembles that of other c-mitotic agents such as colchicine, podophyllotoxin, and the vinca alkaloids in a number of ways, it differs in that microtubule assembly is not affected. Griseofulvin shows an effect on mammalian cell mitosis which resembles that of the herbicide isopropyl-*n*-phenylcarbamate (IPC) in plant cells. Treatment of *Hemanthus katherinae* endosperm with IPC resulted in loss of spindle birefringence, and formation of multipolar spindles, and electron microscopy revealed the presence of normal but disoriented microtubules[9]. As with griseofulvin, the microtubules were associated with the kinetochores in a normal fashion (*Hemanthus* does not contain centrioles). Significant differences in the mechanisms of action of these two agents must exist, however, because IPC was found to be totally ineffective in producing mitotic arrest in HeLa cells, even at $10^{-4}$ M.

How does griseofulvin inhibit mitosis? Although the drug does not affect the assembly of microtubules, it may affect some aspect of microtubule function, possibly a process vital to the sliding of microtubules, which has been proposed to be necessary for separation of chromosomes[10]. Alternatively griseofulvin may not affect microtubules directly, but may act on other processes essential for normal completion of cell division. Further understanding of the

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mechanism of action of griseofulvin on mitosis may provide new insight into the process of cell division in eukaryotic cells.

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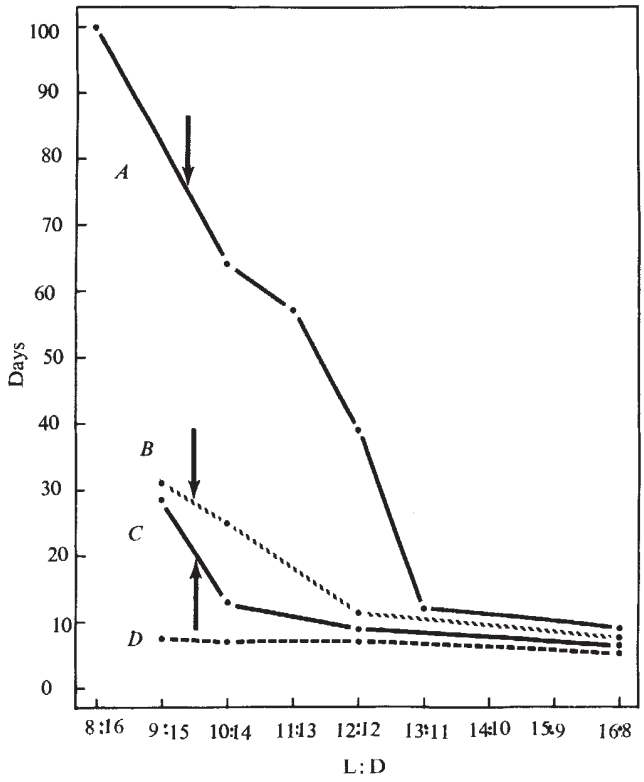


**Fig. 1** Response (mean number of days to initiate oviposition) after overwintering *Chrysopa carnea* adults were transferred to various photoperiods. Sample dates: *A*, October 25, 1971; *B*, December 22, 1971; *C*, January 22, 1972; *D*, February 23, 1972. Arrows, results obtained from groups transferred to the greenhouse on the *A*, *B* and *C* sampling dates. $\bar{x} \pm$ s.d., sample no. in parentheses: curve *A*——99.2±27.8 (8), 64.0±9.2 (10), 57.0±10.9 (10), 39.1±10.1 (12), 11.5±1.5 (8), 9.0±0.5 (6), greenhouse (arrow) 75.3±9.4 (12); curve *B*——30.7±6.5 (10), 25.2 ±1.5 (8), 12.4±2.3 (6), 8.3±0.7 (6), greenhouse (arrow) 27.7 ±3.3 (8); curve *C*——28.6±10.2 (12), 13.1±7.3 (12), 8.8±0.8 (10), 6.6±0.7 (6), greenhouse (arrow) 20.0±8.4 (10); curve *D* ——7.6±0.9 (10), 7.0±1.5 (10), 6.6±2.4 (10), 5.8±0.8 (10). Temperatures: incubators 24°±1° C; greenhouse 23°±4° C.

# Quantitative Response to Daylength during Diapause in Insects

WITH few exceptions investigations into photoperiodically controlled seasonal activity in insects have used stationary light-dark cycles; no experiments known to us have defined the role of increasing or decreasing daylengths in nature. We therefore investigated the influence of natural daylengths on diapause duration and termination, using the green lacewing, *Chrysopa carnea* Stephens (Neuroptera). This species undergoes a reproductive diapause when subjected to short daylengths below a critical photoperiod[1]. A large decrease in stationary daylength above the critical photoperiod can also induce diapause; an increase in stationary daylength below the critical photoperiod can either avert or terminate diapause[2]. It was not clear from these laboratory experiments, however, if *C. carnea* can perceive natural changes in daylength, so we tested (*a*) whether the naturally decreasing daylengths in autumn have a role in maintaining diapause and (*b*) whether increasing daylengths after the winter solstice hasten the end of diapause.

To answer the first question we reared *C. carnea* outdoors in Ithaca, NY (42° 27″ N). On October 25, approximately three weeks after the animals had reached the full depth of diapause[3], we transferred samples from this overwintering population into seven photoperiodic conditions: six with stationary daylengths and one under natural illumination in a heated greenhouse. The animals remained under their respective regimes and were examined daily until diapause ended[3] and reproduction began.

The results (curve *A*, Fig. 1) show (*a*) that at photoperiods of 13 h or less there is an inverse, linear relationship between diapause duration and photoperiod, and (*b*) that when the diapause duration of the greenhouse sample (75 d) is plotted against photoperiod (arrow, curve *A*), a 9.5 h photoperiod is read on the abscissa. As this daylength is equivalent to the shortest day of the year at our latitude, it seems that the *C. carnea* perceived and responded to the duration of natural daylengths occurring through autumn and early winter. Thus, as the autumn days became shorter, between the sample date and the winter solstice, the rate of diapause development[4] slowed. Although, in the laboratory, *C. carnea* is capable of responding to decrease in daylength *per se*, our data here (curve *A*) indicate that the animals responded quantitatively to the absolute duration of autumn and early winter daylengths. These experiments are the first to demonstrate a quantitative relationship between diapause duration and daylength. In other species it has been shown that the rate of morphogenesis is quantitatively related to photoperiod[5–7] and that natural photoperiods may maintain diapause longer than a single stationary short daylength[8].

Our earlier results have shown that an abrupt increase in daylength, below the critical photoperiod, will break diapause in *C. carnea* in the laboratory[2] and that chilling does not accelerate diapause development[3]. To determine whether diapause in this species is terminated by increasing daylengths in nature, we sampled an outdoor diapausing population at the winter solstice and twice afterwards. We transferred samples into various stationary photoperiods and into a heated greenhouse under natural illumination. Our data (Fig. 1) show that within each sample period the various stationary photoperiods produced different responses. Also, the changes in the slopes of curves *A*, *B*, *C*, and *D* indicate that diapause in *C. carnea* is a dynamic state. Between January 22 (curve *C*) and February 23

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