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Effect of *cis*-Platinum(II)diamminodichloride on Cell Division of *Hyphomicrobium* and *Caulobacter*[1]

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Low concentrations of the radiomimetic agent *cis*-platinum(II)diamminodichloride (PDD) inhibited cell division in *Caulobacter crescentus* (0.1 μg/ml) and *Hyphomicrobium* sp. strain B-522 (1.0 μg/ml) without altering the length of prosthecae. After exposure, cells of *C. crescentus* appeared as long filaments, whereas only the bud portion of *Hyphomicrobium* underwent elongation. PDD-treated cells of both species were multinucleated. After the removal of PDD by washing, filaments of *C. crescentus* fragmented unequally and then normal growth resumed. In *Hyphomicrobium* (where division involves release of swarmer cells that arise as buds on the distal ends of hyphae), potential septation sites formed in the presence of PDD remained inactive after washing. Reinitiation of cell division in this species was dependent upon the synthesis of new hyphae that could arise from either end of the elongated bud. This finding suggests that the PDD-induced lesion at a given septation site is irreversible and, upon removal of this compound, alternate sites must be synthesized for the subsequent occurrence of cell division.

cis-Platinum(II)diamminodichloride (PDD) is an antitumor agent (6, 24, 27) that, by generating interstrand cross-links (11, 22), can selectively inhibit synthesis of deoxyribonucleic acid (DNA) in eukaryotic cells (9, 13, 14). PDD does not irreversibly inhibit DNA synthesis in prokaryotes, as judged by the ability of *Escherichia coli* to grow in its presence in the form of very long, multinucleated filaments (1, 12, 23, 25, 26). At least some of the mechanisms known to be necessary for the removal of interstrand cross-links from DNA (7) were operative in treated cells of *E. coli* as shown by detection of PDD-dependent degradation of DNA in vivo, acute sensitivity of DNA repair-deficient mutants to PDD, and ability of PDD to induce mutations by causing base substitutions (1, 2). PDD has not yet been reported to significantly affect the morphology of gram-positive bacteria.

To obtain more information regarding the ability of PDD to promote filamentation, we examined its effect on the morphology of *Caulobacter crescentus* and *Hyphomicrobium* sp. strain B-522. Both of these gram-negative, prosthecate bacteria exhibit an ordered life cycle in which the production of cellular appendages is requisite to septation. For example, swarmer cells of *C. crescentus* form stalks during maturation into forms capable of cell division. The stalks serve to attach the cells to a surface, and division of the main cell body occurs by a typical process of transverse fission (8, 19, 28, 29). In contrast, maturation in *Hyphomicrobium* requires growth of a hypha that forms a terminal bud. Upon concomitant enlargement and acquisition of a daughter nucleoid through the hypha, the bud is released as a swarmer cell (15–17). The consequences of altering these life cycles by exposure to PDD are described in this report.

MATERIALS AND METHODS

Organisms and cultivation. *Hyphomicrobium* sp. strain B-522, obtained from P. Hirsch, was grown in medium 337a as described by Moore and Hirsch (15). *C. crescentus* strain CB2 was kindly provided by J. Staley and was cultivated, without supplemental vitamins, in the MMB medium of Staley and Mandel (31). Both organisms were incubated at 30 C in liquid cultures and aerated on a water bath shaker in 30 ml of medium contained in 250-ml nephelometer flasks. The same medium containing 1.0% Noble agar (Difco Laboratories, Detroit, Mich.) was used for agar slide cultures and determination of viable cells. Growth in liquid media was determined by measuring turbidity with a Klett-Summerson colorimeter fitted with a red (660 nm) filter.

Microscopy. Photomicrographs were made with a

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Zeiss Photomicroscope II equipped with bright-field, phase, and Normarski differential interference optics.

RESULTS

As shown by determining maximum turbidity after exponential growth (Table 1), cells of *C. crescentus* were significantly more sensitive to PDD than were those of *Hyphomicrobium*. Despite increases in turbidity, viability generally decreased in the presence of PDD. The time required to reduce the number of viable cells of *C. crescentus* and *Hyphomicrobium* to 37% in the presence of 1.0 μg of PDD per ml of medium was 1.5 and 2.5 h, respectively (Fig. 1).

The apparent discrepancy between viability and cell mass was caused by the death of a portion of the population, accompanied by elongation. The largest proportion of filaments occurred in cultures containing low concentrations of PDD, although more rapid elongation of a smaller number of cells was observed at higher levels of the compound. With *C. crescentus*, 42% of the cells underwent filamentation in the presence of 1.0 μg of PDD per ml of medium. Representative cells of this species, which had grown to their near-maximal length, are shown in Fig. 2. The remaining organisms are seen as shorter, normal-sized cells, which were either inhibited or killed before elongation occurred. The morphology of the cellular stalks of *C. crescentus* was not modified by growth in the presence of PDD.

Near the end of the exponential phase of growth, approximately 70% of the cells in untreated cultures of *Hyphomicrobium* exhibited a hypha or a hypha with a bud at some stage of development. The remaining portion of the population was composed of young swarmer cells (16). In comparable cultures containing 5 μg of PDD per ml of medium, 4% of the population was comprised of swarmer cells, whereas 26% of the bacteria possessed a hypha

TABLE 1. *Inhibition of bacterial growth by PDD*

Organism	PDD (μg/ml)	Inhibition[a] (%)
C. crescentus strain CB2	0.1	8
	0.2	12
	0.5	30
	1.0	58
Hyphomicrobium sp. strain B-522	1.0	8
	5.0	15
	10.0	19

[a] Maximum turbidity obtained during 48 h of incubation in cultures containing the indicated quantities of PDD compared with control cultures lacking PDD.

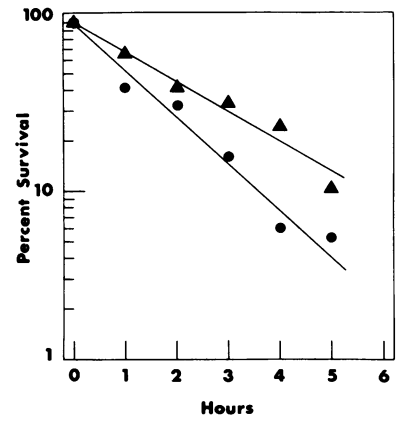

FIG. 1. *Survival (colony-forming ability) of cells of Hyphomicrobium (▲) and C. crescentus (●) during incubation with 1.0 μg of PDD per ml of medium; initial concentrations of cells were 3 × 10⁸ and 4 × 10⁸ per ml, respectively.*

alone or a hypha with a typical bud. The remaining organisms were unique in the sense that a cellular unit ressembling a mature form was attached by a hypha to a second cellular unit that had undergone significant filamentation.

To determine whether the bud or the mother cell had become elongated, the growth of approximately 1,000 individual bacteria was observed on agar slide cultures containing PDD (5 μg/ml). The appearance of normal organisms at the time of inoculation is shown in Fig. 3a, and the same field after 48 h of incubation is illustrated in Fig. 3b. As shown by examination of the comparable cells of the two photomicrographs, it is evident that PDD did not significantly interfere with normal growth of hyphae or prevent initiation of bud formation. However, those buds that were produced in the presence of PDD continued to grow in length and failed to become separated from the hypha of the mother cell.

Giemsa-stained preparations were examined by bright-field microscopy to determine whether PDD-treated cells were multinucleate. Whereas the buds of untreated cells contained zero to one nucleoids (17), four or five nucleoids could be detected in the elongated buds of *Hyphomicrobium* (Fig. 4a). An increased number of nucleoids was also observed in the filaments of *C. crescentus* (Fig. 4b).

To examine the effect of the removal of PDD on the growth of cells, filaments of *C. crescentus* were washed, placed on PDD-free agar slide cultures, and observed by differential interference microscopy. After 70 h of incubation,

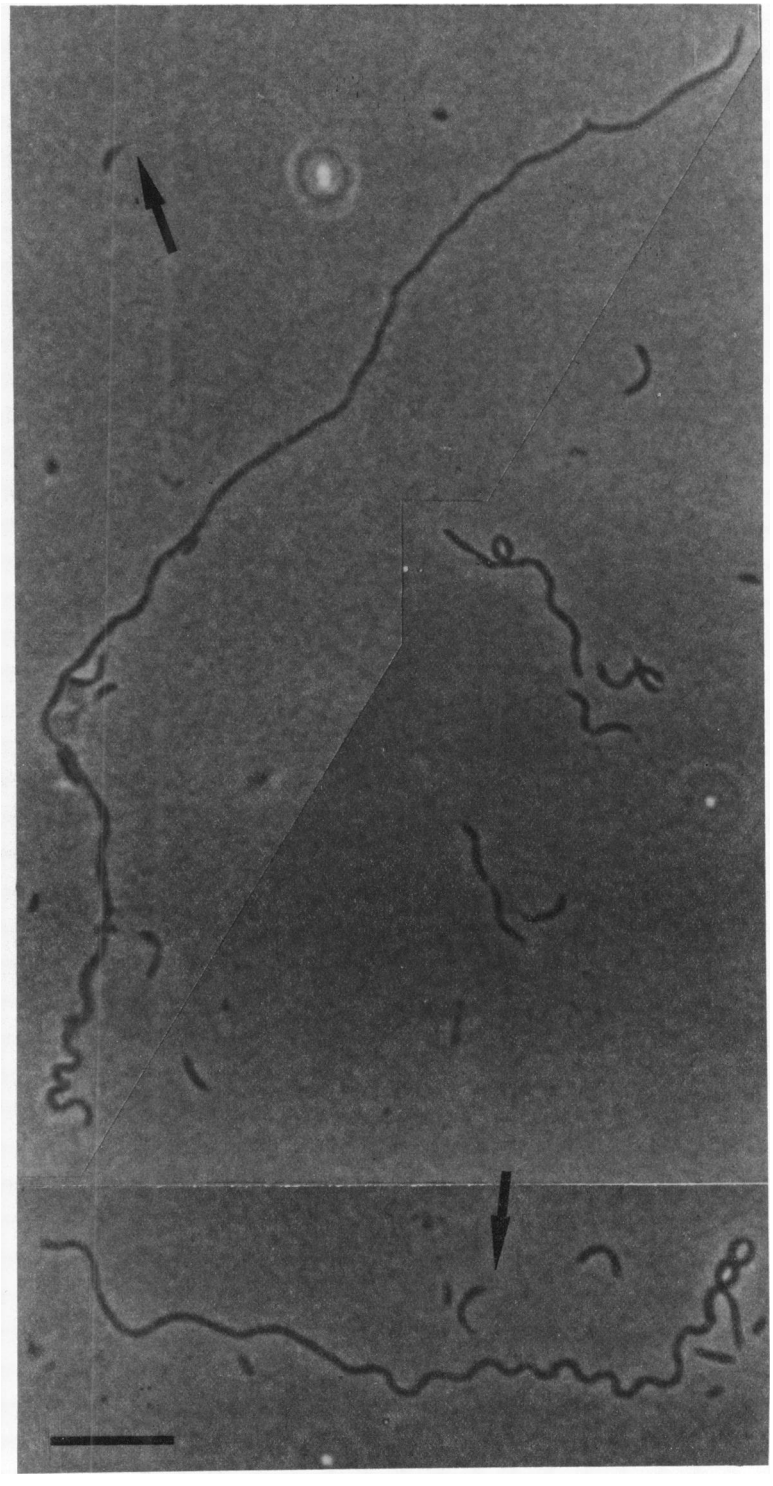

FIG. 2. *Phase photomicrograph of C. crescentus after 95 h of growth with 1.0 μg of PDD per ml of MMB medium. Arrows indicate typical stalks; bar = 10 μm.*

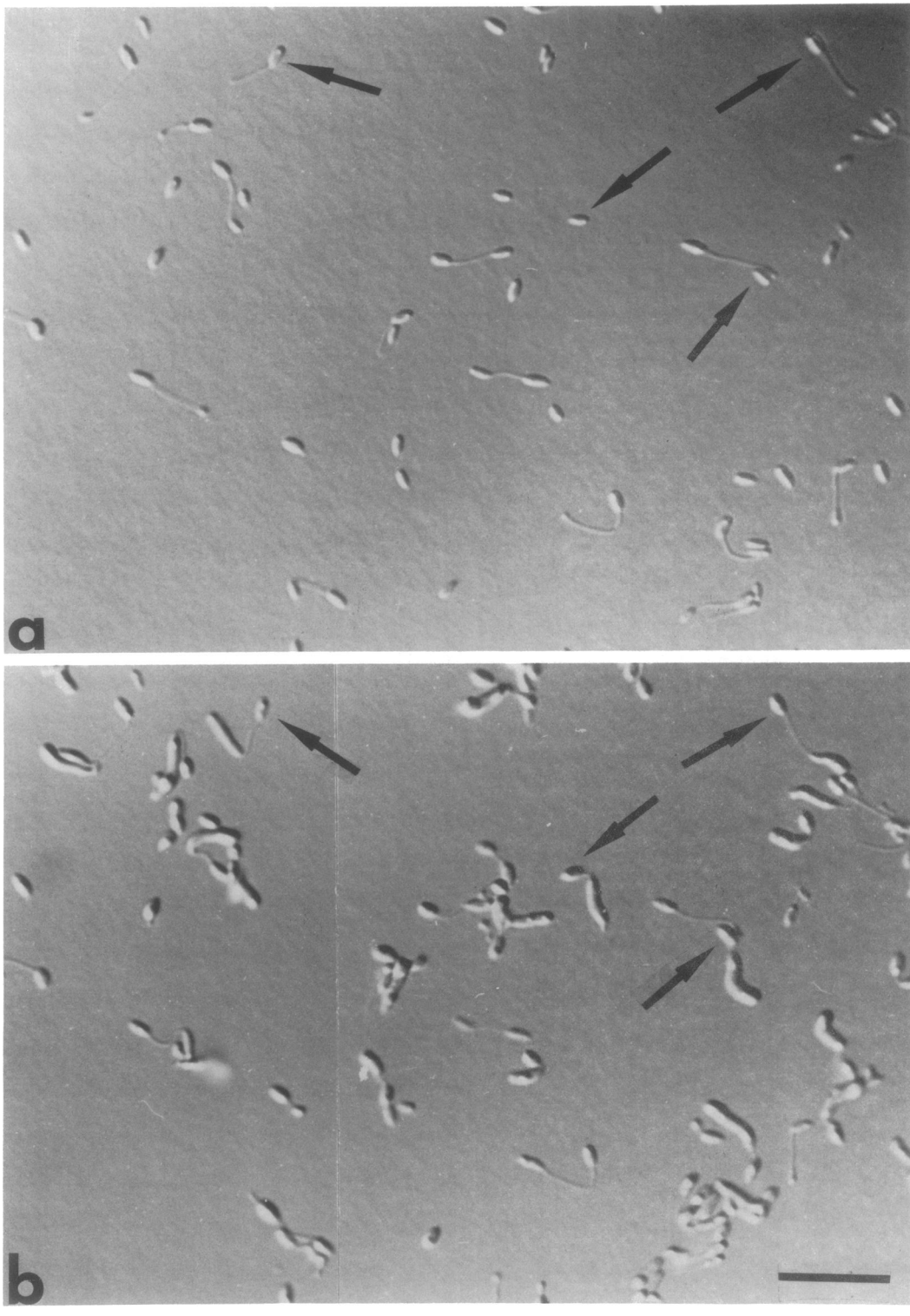

FIG. 3. *Nomarski differential interference photomicrographs of the same field of cells of Hyphomicrobium growing in an agar slide culture containing 5 μg of PDD per ml at (a) the time of inoculation and (b) after 48 h of incubation. The arrows indicate representative cells. Bar = 10 μm.*

microcolonies had developed through unequal fragmentation of filaments and subsequent multiplication of the resulting daughter cells (Fig. 5). However, many filaments still remained, and these continued to increase in length until they eventually fragmented or underwent lysis.

The elongated buds of *Hyphomicrobium* also

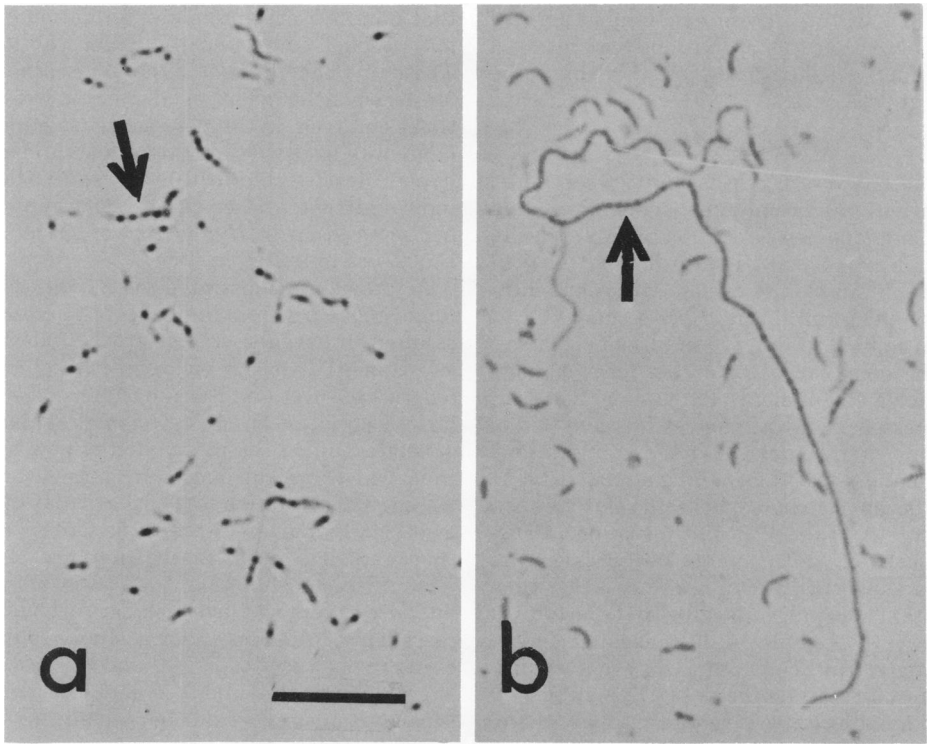

FIG. 4. *Giemsa-stained preparations of (a) Hyphomicrobium and (b) C. crescentus after growth for 48 h in the presence of 5.0 μg and 1.0 μg, respectively, of PDD per ml of medium. The arrow in (a) indicates the presence of at least five nucleoids in an elongated bud; that in (b) shows a series of nucleoids in a filament. Bar = 10 μm.*

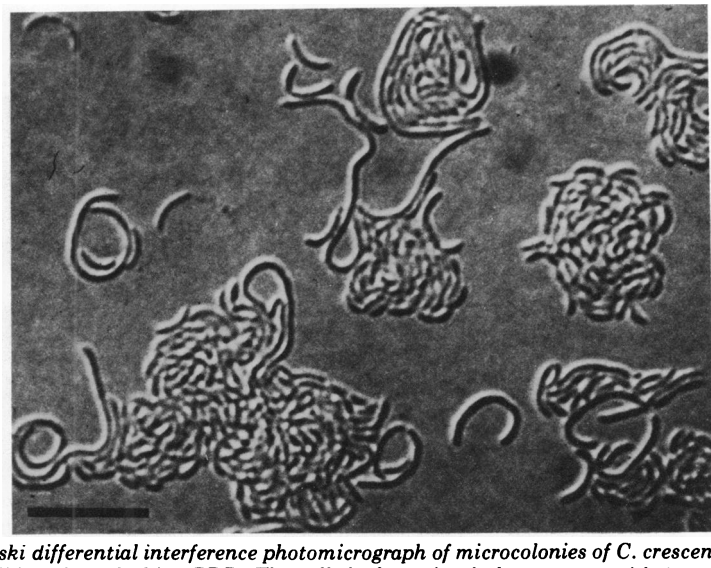

FIG. 5. *Nomarski differential interference photomicrograph of microcolonies of C. crescentus after growth for 70 h in an agar slide culture lacking PDD. The cells had previously been grown with 0.1 μg of PDD per ml of medium for 4 h and were then washed twice by centrifugation with MMB medium. Bar = 10 μm.*

continued to increase in length after the removal of PDD. Rather than undergoing fragmentation, however, a new hypha was produced from either the tip of the elongated bud (Fig. 6a-c) or near the base of the preexisting hypha (Fig. 6d-f). In turn, these new hyphae formed typical buds that, upon division, were released as swarmer cells, thereby reestablishing the normal growth cycle.

DISCUSSION

Accumulation of a critical unit of cell mass, termination of chromosome replication, and subsequent genome segregation are prerequisite to normal division in bacteria like *E. coli* (30) that exhibit simple life cycles. Mutations in *E. coli* that block cell division per se may not, at least initially, prevent accumulation of mass; thus, the cells can grow in the form of long, multinucleated filaments (20, 21). In contrast, cells arrested in growth by expression of *dna* mutations or by addition of DNA inhibitors, such as nalidixic acid, generally appear as short filaments that, of course, contain relatively few nucleoids (5, 10). The phenotype of PDD-treated cells of *E. coli* more closely ressembles that expressed by division mutants rather than *dna* mutants, as judged by growth in the form of very long (>50 μm), multinucleated forms. Accordingly, this compound may not significantly inhibit *net* synthesis of DNA in *E. coli* (1), and an alternative mechanism must, therefore, account for its ability to block cell division.

C. crescentus, like *E. coli*, formed long, multinucleated filaments in the presence of PDD. This response was distinct from that of *Hyphomicrobium* which, unlike either *E. coli* or *C. crescentus*, multiplies by septation at the tips of hyphae rather than by binary fission. After the addition of PDD to cultures of *Hyphomicrobium*, the organism formed elongated, multinucleated buds, which were not released from the otherwise typical hyphae. In contrast, Weiner and Blackman (32) showed that nalidixic acid promoted formation of very long hyphae in *Hyphomicrobium neptunium*. These hyphae did not exhibit terminal bud formation although atypical swelling was sometimes observed. In this case, no newly replicated DNA was available for migration through the hypha. Results obtained in this study (Fig. 4a) indicated that neither chromosome replication nor segregation in *Hyphomicrobium* sp. strain B-522 was inhibited by PDD.

As previously illustrated for *E. coli* (23), the removal of PDD from filaments of *C. crescentus* resulted in irregular fragmentation followed by subsequent divisions yielding cells of normal length. However, reinitiation of division in similarly washed cells of *Hyphomicrobium* was dependent upon the production of new hyphae from which typical buds were formed and released. This situation was of particular interest as it clearly indicated that those septation sites that were laid down in the presence of PDD were unable to function, even after this compound was removed by washing. Although it is reasonable to assume that a similar situation exists in the cases of *E. coli* and *C. crescentus*, irreversible inactivation of septation sites in these organisms would be technically more difficult to detect.

It should be noted that cell division in bacteria is generally quite sensitive to deleterious environmental influences, and a large number of chemically unrelated compounds can promote the formation of filaments (30). Accordingly, PDD is not unique in this respect, although the

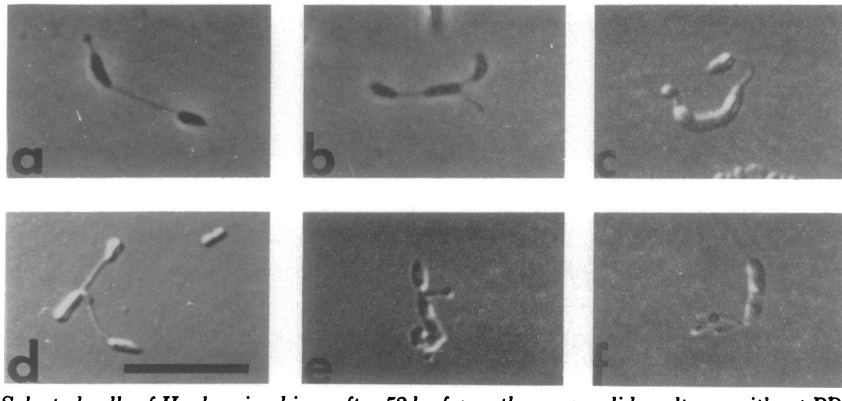

FIG. 6. *Selected cells of Hyphomicrobium after 52 h of growth on agar slide cultures without PDD. The organisms had previously been grown for 12 h with 5.0 μg of PDD per ml of medium and were then washed twice by centrifugation in medium 337a. Hyphae with buds in various stages of development extend from the tip of elongated old bud cells (a-c) or arise from or near the base of preexisting hyphae (d-f); phase (a-b) and Nomarski differential interference (c-f) photomicrographs. Bar = 10 μm.*

low concentrations at which this compound can inhibit division may be unusual. The presence of tightly bound, heavy metals, such as platinum, on the cell surface might, in itself, be sufficient to prevent cell division. It is known, however, that gratuitous induction of DNA repair enzymes can also suppress the initiation of septation (3, 4, 18, 30). DNA repair activity observed during cultivation with PDD (1, 2) could, therefore, account for the formation of filaments. In any any event, the general ability of this compound to promote enlargement of gram-negative organisms may prove useful to those concerned with species in which division mutants have not yet been isolated or in isolates in which such mutations would be lethal.

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