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# Effect of Microtubule-Disrupting Drugs on Protein and RNA Synthesis in *Physarum polycephalum* Amoebae

Victor A. Bernstam, Robert H. Gray, and Isadore A. Bernstein

Environmental Cellular Chemistry Laboratory, Department of Environmental and Industrial Health, School of Public Health, The University of Michigan, Ann Arbor, Michigan 48109, USA

**Abstract.** The effects of the microtubule-disrupting drugs, colchicine, vinblastine, podophyllotoxin, griseofulvin, and lumicolchicine ($10^{-5}$ M), on protein and RNA synthesis were studied in *Physarum polycephalum* amoebae in culture. All, except lumicolchicine, were found to simultaneously reduce the rate of protein synthesis and stimulate RNA synthesis. These results parallel the effects seen in cells exposed to heat shock. Treatment of the cells with a microfilament-disrupting drug, cytochalasin B (10 μg/ml in ethanol), resulted in a reduced rate of protein synthesis after 2 h compared to a similar effect by vinblastine in 5–15 min. A morphological abnormality, microtubule paracrystals, were seen associated with centrioles in vinblastine-treated cells in which protein synthesis had been reduced by 50%. Vinblastine and podophyllotoxin were shown to interfere with the recovery of protein synthesis after inhibition by low or elevated temperatures. The possible role of microtubules in regulating the translational response of a cell to an external environmental stimulus is discussed.

**Key words:** Protein – RNA synthesis – Microtubules – Microfilament-disrupting drugs – Heat – Cold shock – Recovery.

Alteration of cellular functions intimately associated with the cell surface seems to be the earliest effect of a variety of environmental influences. This is manifested in changes of macro-and microtopography of the cell surface (McGroarty et al., 1973; Shelton and Orenstein, 1976), alteration of the cell's adhesive properties and its interaction with other cells (Moscona and Moscona, 1966; Sternberg et al., 1973; Ueda et al., 1976) and derangements in the cell's selective permeability (e.g. Russell and Harries, 1968; Bernstam, 1974, and refs. therein).

Analysis of the sensitivity of various cellular functions to graded doses of non-specific environmental stressors such as supraoptimal temperatures, high hydrostatic pressure, desiccation, proteolytic attack, etc., revealed that, in contrast to other functions (e.g. respiration, RNA synthesis), the initiation of protein synthesis exhibits early injury which coincides with or immediately follows alterations at the cell surface (Bernstam, 1978). Although the two environmental effects might occur coincidentally and not be directly related, the selective high sensitivity of translation and cell surface functions – two cellular activities spatially separated from each other – implies that either a common highly labile component critical for both functions is affected by an environmental stress or a signal is transmitted via a communicative link. This link can be represented by either a diffusible molecule(s) such as a cyclic nucleotide (e.g. Zick et al., 1978), $Ca^{2+}$ (Kretsinger, 1976; Loewenstein, 1976) or by a structual component extending inward from the plasma membrane to the sites of protein synthesis. Transfer of information along this communicative link is thought to occur in both directions.

The function of polysomes in cells has been related to the state of membranes of the endoplasmic reticulum with which they are associated in eukaryotic cells. An elaborate hypothesis for translational regulation of genetic expression visualizing the polysome-membrane interactions as crucial in controlling protein synthesis has been developed by Shires et al. (1974). At the same time, the presence of tubulin and actin in or associated with cell membranes (Nath and Flavin, 1978) makes it difficult to hold a single type of individual cellular component solely responsible for the observed effects produced by an environmental stressor. However, the dynamic nature of the MT-microtrabeculae network (Ockleford and Tucker, 1973; Byers and Porter, 1977), its high sensitivity to environmental stressors such as adverse temperature and high hydrostatic pressure

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(Tilney and Porter, 1967; Borisy et al., 1974), its association with both the surface membrane structures and polysomes (Bensch and Malawista, 1969; Krishan and Hsu, 1969; Dales et al., 1973; Wolosewick and Porter, 1976, 1979) led Bernstam (1978) to suggest that a component of the cytoskeletal structure (possibly the MT-microtrabecular network) can serve as a transmitter of external signals to the sites of protein synthesis. In fact, cytoskeletal structures have been considered as involved in the modulation of cell surface properties such as those affected by lectins (Edelman, 1976; Edelman and Yahara, 1976) and in cellular differentiation (Puck, 1977). No studies have been reported, however, on the involvement of cytoskeletal structures in the transduction of non-specific environmental signals into a specific cellular response, e.g. modification of protein synthesis in cells exposed to changes in the external environment. In support of the postulated role of the cytoskeleton in the control of protein synthesis, data are reported here which show that the exposure of *Physarum polycephalum* amoebae to MT-disrupting drugs results in suppression of the rate of overall protein synthesis accompanied by stimulation of RNA synthesis. This response is strikingly similar to that which is seen in cells exposed to supraoptimal temperatures (Bernstam, 1974). Drugs which affect microfilaments (MF) do not appear to produce the same effect.

## Materials and Methods

*Chemicals.* Colchicine (Ch) and vinblastine sulfate (Vb) were obtained from Sigma Chemical Co. (St. Louis, MO); cytochalasin B (CB), podophyllotoxin (Pd) and griseofulvin (Gr) were from Aldrich Chemical Co. (Milwaukee, WI); [$^3$H]-uridine (sp. act. 27.6 Ci/mmole) from New England Nuclear (Boston, MA); [$^{14}$C]-leucine (sp. act. 309 mCi/mmole) and [$^{14}$C]-reconstituted protein hydrolysate from Schwarz-Mann (Orangeburg, NY). Lumicolchicine was prepared from Ch as described (Wilson and Friedkin, 1966); [$^3$H]-Vb (sp. act. 9.2 Ci/mmole) was from Amersham (Arlington Heights, IL).

*Cells.* Myxamoebae of the slime mold *Physarum polycephalum* were grown in axenic culture in a soluble semi-defined medium described by Goodman (1972) in shake cultures and harvested in log phase on the third day after transfer to a fresh medium. Incubation of the cell suspension at elevated temperature was performed in a temperature controlled water bath (Ultra-Kryomat TK-30, Lauda Instruments, Westbury, NY).

*Assay of Drug Effects on the Rate of Protein and RNA Synthesis.* Cells in the growth medium ($1 \times 10^6$ cells/ml) were exposed to the drugs (Ch, Vb, Pd, Gr, CB, lumicolchicine) at specified concentrations for various periods of time after which [$^{14}$C]-leucine or [$^{14}$C]-protein hydrolysate (1 μC/ml) and [$^3$H]-uridine (10 μCi/ml) were added in the presence of the drug being tested and the cells were allowed to incorporate the tracers for 15 min. Incorporated radioactivity was determined in material which was precipitable in cold 10% trichloroacetic acid (TCA)-50% acetone for 30 min (proteins and nucleic acids) or which remained TCA-insoluble after hydrolysis at 90°C for 20 min in 10% TCA (proteins). The precipitates were transferred to Whatman GF/934 AH filters and washed 5 times with 10% TCA-50% acetone followed by cold ether-ethanol (1:1), dried and counted in 5 ml of PCS (Amersham, Arlington Heights, IL) in a Packard scintillation counter. The acid-soluble radioactivity was measured in cells briefly, washed with cold growth medium followed with suspension and disruption of the cells in a glass-teflon homogenizer in 10% TCA-acetone. Aliquots of the homogenate were counted for total radioactivity. Acid-insoluble radioactivity determined in an aliquot of the cells was subtracted from total radioactivity, the difference representing TCA-soluble radioactivity.

*Assay of [$^3$H]-Vb Uptake.* The kinetics of uptake of [$^3$H]-Vb in amoebae was determined by incubating the cells with 1.0 μCi/ml of [$^3$H]-Vb in the presence of $10^{-6}$, $10^{-5}$ and $10^{-4}$ M of non-radioactive Vb. At specified times 0.3 ml aliquots of cell suspension in triplicate were collected on filters and washed twice with 20 ml of growth medium, dried in the air and counted in PCS in a scintillation counter.

*Electron Microscopy. Physarum polycephalum* amoebae were sedimented and fixed in 3% glutaraldehyde – 0.1 M sodium cacodylate buffer containing 10 mM KCl and 5 mM $MgCl_2$, pH 7.3 for 1 h, washed twice for 1 h each and then overnight in 0.1 M sodium cacodylate buffer containing 0.2 M sucrose. The specimens were then post-stained in 2% $OsO_4$ in 0.1 M sodium cacodylate buffer, washed in 0.1 M sodium cacodylate buffer and in distilled water, dehydrated in an ethanol series followed by propylene oxide and embedding in Epon. Sections were prepared with a diamond knife on a Reichert OmU2 microtome. The sections were post-stained with lead citrate and uranyl acetate and examined with an AEI Corinth 275 electron microscope.

## Results and Discussion

Exposure of amoebae to the MT-disrupting drugs, Ch, Vb, Pd or Gr at $10^{-5}$ M or to the MF-disrupting drug, CB, at 10 μg/ml in ethanol for various periods of time revealed that the two types of drugs had essentially different effects on the rates of protein (Fig. 1A) and RNA (Fig. 1B) synthesis. The MT-disrupting drugs enhanced the synthesis of RNA and depressed the synthesis of protein. The rate of incorporation of [$^3$H]-uridine into RNA, reached a maximum by 15 min of incubation and then returned to control level or was suppressed. The decrease in the rate of protein synthesis took 45 min to become apparent except for Vb, which produced an obvious depression within 5 – 15 min and a maximal effect by 30 min. The MF-disrupting drug, CB, had no effect on RNA synthesis within 2 h, and produced a reduction in the rate of protein synthesis with respect to control only after 2 h of exposure.

In view of the reported impairment of membrane transport systems by an MT-disrupting drug (Wilson et al., 1974), it was necessary to demonstrate that the decreased incorporation of radioactive tracers was not a function of their decreased entry into the cell. The effects of Ch and Vb on the uptake of [$^{14}$C]-leucine and [$^3$H]-uridine into the TCA-insoluble fraction of the cell were therefore measured. At the concentrations of Ch and Vb which were effective in altering the rate of protein and RNA synthesis ($10^{-5}$ M), the radioactivity in this fraction was above control (Table 1). Thus, the changes in the apparent rate of incorporation of protein and RNA precursors which are produced by MT-

**Table 1.** Uptake of [$^3$H]-uridine and [$^{14}$C]-leucine during exposure (15 min) of amoebae to Ch and Vb ($10^{-5}$ M)

| | [$^3$H]-uridine | | | | [$^{14}$C]-leucine | | | |
|---|---|---|---|---|---|---|---|---|
| | TCA soluble[a] | | TCA insoluble | | TCA soluble[a] | | TCA insoluble | |
| | cpm | % | cpm | % | cpm | % | cpm | % |
| 26°C | 1624 | 100 | 1252 | 100 | 412 | 100 | 358 | 100 |
| 26°+Ch | 3367 | 207 | 1628 | 130 | 1134 | 275 | 366 | 102 |
| 26°+Vb | 3758 | 231 | 2252 | 180 | 1033 | 250 | 262 | 73 |
| 26°+Ch+Vb | 8646 | 532 | 1457 | 116 | 1769 | 429 | 252 | 70 |

[a] Soluble radioactivity determined as the difference between total radioactivity − TCA insoluble radioactivity. For technical details, see Methods

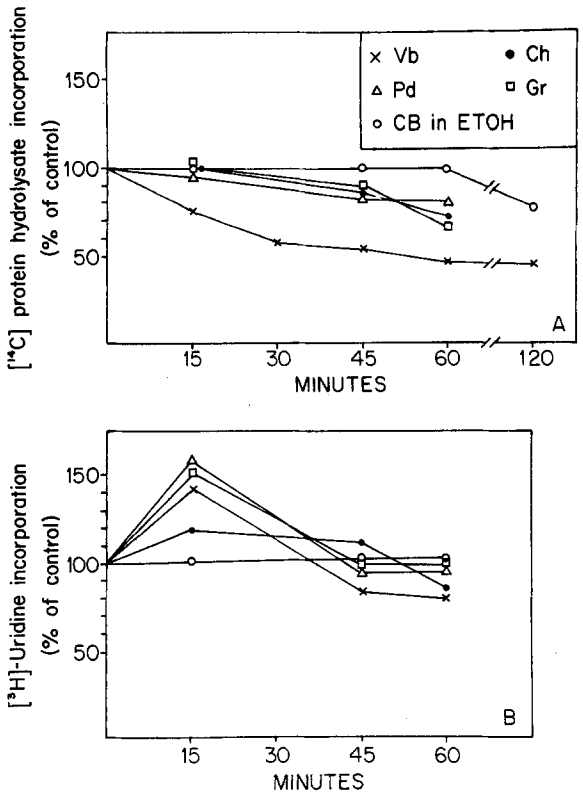


**Fig. 1A and B.** Effects of drugs on protein and RNA synthesis. Time course of the effects of Ch, Vb, Pd, Gr ($10^{-5}$ M each), and CB (10 µg/ml) on pulse (15 min) incorporation of [$^{14}$C]-protein hydrolysate (**A**) and [$^3$H]-uridine incorporation (**B**) into TCA-precipitable material

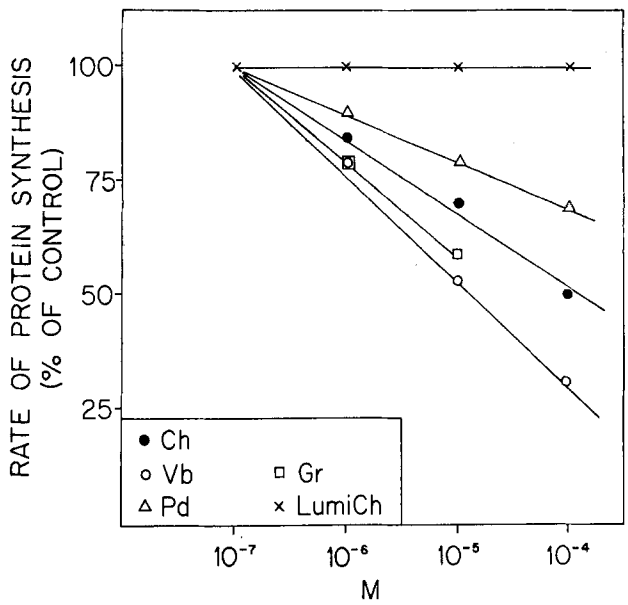


**Fig. 2.** Effect of dose of the drugs on protein synthesis. Concentration dependence of the decrease in the rate of [$^{14}$C]-protein hydrolysate incorporation by Ch, Vb, Pd, Gr and lumicolchicine. The cells were incubated with the drugs at indicated concentrations for 45 min then the label was added for another 15 min and TCA-insoluble radioactivity was determined

disrupting drugs do not appear to be attributable to disturbances in the transport systems resulting from impairment of the cellular membranes.

Figure 2 shows that the rate of protein synthesis as measured by the incorporation of [$^3$H]-leucine was reduced in a dose-dependent manner by exposure of cells to greater than $10^{-7}$ M concentrations of Vb, Pd, Gr or Ch. Vb was most effective producing a 75% inhibition at $10^{-4}$ M. As revealed by electron microscopy, exposure to $10^{-5}$ M Vb, which resulted in a 50% inhibition of protein synthesis (cf Fig. 1), produced a morphological change of microtubules best seen associated with the MT-containing centriole (Fig. 3). This abnormality, designated as MT-paracrystals, is similar to that seen in other cells exposed to MT-disrupting drugs (Bensch and Malawista, 1969; Krishan and Hsu, 1969; Dales et al., 1973), and was not seen in the unexposed amoebae. No other ultrastructural abnormalities were observed in treated cells.

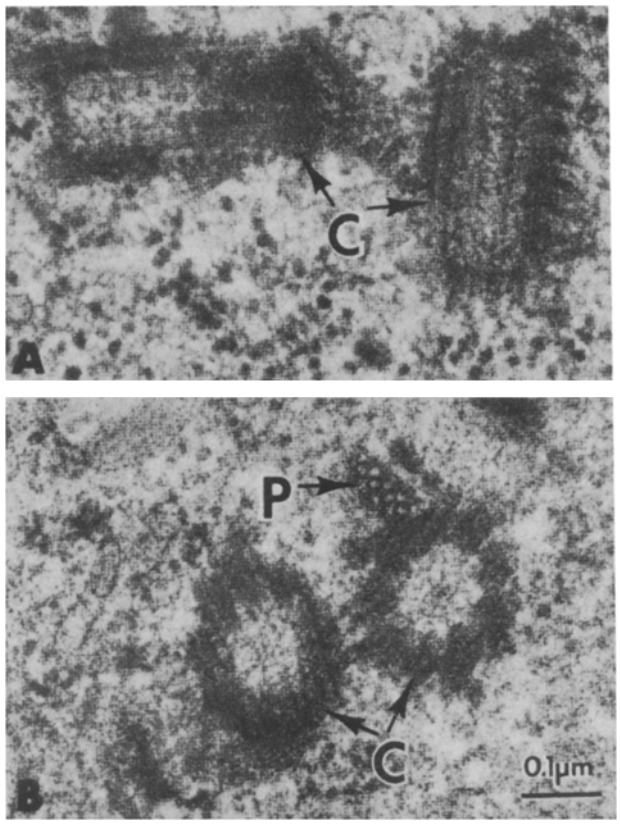


**Fig. 3A and B.** Centrioles of *Physarum polycephalum* amoebae. **A** Control amoebae. ×100,000. **B** Vinblastine treated ($10^{-5}$ M for 30 min) amoebae showing microtubule-paracrystals associated with centriole (*P*). ×100,000

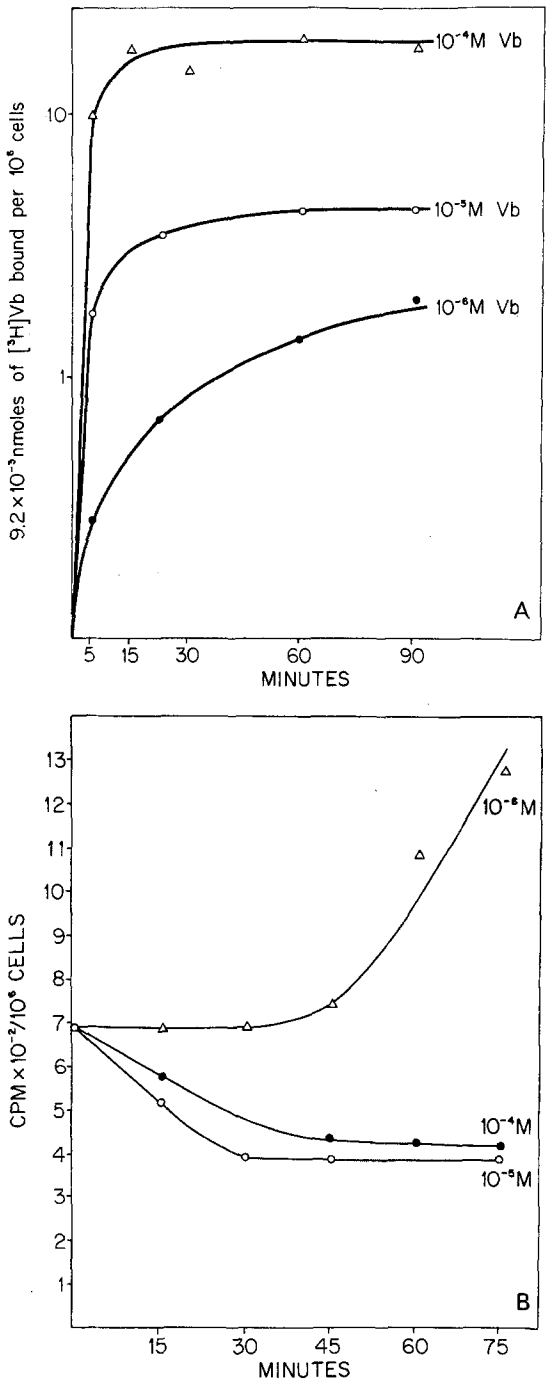


**Fig. 4A and B.** Effect of Vb on protein synthesis. The kinetics of uptake of [$^3$H]-Vb (1 μCi/ml) in the presence of "cold" Vb ($10^{-4}$, $10^{-5}$, $10^{-6}$ M) (**A**) and the effect of Vb ($10^{-4}-10^{-6}$ M) on the pulse incorporation of [$^{14}$C]-leucine (1 μCi/ml) (**B**)

Figures 4A and B compare the cellular binding of Vb with the resultant inhibition of protein synthesis. At $10^{-5}$ and $10^{-4}$ M Vb the binding and inhibition reached a plateau by about 30 min. The binding appeared to preceed the inhibition of protein synthesis. At low concentrations, these drugs have been reported to affect MT and interfere with the secretion of proteins before they affect synthesis of proteins (Ehrlich et al., 1974; Redman et al., 1975; Williams and Lee, 1976; Marks and Labourdette, 1977). At $10^{-6}$ M Vb the binding was much slower and the effect on protein synthesis was extremely variable so that in some cases it was a stimulation rather than an inhibition as shown in Fig. 4B. It should be emphasized that even [$^3$H]-Vb binding experiments provide no information as to the absolute concentration of the drug inside the cell, which can be substantially lower than that in the medium and, to our knowledge, no reports are available relating the external concentration of MT-disrupting drugs to the effective intracellular concentration. Differences in the permeability of MT-disrupting drugs in *Physarum* amoebae and other cells might account for the seemingly higher concentration of the drugs required in *Physarum* amoebae as compared with other cells in order to achieve a similar level of inhibition of protein synthesis.

Despite relatively rapid binding of [$^3$H]-Vb by *Physarum* amoebae, accompanied by a rapid inhibition of protein synthesis, the reversibility of Vb-induced

inhibition of protein synthesis was a slow process apparently related to slow removal of the drug from the cells. As noted in Table 2 the inhibition of protein synthesis by $10^{-5}$ M Vb returned to normal by 16 h after the cells were washed and resuspended in a drug-free medium.

The involvement of MT-related structures in the control of protein synthesis was further studied in amoebae recovering from exposure to heat and cold. Recovery of heat-induced inhibition of protein synthesis occurs within a matter of minutes after a shift-down of *Physarum polycephalum* plasmodium back to normal growth temperature (Bernstam, 1974). The same proved to be the case with *Physarum* amoebae. Thus, recovery of protein synthesis after exposure of amoebae to 44° C for 15 min begins almost instantaneously upon transfer of the cells to 26° C (Fig. 5). In the presence of Vb or Pd ($10^{-5}$ M each) added simultaneously with transfer to 26° C the recovery was delayed and suppressed. Considering the high speed of recovery in the absence of the drugs and the fact that drug-induced inhibition of protein synthesis was shown to be time-dependent, the incomplete block of the recovery of protein synthesis may be explained by the partial recovery of protein synthesis before the drugs begin to exert their inhibitory action. In fact, after 15 min in the presence of the drugs, recovery is almost completely inhibited. The very low inhibitory effect of Ch on the recovery of protein synthesis during 30 min at 26° C following exposure at 44° C for 15 min (experiments not shown) is consistent with this explanation, since it has been shown that Ch begins to inhibit protein synthesis only after 45–60 min of exposure to the drug (Fig. 1).

**Table 2.** Reversibility of Vb effect on pulse incorporation (15 min) of [$^{14}$C]-protein hydrolysate (% of incorporation into untreated cells)

| $10^{-5}$ M Vb, 30 min followed by removal of Vb | 5 h after removal of Vb | 16 h after removal of Vb | Reexposure to $10^{-5}$ M Vb, 30 min, at 16 h after removal |
|---|---|---|---|
| 50–65 | 55–67 | 100 | 52–60 |

For technical details, see Methods

Furthermore, the disruptive effect of low temperatures on MT has been well documented in various cell types (Tilney and Porter, 1967; Frankel, 1976; Schliwa et al., 1978). At the same time, convincing evidence is available to show that cold shock, similar to elevated temperature (Goldstein and Penman, 1973), blocks protein synthesis at the initiation step (Friedman et al., 1971; Craig, 1975; Oleinick, 1976). Therefore, one could expect that MT disruption by tubulin-specific drugs would interfere with recovery of protein synthesis after a cold shock. In fact, $10^{-5}$ M Vb or Pd added to amoebae immediately after the temperature of the culture was returned to 26° C after exposure to 0° C for 15 min significantly reduced recovery of protein synthesis (Fig. 6).

Our contention that MT-related structures are involved in the response of protein synthesis to environmental influences is supported by the following: Firstly, various MT-disrupting drugs, which differ in their sites of action, all decrease the rate of protein – but not RNA synthesis in a dose-dependent manner although at low concentrations they may stimulate protein synthesis. Stimulation of a cellular process by low doses of a stressor which inhibits the same process at higher doses has previously been reported (see Alexandrov, 1977). Removal of the drug reverses the inhibition of protein synthesis. Secondly, lumicolchicine, a photoinactivated derivative of Ch, does not bind to tubulin (Wilson and Friedkin, 1966) and has no effect on protein synthesis. Thirdly, Vb at a concentration which inhibits protein synthesis by 50%, results in an ultrastructural abnormality in an organelle composed of MT. Fourthly, for a microfilament-disrupting drug, CB, to affect protein synthesis, considerably longer exposures were needed.

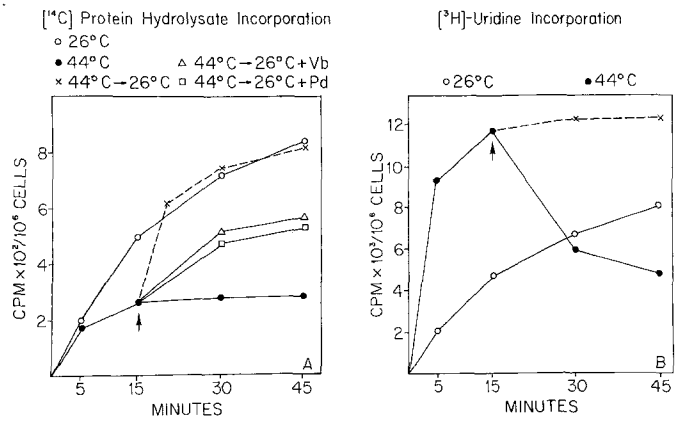


**Fig. 5A and B**
Effect of high temperature on protein and RNA synthesis. **A** Continuous incorporation of [$^{14}$C]-protein hydrolysate into TCA-insoluble material at 26° C (○), 44° C (●) and upon transfer (*arrow*) of amoebae to 26° C (×), in the presence of Vb ($10^{-5}$ M) (△) or Pd ($10^{-5}$ M) (□). **B** Continuous incorporation of [$^{3}$H]-uridine into TCA-insoluble material at 26° C (○), 44° C (●) and upon transfer (*arrow*) of amoebae to 26° C (×)

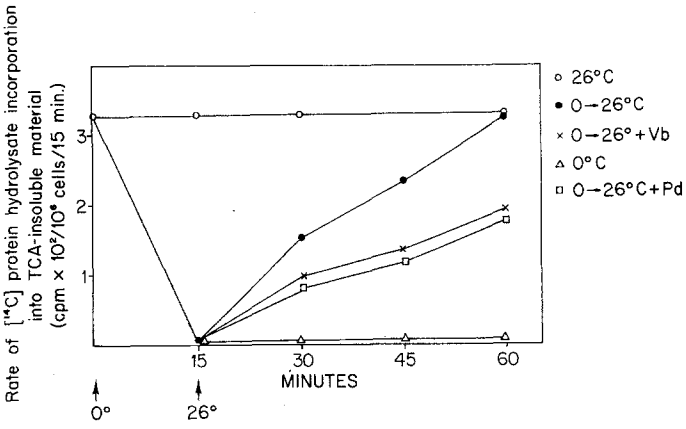


**Fig. 6.** Effect of cold shock on protein synthesis. Pulse incorporation (15 min) of [$^{14}$C]-protein hydrolysate into TCA-insoluble material at 26°C (○), 0°C (△), upon transfer of amoebae to 26°C (○) in the presence of Vb ($10^{-5}$ M) (×) and Pd ($10^{-5}$ M) (□)

Additional support for this concept is provided by a) the structural association of polysomes with MT (Bensch and Malawista, 1969; Krishan and Hsu, 1969; Dales et al., 1973) and the cell surface with MT (Edelman and Yahara, 1976; Wolosewick and Porter, 1979), b) the high sensitivity of MT to injury from adverse temperature or high hydrostatic pressure (Tilney and Porter, 1967; Borisy et al., 1974), c) our finding that the recovery of protein synthesis from a cold or heat shock cannot be achieved when reassembly of MT is blocked by $10^{-5}$ M Vb or Pd, and d) by the preliminary data developed in this laboratory which indicate that binding of phytohemagglutinin, soy bean and gorse lectins to *Physarum* amoebae can stimulate the rate of protein synthesis in a concentration dependent manner – an effect which can be prevented by exposure of the cells to MT-disrupting drugs in concentrations affecting translation in either untreated cells or in cells recovering from a cold or heat shock.

It should be emphasized that relating cytoskeletal alterations produced by chemical agents with changes in protein synthesis as reported by other workers and in the present paper rests on the assumption that the drugs tested specifically affect the MT or MF. While this view is strongly supported by indirect observations such as correlations between the binding pattern of labeled drugs and the inhibition of protein synthesis, and the exclusion of permeability disturbances as causes of the observed changes in protein and RNA synthesis, one cannot, at this point, rule out the possibility that the drugs may directly affect the mRNA, tRNA, ribosomes or the soluble factors required for translation.

On the other hand, MT-disrupting drugs have long been used to synchronize cells with respect to mitosis. This process typically requires exposures lasting for several hours. However, effects of the same drugs on cytoplasmic protein synthesis appear much sooner, suggesting that cytoplasmic MT are much more sensitive than is the mitotic spindle. The early effects of these drugs on translation may be a function, among other things, of the formation of MT-polyribosome complexes. The resulting distortions in the spatial arrangement of polysomes in the cytoskeleton network would certainly affect both the efficiency and specificity of translation. In fact, an indication to the latter possibility was obtained in SDS-gel electrophoresis of proteins from amoebae exposed to different concentrations of vinblastine after pulse labeling with radioactive amino acids. Changes were observed in the band patterns as compared with those from untreated cells (our unpublished observations). At the same time, association of polysomes with the cytoskeleton in HeLa cells was thought to occur via mRNA (Lenk et al., 1977). However, in that study the microtubular network had been destructed and, thus, its role in polysome-cytoskeleton association was not estimated.

Investigation of the above mentioned possibilities is in progress.

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