# REVERSAL BY LIGHT OF THE ACTION OF *N*-METHYL *N*-DESACETYL COLCHICINE ON MITOSIS

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## INTRODUCTION

Colchicine, in low concentrations, inhibits cell division in a wide variety of cell types through an effect which results in dissolution of the spindle (1, p. 62 *et seq.*) This is especially clearly shown in the work of Inoué (2).

When exposed to sunlight, colchicine undergoes a photochemical rearrangement to give $\alpha$, $\beta$, and $\gamma$ lumicolchicines (3, 4) (Fig. 1). There is also evidence that exposure to sunlight and air reduces the biological activity of colchicine (1, p. 374). This suggests the possibility of photochemically reversing colchicine effects in living cells.

The *N*-methyl derivative of *N*-desacetyl colchicine (Ciba's trade name is Colcemid) was used in this study in preference to colchicine because of its effectiveness at lower external concentrations and because of fewer irreversible effects.

## MATERIALS AND METHODS

*N*-methyl *N*-desacetyl colchicine[1] (Ciba Colcemid lot No. M 1168) had a molar extinction coefficient of $1.7 \times 10^4$ at 355 nm in water (assuming a molecular weight of 371) and an $\epsilon_{290}/\epsilon_{355}$ ratio of 0.25. For irradiation, either a G.E. AH-4 or an Osram HBO-200 mercury arc was used and the 366 nm complex was isolated by a 2 mm Jena glass UG-1 filter or a Kodak 18 A filter. These were used in conjunction with a 3 mm KG-1 heat filter and with a 2 mm BG 38 filter.

Quantum efficiencies were determined by ferric oxalate actinometry (5) with a Zeiss Model PMQ II spectrophotometer. The absorption curves shown in Fig. 2 were obtained with a Beckman Model DB-G spectrophotometer.

Several species of sea urchin, *Strongylocentrotus drobachiensis*, *Strongylocentrotus purpuratus*, and *Lytechinus variagatus*; one species of starfish, *Pisaster ochraceous*, and the annelid *Pectinaria gouldii* were used. These specimens were obtained from commerical sources and shipped to Philadelphia for use during the spring of 1969. Maturation in the starfish was induced by motor nerve extract (6).

Artificial seawater prepared according to the Woods Hole Marine Biological Laboratories formulation was used throughout.

Microscope observations on *L. variagatus* and *P. gouldii* were made at room temperature (22°C) and on *P. ochraceous* below room temperature (13–17°C) with a Leitz polarizing microscope and objective (UMK 32. NA about 0.4) in conjunction with an American Optical Company (Southbridge, Mass.) "Super Bio" rectified condenser.

## RESULTS

### *Chemical*

Irradiation of Colcemid at 366 nm caused a large decrease in absorbance at 355 nm which was comparable to that seen on irradiating colchicine (Fig. 2).

The quantum efficiency of this reaction is about 0.003 in water and is 12 times greater in *n*-butanol containing ca. 3% of water. It may be possible to estimate the quantum efficiency for Colcemid bound to the cell in vivo which would give information about the binding site. An order of magnitude value is 0.003 based on an OD of 0.07 for the egg in $10^{-6}$ M Colcemid, an incident intensity at 366 nm of $4 \times 10^8$ quanta/$\mu^2$ per sec and assuming, on the basis of birefringence recovery, that 1 min of irradiation inactivates half the bound Colcemid.

### *Cell Irradiation*

Cleavage of sea urchin eggs was inhibited, when they were placed, soon after fertilization, in Colcemid at a concentration of $3 \times 10^{-7}$ M or greater. Continuous exposure to 366 nm radiation from a 100 watt mercury arc[2] reversed this inhibition with

[1] *N*-methyl *N*-desacetyl colchicine is listed as demecolcine in the Merck Handbook, 7th edition, and is sold by Ciba Pharmaceutical Company, Fairlawn, N.J., under the trade name of Colcemid[R]. Since the name Colcemid is in fairly common usage, we have used it throughout this paper.

[2] We used a 100 watt G.E. AH-4 mercury arc lamp and a Kodak 18A filter mounted in a Spencer 370-A microscope lamp housing and imaged about 15 in. away.

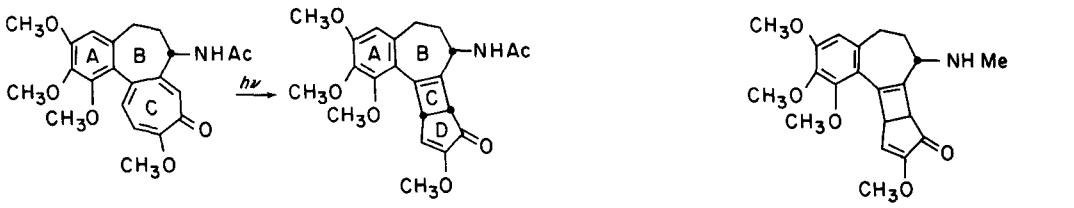



FIGURE 1 Formulae for photoconversion of colchicine to β-lumicolchicine and formula of Colcemid. α- and γ-lumicolchicine, dimer and isomer of β-lumicolchicine, are also found on irradiation.

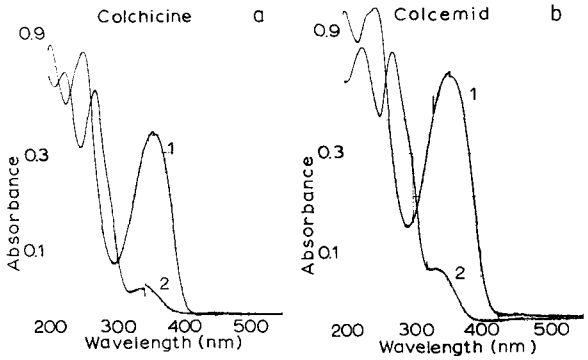



FIGURE 2 Changes in absorbance seen when solutions of colchicine and of *N*-methyl *N*-desacetyl colchicine (Colcemid) were irradiated at 366 nm; (*1*) before irradiation; (*2*) after irradiation. The spikes at about 330 nm and the discontinuities at 355 nm are instrument artefacts. (*a*) 2 x $10^{-5}$ M colchicine in water. (*b*) 3 x $10^{-5}$ M *N*-methyl *N*-desacetyl colchicine in water.

no delay for eggs in 1 × $10^{-6}$ M Colcemid, with a slight delay for eggs in 3 × $10^{-6}$ M Colcemid, and had no obvious effect on eggs in 3 × $10^{-5}$ M Colcemid. Irradiation of eggs in Colcemid-free seawater under the same conditions did not cause a decrease in the number of cleaving eggs or a definite change in the time of cleavage.

Most of the cytological observations were made on the second cleavage division of *L. variagatus* eggs. We routinely used artificial seawater containing 3 × $10^{-5}$ M Colcemid for 5 min to destroy the spindle birefringence, followed by 1 × $10^{-6}$ M Colcemid in artificial seawater to provide a defined background concentration. Healthy cells already in anaphase continued through cleavage, while cells in other stages were blocked from cleavage or spindle formation. *L. variagatus* spindles showed a major decrease in length and in birefringence within 2 min in 3 × $10^{-5}$ M Colcemid which gives an indication of the time to affect the spindle. In the absence of Colcemid, the time from nuclear membrane breakdown to anaphase was about 7 min.

A specific cell was followed under the polarizing microscope. As the spindle in this cell reached the desired stage (usually midmetaphase), Colcemid was applied by perfusion, and the resulting decrease in birefringence was observed. When the birefringence loss was complete, the cell was irradiated at 366 nm. The general approach was to irradiate for a short time until a localized increase in birefringence was seen; to wait a few minutes for obvious time-dependent effects; to irradiate so as to maximize the birefringence increase; then to irradiate just because it might help the cell pass through anaphase and cleavage. The usual radiation intensity[3] on the cell was about 4 × $10^8$ quanta/$\mu^2$ per sec.

[3] This value was obtained with an HBO-200 lamp using a Leitz lamp housing, microscope base, and Ortholux stand, and with the condenser unimmersed but the diaphragm fully open.

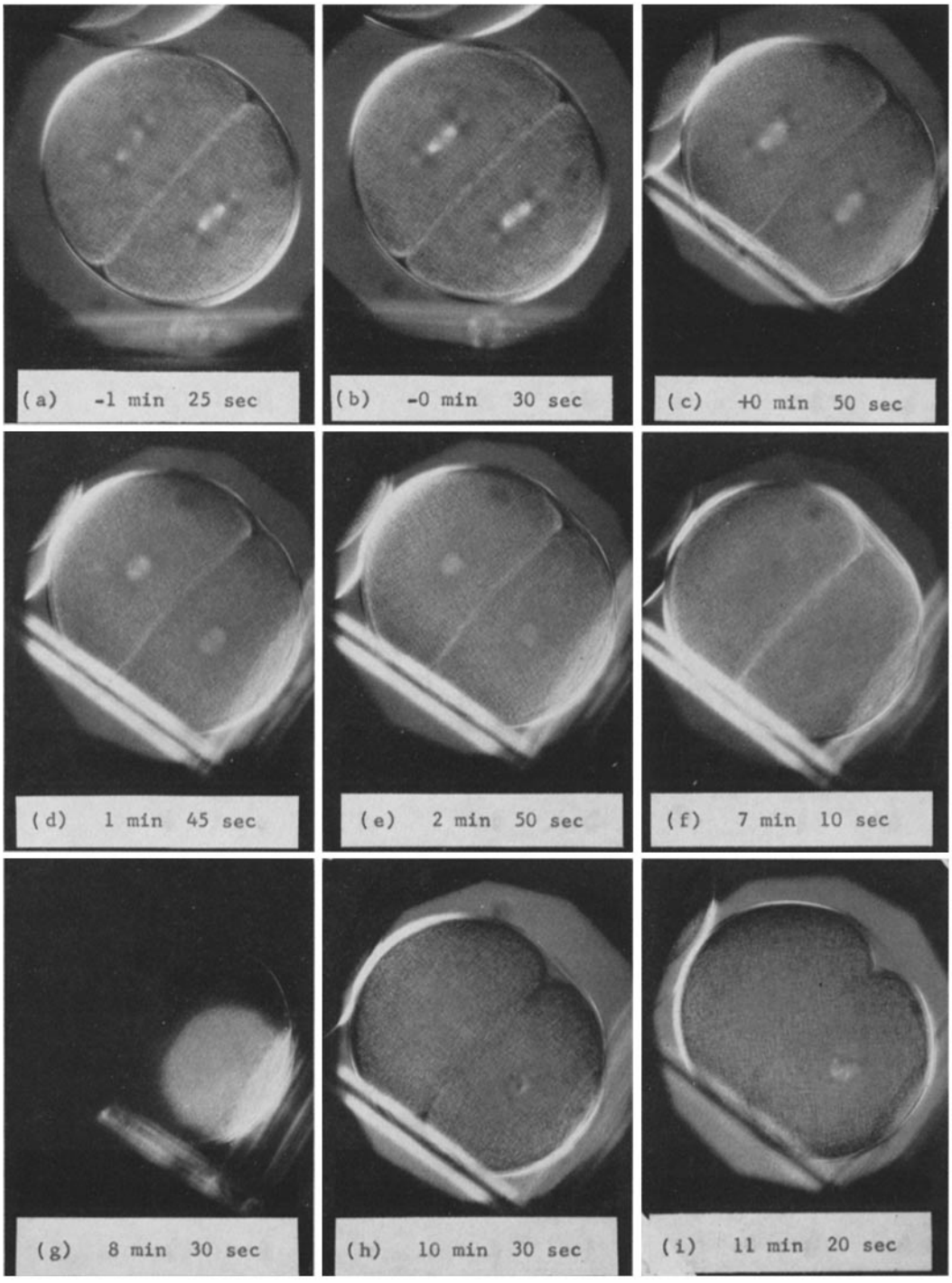
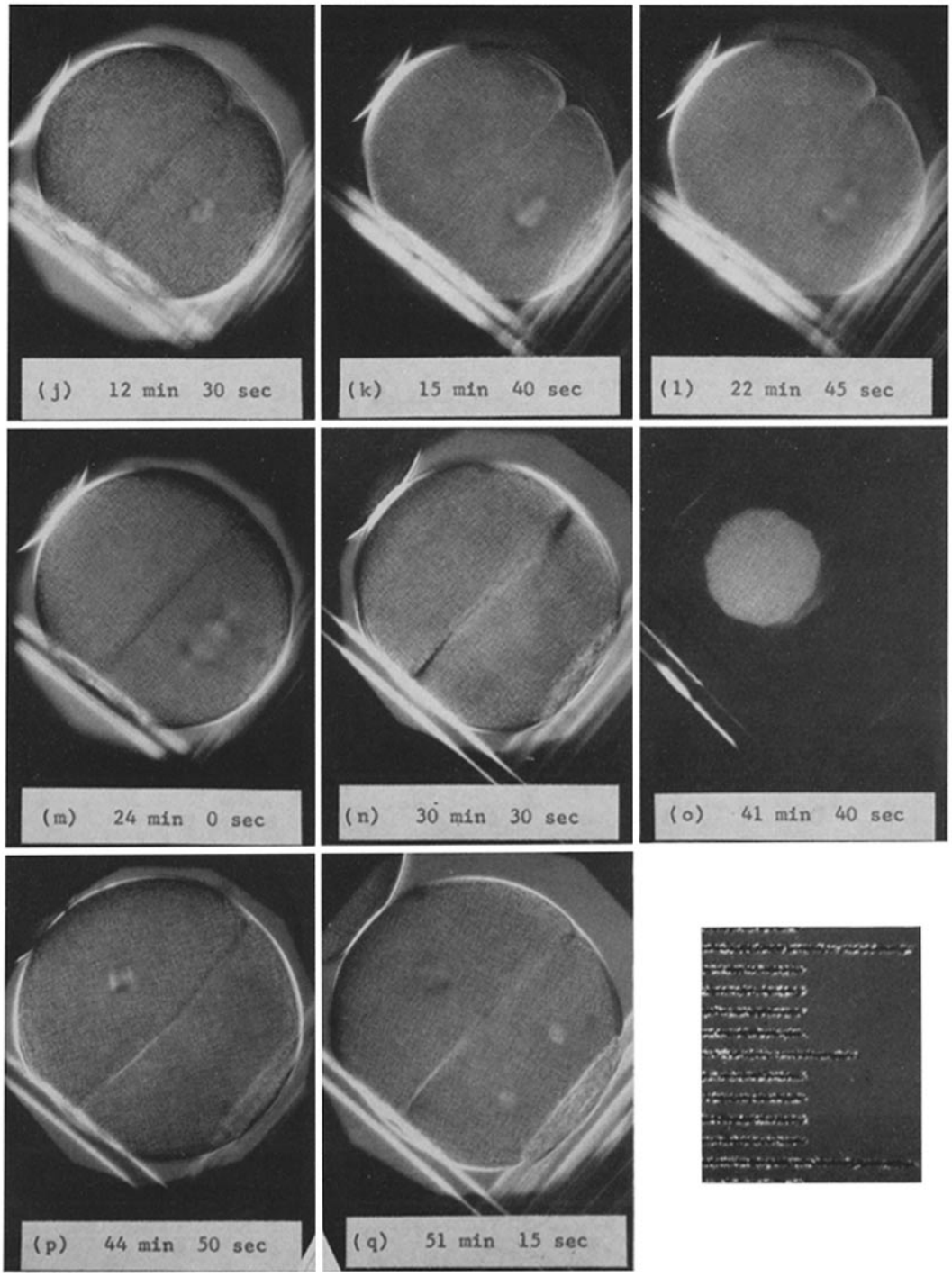



FIGURE 3 Initial development of spindle birefringence centers on the asters (*a*) and develops toward the metaphase plate to give a definitive spindle (*b*). At time *0*, 3 x $10^{-5}$ M Colcemid was added and the changes in the spindle seen in *c–f* were observed. 1 x $10^{-6}$ M Colcemid was added at time *5′20″*, and this level of Colcemid was maintained for the rest of the experiment. *g* shows the area in the lower cell to be irradiated for 30 sec at time *9′15″*, and *h–j* show some of the change observed. Further irradiation




in the region shown in *g*, given at reduced intensity over a period of minutes, carried the irradiated blastomeres through anaphase as shown in *k–n*. There was a suggestion of cleavage only.

The previously unirradiated blastomere was irradiated over the area shown in *o* for 30 sec at time 42′40″. Further low-intensity irradiation was followed by the appearance of the next set of spindles (*q*).

The scale has a spacing of 10 nm. Figs. 3 and 4 show fertilized eggs of *Lytechinus variagatus*.

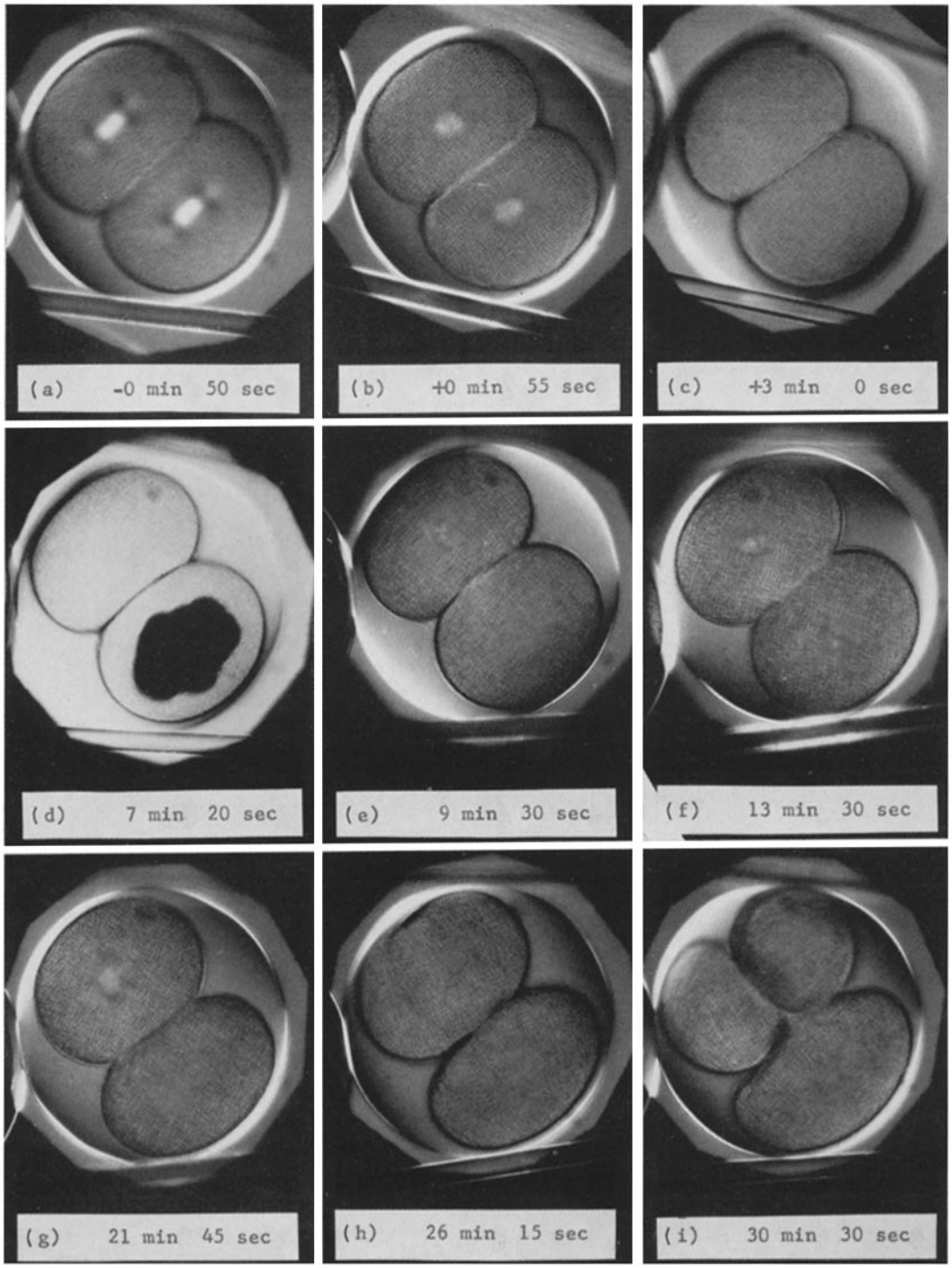



FIGURE 4 An egg at the two-cell stage with well-developed metaphase spindles (*a*) was treated with 3 x $10^{-5}$ M Colcemid at time *0* which was followed by a rapid loss of spindle birefringence (*b* and *c*). The level of Colcemid was reduced to 1 x $10^{-6}$ M at time *5'10"* and kept at this level for the rest of the experiment. For irradiation, an opaque spot in the plane of the field diaphragm was imaged in green light on the former spindle region of one cell (*d*). After 15 sec of 366 nm irradiation at time *7'30"*, there was recovery of birefringence in the unshielded cell and no detectable recovery in the shielded cell (*e*). Further localized irradiation increased the amount of birefringent material in the unshielded cell and caused some birefringence to appear in the shielded region (*f*, *g*). The fully irradiated cell cleaved after being irradiated at a reduced intensity level for about 3 more min over the next 10 min (*h*, *i*). The magnification is the same as in Fig. 3.

For *L. variagatus* eggs, 15 sec of irradiation of the whole cell at 366 nm often brought back detectable spindle fibers within 30 sec. These fibers were usually short with weak retardation and showed some further increase in birefringence during the next minute without further irradiation. This increase is opposed by the finite background level of Colcemid, and the birefringence can be seen to again decrease with time. Further irradiation in many instances increased the number of recognizable spindle fibers, made the existing ones longer, wider, and more strongly birefringent, and improved spindle organization.

The birefringence recovered was usually less and the spindles were smaller than those seen in normal divisions, although, in several instances, irradiation for 3 min gave recovery approaching that expected for a normal spindle. The degree of organization of the radiation-induced spindle was variable; well-formed bipolar spindles were seen, but most were more complex.

A stage comparable to anaphase was recognizable when the spindle was fairly well organized. It was characterized by spindle fibers with a less birefringent middle region whose occurrence was correlated with an increase in spindle length and

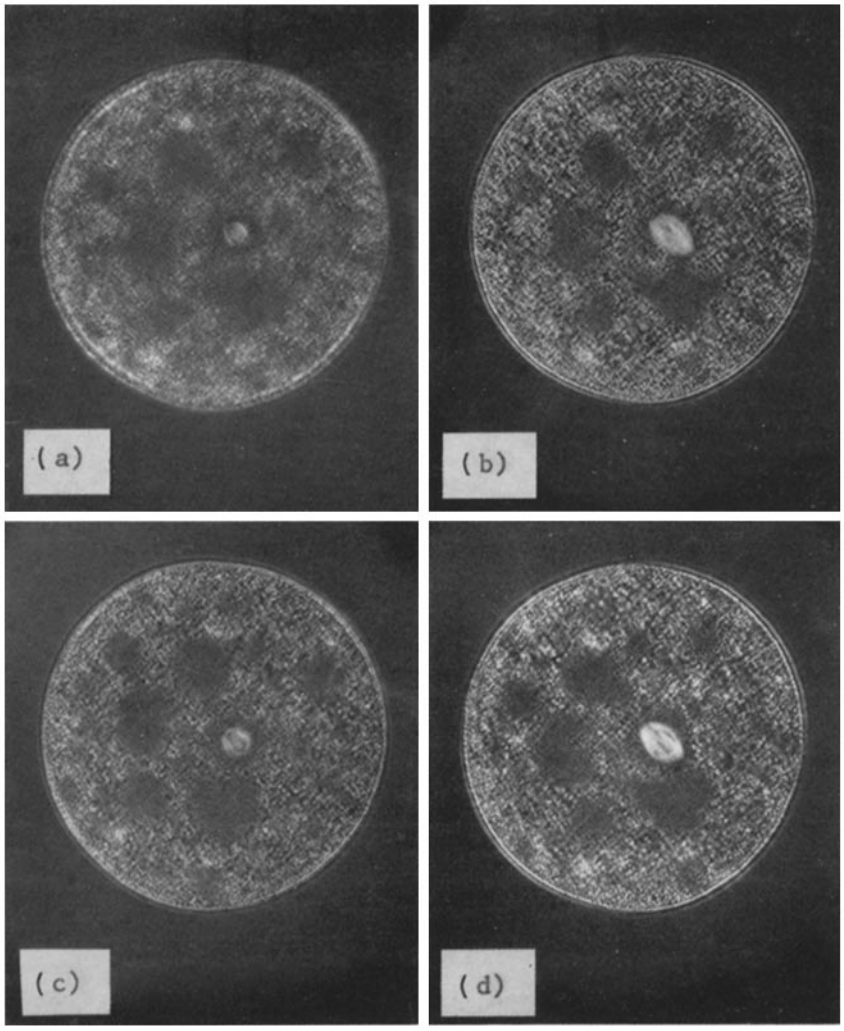



FIGURE 5 This figure shows an egg from *P. gouldii* in 1 x $10^{-6}$ M Colcemid seawater: (*a*) before irradiation, (*b*) then after irradiation at 366 nm, (*c*) then after 25 min in 1 x $10^{-6}$ M Colcemid, (*d*) then 1 min after another irradiation at 366 nm for 10 sec. × 650.

was usually followed by a rapid loss of birefringence and frequently by cleavage. Some of these observations are apparent in Figs. 3 and 4.

Comparable experiments were done with *Lytechinus pictus* eggs in which the spindles were inhibited before forming by putting cells in $3 \times 10^{-6}$ M Colcemid either at 30 min after fertilization or just after first cleavage. Irradiation of these cells over the next 20 min or so routinely gave normal spindles and regular cleavage.

The first meiotic spindles of *P. ochraceous* eggs (starfish) and of *P. gouldii* eggs (annelid) have a prolonged metaphase which makes it possible to demonstrate clearly that the spindle birefringence regained on irradiation is still sensitive to Colcemid. In some experiments of this sort with *Pectinaria* eggs (such as the one shown in Fig. 5), Colcemid was applied before germinal vesicle breakdown so that a normal-size spindle had never been formed. This early application of Colcemid did not decrease the speed of recovery, which, if anything, was faster than in eggs in which the spindle was removed with $3 \times 10^{-5}$ M Colcemid, nor did it obviously affect the final size of the spindle. The recovery of birefringence, as with *L. variagatus* eggs, was rapid with most of the change occurring within 30 sec.

Spindle birefringence did not reappear when Colcemid-blocked cells were exposed to large doses of radiation peaking at 436 nm given at incident intensities greater than those used at 366 nm.

## DISCUSSION

The present work shows that it is possible to reverse the effects of Colcemid on the spindle and on cell division by irradiation. It is likely that this result is due to photo-inactivation of the Colcemid by light absorbed in the 355 nm peak, although we have not yet obtained direct evidence for this, such as an action spectrum and a decrease in absorbance at 355 nm on irradiation.

The cytological results require more work. However, the state of the spindle at the time Colcemid was applied and the time at which irradiation occurred were clearly important parameters. Partial cell irradiation experiments, such as shown in Fig. 4, often resulted in the recovery of birefringence behind the image of the opaque spot, but we are hesitant to interpret this until scattering and focal effects are more carefully ruled out.

If colchicine and Colcemid act on the association-dissociation reaction (7) of the microtubule protein, as seems reasonable from colchicine-binding studies of Borisy and Taylor (8), then the photochemical inactivation of Colcemid should be a means of varying the pool size or the local concentration of functionally effective microtubule protein. On this basis, one might liken the effect of a short exposure to light as a "concentration jump" which is followed by changes whose rate and extent may give information about the in vivo development of microtubules. The rate at which spindle length increased in some of our experiments could be placed at better than 10 $\mu$/min, and this is a low estimate for the initial rate since it is certainly affected by the speed with which Colcemid diffused back in, and probably by diffusion of the microtubule protein by limited monomer pool size and by a possible requirement for nucleating centers.

The delay in or lack of cell cleavage, which often occurred when Colcemid-treated cells were irradiated to give recovery of spindle birefringence, may have resulted from experimental factors related to irradiation or the application of Colcemid or to characteristics of the mitotic cycle. Whatever the explanation, the use of controlled activation of the spindle by light for studying the relation of the metaphase spindle to anaphase and to cell cleavage seems promising.

Other microtubule-associated colchicine-sensitive processes have been described (1, 7), and the approach used on the spindle may be experimentally useful in their study.

## SUMMARY

The colchicine derivative Colcemid$^{R}$ was used for causing dissolution of the spindle and inhibiting cleavage in early divisions of *Lytechinus variagatus* eggs. Irradiation of such Colcemid-blocked cells at 366 nm reversed these effects as determined by the recovery of spindle birefringence and by cleavage. We tentatively conclude that 366 nm radiation causes a photochemical rearrangement in the Colcemid which reduces or destroys its effect on the spindle.

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*Note Added in Proof:* Wilson and Frieden (*Biochemistry.* 1967. **6**:126) have shown that $2 \times 10^{-6}$ M lumicolchicine does not inhibit cell division in grasshopper embryos nor does it inhibit the binding of colchicine in homogenates of such cells.