COLCHICINE INHIBITION OF STALK ELONGATION IN CARCHESIUM SP.: EFFECT OF Ca²⁺ AND Mg²⁺

M. RAHAT, HANNA FRIEDLAENDER AND RACHEL PIMSTEIN
Department of Zoology, The Hebrew University of Jerusalem, Israel

SUMMARY

The effect of colchicine on stalk elongation in the colonial peritrich ciliate Carchesium sp. has been investigated by growing this protozoon in colchicine-containing media. The length of the stalk in control cultures was 0.4-0.9 mm. In the presence of 2.5-12.5 mM colchicine, stalk elongation was inhibited, and stalk length was inversely proportional to colchicine concentration. At concentrations above 7.5 mM colchicine, stalks measured less than 0.1 mm, and sometimes contained imperfect myonemes. The rate of cell fission was retarded in colchicine-containing media, but nevertheless short-stalked colonies with apparently normal zooids were formed. On transfer of such colonies to media without colchicine normal growth was resumed, but only the newly formed branches were of normal length and contractility.

The inhibitory effect of colchicine was annulled by Ca⁺⁺ and Mg⁺⁺ at 10⁻³ and 10⁻⁴ M, respectively. At lower concentrations of Mg⁺⁺, but in the presence of Ca⁺⁺, the effect of colchicine was less conspicuous than at low Ca⁺⁺ concentration in presence of Mg⁺⁺. Lowering Mg⁺⁺ concentration at low Ca⁺⁺ concentration, increased the inhibitory effect of colchicine.

It is concluded that colchicine-sensitive, probably tubulin-like proteins, participate in the formation of the contractile stalk of Carchesium. Ca⁺⁺ and Mg⁺⁺ probably compete with colchicine for a common site in these proteins, or they might reduce the cell’s permeability to this drug.

INTRODUCTION

A close relation has lately been found between the presence of microtubules in cells, and primary motile processes. Such processes include cytoplasmic streaming, cell fission, axopod formation, cell contraction and undulation of cilia and flagella (Tilney, 1971; Margulis, 1973).

Recent studies have shown that colchicine prevents formation of microtubules in the mitotic spindle (Deysson, 1968), axopods (Tilney, 1968) and regenerating cilia (Neviakas & Margulis, 1969; Rosenbaum & Carlson, 1969). Amos (1972) has shown the presence of microtubular structures in the scopular organelles and stalk of Carchesium.

As a means of evaluating the role of colchicine-sensitive microtubular proteins in the formation and function of the contractile stalk of Carchesium, cultures were grown in colchicine-containing media. The effect of this drug on the elongation of the stalk of Carchesium, and the annulment of this effect by Ca⁺⁺ and Mg⁺⁺ are described in the following study. An abstract of this work has been published previously (Rahat & Pimstein, 1973).
MATERIALS AND METHODS

Stock cultures of *Carchesium* sp. were kept in filtered spring water (Aqua-bella, west of Jerusalem), to which 15% v/v of a 0.3% w/v lettuce infusion was added. New cultures were initiated by transferring a large colony with more than 30 zooids into fresh medium. Glass microscope slides were placed in some dishes, and the slides with the attached colonies were conveniently used to initiate new cultures.

For experimental work carchesia were grown as above in small Petri dishes, containing different concentrations of colchicine in 10 ml SWL medium (Rahat, Parnas & Nevo, 1969). An unidentified bacterial flora served as food organisms. After 4–5 days, specimens with 2–3 zooids were detached from the bottom of the dish with the tip of a fine needle, and collected with a micropipette for microscopic measurements. Our observations were limited to such young colonies, as in colonies with more than 10–15 zooids the main stalk loses its contractility and the myoneme degenerates. To evaluate the effect of Ca++ and Mg++ ions on the colchicine inhibition, carchesia were grown in a simplified synthetic medium C, containing: CaCl₂, 10⁻⁴ M; MgCl₂, 10⁻⁴ M; KCl, 10⁻⁴ M; and NaHCO₃, 10⁻³ M, at pH 7.5–8.0. Lettuce infusion was added as above. Concentrations of Ca++ and Mg++ were varied as described for each experiment. All cultures were kept in the dark, at 18–23 °C. Colchicine from BDH, England, and Fluka, Switzerland, gave similar results.

RESULTS

Parameters of normal growth

Normal growth of a *Carchesium* colony is shown in Figs. 5–8. Apical zooids of a mature colony eventually form migratory cells, detach themselves from their stalk, swim around for some time and settle on the bottom of the dish, or beneath the surface film of the medium. The settled zooid grows a new stalk, multiplies by fission, and each zooid grows at the end of its own branch. Occasionally teletrochs are formed, from which new colonies develop.

Development of a normal population of *Carchesium* colonies is shown graphically in Fig. 1. A mature colony (Fig. 7) was placed in fresh medium. Over a period of about 16 days it released migratory cells to form new colonies. The number of zooids in each colony increased with time, but there was no synchrony in the formation of new zooids.

Fig. 2 shows the relation between the number of zooids in a colony, and the length of its primary stalk up to the point where it branches. Stalks measured up to 0.9 mm, and none was less than 0.4 mm. The latter length was taken as a measure for determining the effect of colchicine on stalk elongation. During maturation of the colonies there was slight additional growth of the main stalk, after its first branching, and the mean length of the main stalk in colonies with more than 5 zooids was usually about 0.7 mm.

Effect of colchicine on elongation of stalk

Colchicine inhibited the formation of normal stalks (Fig. 9). At 5 mM colchicine or less, there was only a slight effect, but at higher concentrations, up to 12.5 mM, colonies with very short stalks or even stalk-less colonies were formed. In some carchesia grown with 10.0–12.5 mM colchicine the myonemes formed were apparently imperfect (Figs. 10, 11), and such stalks were unable to contract. However, when an
Colchicine inhibition in Carchesium

Fig. 1. Development of a normal population of Carchesium sp. in a small Petri dish containing 10 ml SWL medium. The culture was initiated by a mature colony with about 50 zooids. Numbers within figure represent total counts of colonies with respective numbers of zooids (i.e. on the 19th day, there were: one colony with 35 zooids, one with 20 zooids, two colonies with 12 zooids, etc., out of a total of 22 colonies).

Fig. 2. Relation between number of zooids in colony, and length of the primary stalk, up to the point of branching. A total of 74 measurements is given.
M. Rahat, H. Friedlaender and R. Pimstein

intact myoneme was visible in even a short branched colony (Fig. 13), contractility was not impaired.

Carchesia were grown in colchicine-containing media for up to 3 weeks. In such cultures, especially at the higher concentrations of colchicine, growth rate and number of zooids per colony were only about half those of organisms grown in media without colchicine (Fig. 1). Nevertheless, short-stalked colonies with up to ten zooids were obtained (Figs. 12, 13, compare with Figs. 7, 8). No apparent abnormality in cell morphology or ciliature could be seen in such colonies, except for the length of the stalk and branches. At concentrations of colchicine above 7.5 mM, stalk-less zooids were sometimes found on the bottom of the dish, besides the short-stalked colonies. Among these zooids, abnormal cell forms and vacuoles were observed, probably caused by cytotoxic effects of colchicine. Even in these cells, the ciliature seemed to be normal. No colonies were formed from the abnormal cells, and they disintegrated after several days.

**Release of colchicine inhibition**

Colchicine-inhibited short-stalked colonies were transferred into colchicine-free media. Normal growth was resumed, but only the newly formed branches were of normal length and contractility (Figs. 14, 15). The stalk and branches formed in the colchicine-containing medium did not resume their growth.

![Fig. 3. Effect of Ca²⁺ and Mg²⁺ on the colchicine-inhibitory effect. Cultures grown in C medium to which Ca⁻ and Mg⁻ were added at the concentrations shown. Each dot represents the mean ± the standard error of the given number of measurements (shown right of vertical line). A and C, without colchicine; B and D, with 12.5 mM colchicine.](image-url)
Effect of Ca$^{2+}$ and Mg$^{2+}$ on colchicine inhibition

In some experiments done with media containing natural spring water reproducibility was poor, and sometimes no significant inhibition of stalk elongation could be obtained even with 12.5 mM colchicine. At higher colchicine concentrations growth of Carchesium was arrested, and no new colonies were formed. The presence of an interfering ion was therefore suspected.

![Graph showing the effect of Ca$^{2+}$ and Mg$^{2+}$ on colchicine inhibition](image)

Fig. 4. A, colchicine-inhibition in C medium containing 10$^{-6}$ M Ca$^{2+}$, and 10$^{-4}$ M Mg$^{2+}$. B, the same, with Ca$^{2+}$ and Mg$^{2+}$ at 10$^{-8}$ and 10$^{-6}$ M, respectively. Each dot represents the mean ± the standard error of the given number of measurements (shown right of vertical line).

To evaluate the possible effects of Ca$^{2+}$ and Mg$^{2+}$ ions on the colchicine inhibition, carchesia were grown in a simplified synthetic medium C (see Methods), to which lettuce infusion was added. The concentrations of Ca$^{2+}$ and Mg$^{2+}$ in this medium were changed independently, in the presence and absence of 12.5 mM colchicine (Fig. 3). Change of concentration of these ions in itself, in the absence of colchicine, had no effect on stalk elongation (Fig. 3A, C). There was, however, a definite and reproducible colchicine-inhibitory effect below 10$^{-4}$ M Ca$^{2+}$, which was reversed at
higher Ca$^{2+}$ concentration (Fig. 3B). When Mg$^{2+}$ concentration was lowered from $10^{-4}$ to $10^{-7}$ M, in the presence of colchicine (Fig. 3B), there was only a slight inhibitory effect, at and below $10^{-6}$ M Mg$^{2+}$.

In C medium containing $10^{-6}$ M Ca$^{2+}$, inhibition of stalk elongation was proportional to colchicine concentration (Fig. 4A). This effect was even more conspicuous when the Mg$^{2+}$ concentration was concomitantly lowered to $10^{-6}$ M (Fig. 4B).

**DISCUSSION**

For our experiments on inhibition of stalk elongation in *Carchesium*, we had to use rather high concentrations of colchicine, up to 12.5 mM. Such concentrations were also used by Tilney (1968) and Rosenbaum & Carlson (1969) in their work on *Actinosphaerium* and *Tetrahymena*, respectively. These concentrations of colchicine are probably responsible for the cytotoxic effects noted in some *Carchesium* cells. Nevertheless, other cells were less sensitive, and reacted to colchicine by slower fission rate, inhibition of stalk elongation and imperfect myoneme formation. In all other aspects, e.g. ciliature and cell contractility, these cells were apparently normal.

How does colchicine inhibit elongation of the *Carchesium* stalk, and how do Ca$^{2+}$ and Mg$^{2+}$ interfere with this inhibition? The inhibitory effect of colchicine on the regeneration of cilia (Neviackas & Margulis, 1969; Rosenbaum & Carlson, 1969) can be correlated with its binding to proteins isolated from the latter (Borisy & Taylor, 1967a, b). Likewise, if similar proteins participate in the formation of the *Carchesium* stalk, its elongation should be inhibited by colchicine. There was a great fluctuation in length of the primary stalk of normal *Carchesium* colonies. This length, however, was never below 0.4 mm, and could therefore be taken as a parameter for the assay of the colchicine effect. Fig. 9A shows this effect in a somewhat idealized form, while actual measurements are given in Fig. 9B. At colchicine levels which prevent normal stalk elongation (Figs. 10–13), cell fission and cilia formation still occur. The sites at which the mitotic apparatus and the cilia of *Carchesium* are formed are therefore less colchicine-sensitive or permeable, than the site of stalk formation. Similarly, as stalks with defective myonemes but intact sheaths were formed (Figs. 10, 11), the site of myoneme assembly should be more sensitive than that of sheath formation. Normal contractility was retained in the shorter stalks formed in the presence of colchicine, in which intact myonemes were visible. These stalks therefore also retained their biochemical and ultrastructural integrity. Indeed, no ultrastructural changes could be found in colchicine-treated stalks, as compared to untreated ones (D. Kafri, M.Sc. Thesis, in Hebrew, unpublished).

The colchicine effect in an inhibited zooid cannot be reversed, and inhibited stalks will not resume their growth in colchicine-free media. New cells, however, formed by fission of colchicine-treated zooids, will grow normal stalks. Generation of a new stalk-forming site in *Carchesium* is therefore not affected by colchicine; only the function of such a site is inhibited.

Concentrations of Ca$^{2+}$ and Mg$^{2+}$, as present in our synthetic C medium ($10^{-3}$ and $10^{-4}$ M, respectively), were found to nullify the colchicine inhibition. This explains
the irreproducibility of inhibition experiments done in media made up with natural spring water. In such media the ionic composition probably differs from batch to batch.

How do these ions affect the colchicine-inhibition? Divalent ions have been shown to have a stabilizing effect on labile microtubules under certain conditions (Goode & Roth, 1969; Shigenaka, Watanabe & Kaneda, 1974), and might have a similar effect in Carchesium. In addition to its direct action, \( \text{Ca}^{2+} \) may compete with colchicine for a common site in the Carchesium cell, or it may alter the cell's permeability to colchicine. The effect of \( \text{Mg}^{2+} \) on the inhibition by colchicine in our experiments is less clear-cut, although it had a synergistic effect at low concentrations of \( \text{Ca}^{2+} \).

Different batches of lettuce infusion, added to our C medium, probably contain various amounts of \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \), as well as some other factors like B vitamins. The latter were claimed to annul the effect of colchicine (Margulis, 1973). The exact effect and mode of action of the above ions, and other factors, on stalk elongation and colchicine inhibition in Carchesium cannot therefore be evaluated until a complete synthetic medium for culturing Carchesium is developed.

From the data presented in this and other studies we conclude that colchicine-sensitive, probably tubulin-like proteins, participate in the formation of the Carchesium stalk. The biochemical evaluation of these proteins requires a relatively large number of Carchesia. Towards this end, synchronized mass cultures of Carchesium are now being developed in our laboratory.

REFERENCES


(Received 28 February 1975)
Figs. 5–8. (As a reference for all figures, diameter of stalk equals 15 μm.) Normal growth of a *Carchesium* colony.

Fig. 5. First branching of a young colony, myoneme intact.

Fig. 6. Young colony, myoneme in main stalk broken.

Fig. 7. Mature colony, expanded.

Fig. 8. Same colony contracted. Contractility is lost in main stalk and in some secondary branches where myoneme is broken.
Fig. 9. Inhibitory effect of colchicine on stalk elongation. A, Carchesia collected from cultures grown in SWL, at colchicine concentrations corresponding to those given in B. B, actual measurements of such Carchesia. Each point represents the mean of 15-34 measurements ± the standard error.

Figs. 14, 15. Release of colchicine inhibition in a short-branched colony transferred into colchicine-free medium.

Fig. 14. Inhibited branches do not resume growth, but new branches are of normal length.

Fig. 15. Same colony contracted.
Colchicine inhibition in Carchesium

10

11

12

13

14

15