THE EFFECT OF COLCHICINE ON FIBRILLAR MATERIAL IN WHEAT MEIOCYTES

M. D. BENNETT AND J. B. SMITH

Cytogenetics Department, Plant Breeding Institute, Trumpington, Cambridge CB2 2LQ, England

SUMMARY

Wheat meiocytes contain intranuclear bundles of microfibres at stages from pre-meiotic mitosis until zygotene. This includes early pre-meiotic interphase, the stage when colchicine induces univalents at subsequent metaphase I. This paper reports the effect of colchicine on the structure and distribution of such fibrillar material. Many normal-looking bundles of intranuclear fibrillar material formed after prolonged colchicine treatment (up to 5 days) which totally suppressed microtubule assembly in meiocytes. Thus, fibrillar material is unlikely to comprise tubulin. However, the formation of intranuclear fibrillar material was retarded in colchicine-treated meiocytes, and fibrillar material was absent during the colchicine-sensitive stage for univalency. The occurrence of cytoplasmic fibrillar material was delayed and greatly increased in colchicine-treated meiocytes compared with controls. A novel curved form of fibrillar material was seen in colchicine-treated meiocytes. It is concluded that colchicine had significant effects on the assembly and distribution of fibrillar material. These results are, therefore, compatible with the possibility that colchicine causes univalency in wheat via its effects on fibrillar material.

INTRODUCTION

Two major effects of colchicine on meiotic chromosomes are known in higher plants. First, as in mitotic cells, colchicine suppresses the formation of the spindle, thus preventing the normal chromosome movements of congression and separation at first and second meiotic divisions, and inducing restitution monads instead of tetrads. This effect, noted in Tradescantia (Walker, 1938), Rhoeo (Derman, 1938), Allium (Levan, 1939), Fritillaria (Barber, 1942), Triticum (Dover, 1972) and Lilium (Toledo, Bennett & Stern, 1979) is presumably due to colchicine binding tubulin subunits and thus preventing their polymerization to form the microtubules of the meiotic spindle, and seems well understood. By comparison, the second effect of colchicine in reducing chiasma formation in meiocytes is less well understood. This effect, first demonstrated by Walker (1938) and Derman (1938) in pollen mother cells (PMCs) of Tradescantia and Rhoeo, respectively, and soon after by Levan (1939) in Allium, and Barber (1942) in Fritillaria, has more recently been studied in Lilium (Shepard, Boothroyd & Stern, 1974; Bennett, Toledo & Stern, 1979), Secale (Bowman & Rajhathy, 1977), Triticale (Thomas & Kaltsikes, 1977) and Triticum (Driscoll, Darvey & Barber, 1967; Dover & Riley, 1973). The stage of colchicine sensitivity apparently differs between Lilium and Triticum. Univalents were induced by colchicine only during early pre-meiotic interphase in Triticum
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(Dover & Riley, 1973), but at stages from mid pre-meiotic interphase to zygotene in Lilium (Shepard et al. 1974; Bennett et al. 1979). Colchicine has no effect on the ultrastructure of already formed synaptic regions (Shepard et al. 1974), and it is generally agreed that the drug does not interfere with chiasma formation but with some step early in the synaptic process and perhaps the initiation of pairing (Levan, 1939; Driscoll & Darvey, 1970; Shepard et al. 1974; Bennett et al. 1979). The mechanism of the effect remains unknown, but as noted above, its timing in wheat is early pre-meiotic interphase.

Ultrastructural studies of Triticum aestivum cv. Chinese Spring anthers (Bennett, Simpson, Smith & Wells, 1979) revealed numerous bundles of microfibres in pollen mother cells (PMCs). The distribution of such fibrillar material in anthers was exclusive to PMCs, and normally to their nuclei. Fibrillar material increased in abundance throughout pre-meiotic interphase, reaching a peak around leptotene before decreasing during zygotene and disappearing by pachytene. Thus the material was present before synaptonemal complex and reached its peak of abundance before the synaptonemal complex did at pachytene. A striking feature of bundles of fibrillar material was the ability to form links at their ends either between 2 masses of chromatin, or between chromatin and the nuclear membrane, separated by up to 3.0 μm. It was suggested that fibrillar material may be of significance for the meiotic process, and may function before and over greater distances than the synaptonemal complex in establishing or maintaining the co-orientation of chromosome prerequisite for normal chromosome pairing.

As fibrillar material was present in wheat PMCs at, and immediately after, the stage when colchicine treatment causes univalency, it was questioned whether the drug might have its effect by affecting the formation of the material. This paper presents the results of experiments which compared the distribution and ultrastructure of fibrillar material in PMCs treated or untreated with colchicine.

MATERIALS AND METHODS

The material used in the present work was breadwheat (Triticum aestivum cv. Chinese Spring: euploid 2n = 6x = 42).

Plants were grown one per pot in a glasshouse until their leading tillers were about 2 weeks before the onset of meiosis in pollen mother cells (PMCs), when they were transferred to a growth room at 20 ± 1 °C and given constant illumination.

When a tiller contained anthers 1−7 days prior to first metaphase of meiosis, a whole spikelet was carefully excised through a small 'window' cut in the leaf sheaths surrounding the young spike, and the time (sampling time) was noted. The window was quickly sealed with a small piece of transparent adhesive tape to minimize desiccation of the spike, and the tiller was numbered and tagged. The sampled spikelet was fixed in a correspondingly numbered tube containing 1:3 glacial acetic acid: absolute ethanol for at least 2 h. After fixing the 3 anthers were carefully dissected from the first, second, third and fourth floret of each spikelet, and their lengths measured using a Vickers moving-scale micrometer eyepiece. Next, the developmental stage of archesporial cells PMCs was estimated in single anthers stained with either aceticarmine or leucobasic fuchsin as described previously (Bennett, Rao, Smith & Bayliss, 1973).

Within 5 min of sampling time, sampled tillers were cut under distilled water just below the node of the penultimate leaf, and transferred to a wide-necked 250-ml conical flask containing 200 ml of 0.05% colchicine (w/v). During transfer the cut end of the tiller was sealed
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from the air by a hanging drop of distilled water. The leaf sheaths were inside the flask but not the leaf blades, which projected above the neck. Flasks were wrapped in aluminium foil to prevent lumicolchicine forming. Flasks containing up to 10 tillers were stood in a growth room at 20 ± 1 °C with continuous illumination for up to 120 h. Colchicine uptake was signified by a large drop in the level of solution seen each day. However, the level of colchicine solution never fell below the cut ends of tillers because it was topped up to the original volume with 0.05 % colchicine solution daily.

After a known interval of continuous exposure to colchicine of from 22 to 120 h (i.e. at fixing time), tillers earmarked for timing meiotic development were transferred to the laboratory. The spike was excised and placed on filter paper moistened with distilled water in a glass Petri dish under a Wild stereozoom dissecting microscope. Four spikelets (identified as Sp.1 to Sp.4 according to their positions relative to the spikelet taken at sampling time (Sp. o) – see Fig. 1, p. 36) were excised and fixed. After fixing, the 3 anthers from the first, second, third and fourth floret in each spikelet were carefully excised, and the length of one anther from each floret measured as described above. Fixed anthers were stained as described above and the stage of development of archesporial cells or PMCs determined in squashes by light microscopy. A few anthers fixed at sampling time or after a known interval of colchicine treatment were Feulgen stained, and the relative DNA contents of selected PMC and tapetal cell nuclei were determined using a Vickers M86 microdensitometer as described previously (Bennett & Smith, 1972, 1976).

Tillers exposed to colchicine earmarked for ultrastructural studies were treated as above except that only one anther from each floret was prepared for light microscopy. The other two were prepared for electron microscopy. As the 3 anthers from a single floret are approximately synchronous in development, the anthers used for ultrastructural studies should be at the same stage as the single anther taken for light microscopy.

In all, 38 spikes were treated with colchicine and from 11 of them a total of 104 pairs of anthers were embedded for electron microscopy.

Anthers for ultrastructural studies were fixed in 5 % glutaraldehyde in 0.1 M phosphate buffer at pH 6.8 for 4 h at room temperature (about 20 °C), followed by two 15-min changes in buffer alone. Anthers were then stored overnight at 4 °C in fresh buffer before postfixing in 1 % osmium tetroxide in 0.1 M phosphate buffer at room temperature for 2 h. After two 10-min changes in distilled water, they were dehydrated in successive 10-min changes in 50, 70, 90 and 95 % ethanol to absolute ethanol (2 changes). After embedding in Spurr’s standard medium A (Spurr, 1969), silver or gold sections were cut using a diamond knife on an LKB Ultratome III and collected on 2 x 1 mm slot grids using the technique described by Wells (1974). Sections were double stained in a saturated solution of uranyl acetate in 50 % ethanol for 7 min, followed by 2 min in Reynolds’ (1963) lead citrate, and examined using a Philips 201 electron microscope at 80 kV.

The relative abundance of fibrillar material at different developmental stages was estimated, as previously described (Bennett et al. 1979), by scoring for its presence or absence in about 10 consecutive serial sections from each of between 9 and 34 PMCs at each of several stages. No allowance was made for variation in the number of pieces of fibrillar material per cell; nuclei were scored either as having or not having it. Finally the relative abundance was expressed as the percentage of PMCs seen to contain fibrillar material. As only about 5–10 % of the nuclear volume is sampled, this method probably underestimates the proportion of nuclei containing the material. Nevertheless it does give large repeatable differences between stages which allow meaningful comparisons of the relative abundance of fibrillar material during development (Bennett et al. 1979).

RESULTS

Spike and anther survival

The proportion of surviving spikes decreased with increasing exposure to colchicine, being 100, 86, 60, 44 and 33 %, respectively, after 1, 2, 3, 4 and 5 days of treatment. These proportions are similar to those noted previously for cut spikes.
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given water alone (Bennett, unpublished). Surviving spikes contained some dead anthers, their overall proportions in the 4 spikelets (Fig. 1) being: 15.5%, Sp. 1; 8.5%, Sp. 2; 37.8%, Sp. 3; and, 31.7%, Sp. 4. This pattern is consistent with damage caused by excising Sp. 0 being the major cause of anther death in surviving spikes. For example, the proportion of dead anthers on the side of the spike axis sampled at time zero (about 35%) was about 3 times higher than on the other side of the spike. The younger the spike when Sp. 0 was excised, the higher was the subsequent frequency of whole spike and anther death.

Fig. 1. Diagrammatic representation of part of a wheat spike showing the spatial relationship of the spikelet excised at the start of colchicine treatment (Sp. 0) to the 4 spikelets examined after colchicine treatment (Sp. 1 to Sp. 4). The arrow indicates the side of the spike axis most subject to damage at excision, and to dehydration during colchicine treatment.

**Colchicine penetration to anthers**

The concentration of colchicine for total suppression of spindle activity was reached after 6–8 h in anther filament cells; 12–16 h in tapetal cells; 16–22 h in archesporial cells at pre-meiotic mitosis; and 24–36 h in PMCs at meiotic divisions. These results are based on light-microscope observations of the presence or absence of c-metaphase and/or normal anaphase nuclei. Light microscopy cannot show whether all microtubule assembly has been inhibited, so this was checked in thin sections by electron microscopy. Microtubules in untreated anther cells were 22 ± 2 nm in diameter. No microtubules of similar size were seen in any tapetal, archesporial or pollen mother cell fixed 42–116 h after the start of drug treatment. Thus the threshold concentration of colchicine for totally inhibiting the assembly of normal microtubules was reached in these cells at or before 42 h after the start of treatment, and thereafter was maintained.
Anther length and cell size

Effective penetration of colchicine was indicated by treated anthers appearing short in relation to their width after prolonged exposure. Untreated anthers increase in mean length from about 0.1 to 1.3 mm during 7 days prior to meiosis (Bennett et al. 1973). Comparing untreated anthers and anthers treated with colchicine for up to 5 days showed a progressive decrease in mean anther length with increasing intervals of drug exposure for each stage from pre-meiotic mitosis until the end of meiosis. The reduction in mean anther length approached 50% after 5 days of treatment.

Rate of cell development

The durations of the last 3 pre-meiotic cycles in archesporial cells and of the stages of pre-meiotic interphase and meiosis in PMCs of Chinese Spring have been published previously (Bennett et al. 1973). Comparing these with the development of cells exposed to colchicine for different known intervals (up to 120 h) in the present work showed that the rate of development was unaltered or only slightly reduced in 70% or more of the surviving anthers and greatly reduced in the remaining anthers. Examples of cells completing normal or near normal development were first, the appearance of restitution PMC nuclei at early pre-meiotic interphase with the 16C DNA amount 116 h after the start of treatment. Allowing a drug penetration time of 16 h, these must have completed the last 2 archesporial cell cycles (which last 90 h in untreated spikes) in 100 h or less. Secondly, colchicine-treated PMCs passed from early pre-meiotic interphase to become restitution monads (the equivalent of tetrads) in 72-8 h. This development lasts about 68 h in untreated anthers. The present results therefore agree with those recently obtained for Lilium anthers given continuous colchicine treatment for up to 3 weeks (Bennett et al. 1979) in showing a normal, or only slightly reduced, rate of development in treated PMCs.

Effect on meiotic chromosomes

The present work confirmed the previous observations regarding the effect of colchicine on meiosis in Chinese Spring (Dover, 1972; Dover & Riley, 1973). For example, 42-48 h treatment with colchicine starting at mid-pre-meiotic interphase had no effect on chromosome pairing at subsequent first metaphase. Exposure to the drug for 72 to 96 h in PMCs starting at G1 of pre-meiotic interphase resulted in univalents at subsequent first metaphase and later, poreless uninucleate restitution monads. Exposure to colchicine for 96 to 120 h starting during interphase of the last pre-meiotic mitotic cycle resulted in the formation of PMCs with restitution nuclei containing the 8C DNA amount at first meiotic prophase and mainly bivalents at subsequent first metaphase. These results confirm that the colchicine-sensitive stage for univalency in PMCs is indeed after pre-meiotic mitosis and is coincident with early pre-meiotic interphase as suggested by Dover & Riley (1973).

Thin sections provided clear evidence of the formation of restitution nuclei in archesporial cells, PMCs and tapetal cells. Thus the number of cells seen in transverse
Fig. 2. PMCs at early pre-meiotic interphase seen in a transverse section of one loculus of an anther not treated with colchicine. $\times 2380$.

Fig. 3. A transverse section of one loculus of an anther after 93 h of colchicine treatment showing a PMC with a restitution nucleus at early pre-meiotic interphase. $\times 3200$. 
sections of a loculus was progressively reduced after increasing intervals of exposure to colchicine. For example, compare the number of PMCs cut in a typical untreated anther loculus at early pre-meiotic interphase (Fig. 2) with the large PMC almost filling the loculus at the same stage after 93 h of colchicine treatment (Fig. 3).

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Fig. 4. The frequency of occurrence of PMCs with intranuclear or cytoplasmic bundles of fibrillar material at different stages of development in untreated anthers and in anthers treated with colchicine for 69–116 h. Stages: PMM, pre-meiotic mitosis; EPI, MPI, LPI, early, mid and late pre-meiotic interphase, respectively; L/Z, leptotene–zygotene; and P, pachytene. ●, Untreated nuclei; ○, treated nuclei; ■, untreated cytoplasm; □, treated cytoplasm.

Fibrillar material

Fig. 4 compares the relative abundance of nuclear and cytoplasmic fibrillar material in PMCs treated or untreated with colchicine at stages from pre-meiotic mitosis to pachytene. The peak abundance of intranuclear fibrillar material was very similar in treated (81.8%) and untreated (72.4%) nuclei. However, 2 differences between treated and control PMCs should be noted. First, the maximum abundance of cytoplasmic fibrillar material was more than 5 times greater in treated (50%) than in untreated (8.3%) PMCs. Cytoplasmic fibrillar material was uncommon in untreated PMCs but was most abundant at mid-pre-meiotic interphase before the peak abundance of intranuclear fibrillar material. However, in treated PMCs cytoplasmic fibrillar material was most abundant at leptotene–zygotene, as was intranuclear fibrillar material. Secondly, the curves indicate that the formation of intranuclear bundles of the material was delayed in treated PMCs, so that it was present during fewer stages of the meiotic cycle than in untreated PMCs. In particular, fibrillar material was absent from treated PMC nuclei during early to
mid-premeiotic interphase (i.e. the colchicine-sensitive stage for univalency), but present in a significant proportion of untreated PMCs.

Although the maximum abundance of PMC nuclei with one or more bundles of fibrillar material was very similar in control and colchicine-treated PMC nuclei (Fig. 4), the number of bundles per nucleus may differ. Serial-section reconstructions of colchicine-treated nuclei have not been made, so counts of the number of bundles per PMC nucleus are unavailable. However, meaningful estimates of the approximate relative abundance per nucleus were made by counting the number of bundles per PMC nucleus per single section. A few such estimates were made for untreated PMCs at late pre-meiotic interphase, and for PMCs at leptotene fixed after either about 42 or 93–116 h of colchicine treatment. Nearly all PMCs at leptotene after 93–116 h of treatment contained colchicine-doubled restitution nuclei with the 8C DNA amount, while PMCs at leptotene after 42 h of treatment contained undoubled nuclei with the 4C DNA amount. The results (Table 1) suggest that the number of bundles of fibrillar material per 4C DNA amount was unreduced in colchicine-treated nuclei. Measurements of random samples of the bundles in the same material (Table 2) show that the maximum bundle length and width seen was similar in treated and untreated PMCs. However, the results also suggest that, compared with controls, the mean length and width of bundles of fibrillar material may have been reduced in treated undoubled nuclei (expected to exhibit univalency), but increased in doubled restitution nuclei (expected to exhibit bivalent pairing). However, beyond noting that colchicine had no striking effect on bundle size, any further interpretation would be premature before several stages around leptotene are similarly studied.

The appearance of most bundles of fibrillar material in colchicine-treated PMC nuclei (Figs. 5, 6) was indistinguishable from bundles of elongate or amorphous fibrillar material described previously in untreated PMC nuclei (Bennett et al. 1979). However, a novel condition consisting of curved elements was seen in the nucleus after treatment with colchicine for 69 or more hours comprising curved elements (Fig. 7). Microfibres in elongate fibrillar material in untreated PMCs were straight except where they diverged where a bundle of such material was attached to chromatin

Table 1. The number of bundles of intranuclear fibrillar material cut in single median sections of untreated and colchicine-treated PMC nuclei containing the material at its stage of maximum abundance

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of nuclei examined</th>
<th>No. of bundles</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>10</td>
<td>2–7</td>
<td>3.9 ± 1.7</td>
</tr>
<tr>
<td>42 h colchicine treatment</td>
<td>8</td>
<td>4–8</td>
<td>6.0 ± 1.2</td>
</tr>
<tr>
<td>93–116 h colchicine treatment</td>
<td>4</td>
<td>9–12</td>
<td>10.0 ± 1.2</td>
</tr>
</tbody>
</table>
Table 2. Sizes (nm) of bundles of intranuclear fibrillar material cut in single sections of untreated and colchicine-treated PMC nuclei at the stage of maximum abundance of the material

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of bundles measured</th>
<th>Length</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>Untreated</td>
<td>32</td>
<td>72.5</td>
<td>973.4</td>
</tr>
<tr>
<td>42 h colchicine treatment</td>
<td>52</td>
<td>79.5</td>
<td>1038.9</td>
</tr>
<tr>
<td>93–116 h colchicine treatment</td>
<td>25</td>
<td>117.0</td>
<td>1118.5</td>
</tr>
</tbody>
</table>
Fig. 5. Part of a PMC nucleus at late pre-meiotic interphase after 44 h of colchicine treatment showing several bundles of amorphous fibrillar material (arrowed). × 23 500.

Fig. 6. A bundle of elongate fibrillar material (FM) in a PMC nucleus at late pre-meiotic interphase after 43.5 h of colchicine treatment. × 69 900.

Fig. 7. A curved form of FM in a PMC nucleus at early meiotic prophase after 116 h of colchicine treatment. × 102 600.
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(Bennett et al. 1979). The appearance of most cytoplasmic bundles of fibrillar material in colchicine-treated PMCs was also indistinguishable from that of bundles of elongate or amorphous fibrillar material seen in untreated PMCs. For example, Figs. 8 and 9 show a typical elongate bundle of cytoplasmic fibrillar material from a treated PMC comprising parallel fibres about 8.4 nm in thickness and spaced on average 13.7 nm apart from centre to centre. These estimates are within the corresponding ranges for fibrillar material in untreated PMCs previously reported (Bennett et al. 1979). The novel curved form mentioned above also occurred in the cytoplasm of treated PMCs (Figs. 10, 11). The present observation of a novel form only in colchicine-treated PMCs may indicate that colchicine can affect the assembly of subunits to form microfibres and, thereby the structure of bundles of fibrillar material.

No form of fibrillar material was seen in untreated tapetal cells studied either previously or in the present work. However, in the present work a large bundle of microfibres, about 1.6 μm long and 0.2 μm wide, was seen in a colchicine-treated tapetal cell nucleus (Figs. 12, 13) in an anther containing PMCs at mid-pre-meiotic interphase. Whether this bundle comprised fibrillar material is unknown, but its gross appearance resembled amorphous fibrillar material in PMCs previously described (Bennett et al. 1979).

In the large samples of untreated PMCs previously studied a ‘paracrystalline’ condition of fibrillar material was seen very rarely and only in the nucleoplasm at around early pre-meiotic interphase (Bennett et al. 1979). A small proportion of the present colchicine-treated PMCs also displayed intranuclear paracrystalline fibrillar material but, in striking contrast to untreated cells, they also often contained paracrystalline fibrillar material in their cytoplasm (Fig. 14). As in untreated cells, paracrystalline fibrillar material in treated PMCs contained subunits giving parallel lines with a separation of about 16 nm, but repeats of up to 19 nm were also seen in the latter. It should be noted that the repeats seen in the paracrystalline form in PMCs (in the range 16–19 nm) are significantly smaller than those seen in colchicine-induced paracrystals in tapetal cells (in the range 29–48 nm) in the same anthers (Fig. 14) as described in the accompanying paper (Bennett & Smith, 1979).

DISCUSSION

The present results show that bundles of fibrillar material, mostly of normal appearance, were formed in PMC nuclei in the presence of colchicine at a concentration which totally suppressed the assembly of spindle microtubules both in PMCs and tapetal cells. This observation therefore tends to support the view that fibrillar material is unlikely to comprise tubulin, although this possibility cannot be ruled out on the present evidence alone.

The present results also show several clear differences between the appearance and distribution of fibrillar material in colchicine-treated and untreated PMCs, namely: (1) the formation of intranuclear fibrillar material was retarded in treated PMCs so that the material, was present at fewer stages, and absent during the colchicine-sensitive stage for univalency; (2) the formation of cytoplasmic fibrillar
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material was retarded and greatly increased in treated PMCs; (3) colchicine apparently induced the formation of a curved condition of fibrillar material not seen in untreated material; (4) cytoplasmic paracrystalline fibrillar material was common in treated PMCs at early pre-meiotic interphase but absent in untreated PMCs; (5) the mean size of intranuclear bundles of fibrillar material may differ between treated and untreated PMCs.

Taken together these differences indicate that colchicine has a significant effect on the behaviour of fibrillar material in wheat PMCs. While these results do not prove that colchicine affects meiotic chromosome behaviour directly via its effect on the material, they are not incompatible with this hypothesis. Further experiments

Fig. 14. The frequency of occurrence of PMCs with the 'paracrystalline' condition of FM in their nuclei (▲) or their cytoplasm (△), compared with the frequency of occurrence of tapetal cells with cytoplasmic paracrystals during anther development (■). (The key to the stages of development is the same as in Fig. 4. The curve for tapetal paracrystals is taken from Bennett & Smith (1979)).

Fig. 8. Elongate FM in the cytoplasm of a PMC at early meiotic prophase after 116 h of colchicine treatment. × 52 800.
Fig. 9. Detail of microfibres in part of the bundle of FM shown in Fig. 8. × 137 800.
Fig. 10. A curved form of FM in a PMC nucleus at early meiotic prophase after 116 h of colchicine treatment. × 73 400.
Fig. 11. A curved bundle of FM in a PMC nucleus at early meiotic prophase after 116 h of colchicine treatment. × 78 000.
Fig. 12. An interphase tapetal cell containing a bundle of microfibres (arrowed) from an anther at mid-pre-meiotic interphase after 69 h of colchicine treatment. × 7000.
Fig. 13. Detail of the bundle of microfibres arrowed in Fig. 12. × 46 800.
to investigate the possible function of fibrillar material are being made, notably observations of it in wheat genotypes with different pairing behaviour, including some achiasmate conditions of which colchicine-induced univalents may be a phenocopy.

The chemical nature, origin and mode of transport of the subunits of fibrillar material in PMCs are unknown, but if it is a protein, as seems likely, its subunits are almost certainly of non-nuclear origin and are synthesized in the cytoplasm of either the PMCs or even the tapetal cells. The accompanying paper (Bennett & Smith, 1979) reports the presence of numerous large cytoplasmic paracrystals in tapetal cells of colchicine-treated anthers containing PMCs at around early pre-meiotic interphase. Such paracrystals almost disappeared from treated tapetal cells by mid-pre-meiotic interphase (Fig. 14), and were not seen in untreated tapetal cells at any stage. The presence of such paracrystals only in treated tapetal cells strongly suggests that colchicine may interfere with the transport of their constituent subunits within or across the tapetum. Thus it is feasible that the present differences in distribution of fibrillar material between treated and untreated material may result from effects of colchicine on either the transport of subunits in the cytoplasm or their movement across the PMC nuclear membrane. For example, delayed formation of intranuclear fibrillar material in treated PMCs might indicate either delayed or reduced transport of subunits to the nucleus, or loss of subunits from the nucleus into the cytoplasm. The present results do not distinguish between these 2 possibilities, either of which could also account for the observed unusually large amount of cytoplasmic fibrillar material in treated PMCs. Nevertheless, even the possible implication of the PMC nuclear membrane as a site where colchicine may affect such distribution is interesting. This is because the PMC nuclear membrane is known to contain colchicine-binding protein (Hotta & Shepard, 1973), to have bundles of fibrillar material attached to its inner surface (Bennett et al. 1979), and often to be the site of initiation of chromosome pairing at telomeres attached to its inner surface (Gillies, 1975; Rasmussen, 1977). However, colchicine has effects on a wide range of metabolic activities and structures (Hart & Sabnis, 1976), and the influence of the drug on chromosome pairing almost certainly results from an interaction of these (Bennett et al. 1979).

REFERENCES


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