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The Effect of Colchicine on Synapsis and Chiasma Formation in Microsporocytes of *Lilium*

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Abstract. Microsporocytes of *Lilium* that are exposed to colchicine as late as early zygotene show reduced chiasma frequencies and the presence of univalents at Division I. These effects are preceded at pachytene by the appearance of pairing gaps (light microscopy) and by a relatively high ratio of uncomplexed lateral elements/synaptonemal complexes (EM). Chiasma formation thus appears to be reduced by failures in synapsis. Unlike the behavior of wheat, colchicine can disrupt chiasma formation in *Lilium* after cells have entered meiosis.

Introduction

The elimination of chiasma formation by colchicine treatment was first observed by Walker (1938) and Dermen (1938) in microsporocytes of *Tradescantia* and *Rhoeo* respectively. At about the same time, Levan (1939) made a detailed study of the phenomenon in *Allium* and proposed that the drug acted by interfering with the ongoing process of synapsis. Subsequent analyses of colchicine action have confirmed these early observations, but have left open the question of mechanism. The most intensive and revealing studies have been done with wheat and these have led to the conclusion that colchicine affects chiasma formation only if applied prior to the onset of meiosis (Dover and Riley, 1973). An even more accurate timing of the interval of colchicine susceptibility has been provided by the studies of Bennett *et al.* (1973) who found it to extend from about the anaphase of the last premeiotic mitosis to the early part of the premeiotic G_1 phase.

The premeiotic action of colchicine on chiasma formation has generally been attributed to presynaptic homolog alignment. Indeed, Dermen (1938) had already observed some tetraploid cells with complete bivalent formation in colchicine-treated microsporocytes of *Rhoeo*. The exclusive presence of bivalents in these cells is best explained by a persistence of associations between sister-chromatids following premeiotic C-mitosis, an explanation supported by the observations of Brown and Stack on premeiotic pairing in that plant (1968). The demonstration by Driscoll and Darvey (1970) that colchicine does not affect chiasma formation in

isochromosomes of $5D^L$ is particularly strong evidence for its action on prealignment rather than on the ongoing process of synapsis. These observations on the premeiotic action of colchicine thus indicate that the synaptic process begins well before any of the conventional features of synapsis are evident by light or electron microscopy.

The biochemical consequences of colchicine action in meiocytes of *Lilium* have already been described (Hotta and Shepard, 1973). In this paper we examine its cytological consequences. The advantage of *Lilium* for such a study lies in the synchrony of the meiocytes and the long duration of meiotic prophase. The precise stage at which colchicine is administered to the cells can be directly determined, and the cytological progress of affected cells can be traced in considerable detail. It will be seen that wheat and lily meiocytes do not respond identically to colchicine, the major difference lying in the time of cell susceptibility to the achiasmatic effect of the drug.

Methods

Lilium speciosum var. Lucie Wilson ($2n=24$) was the principal source of microsporocytes for these studies. The hybrid cultivar, Sonata ($2n=3x=36$), was used for certain comparisons and will be so indicated. Cells were exposed to colchicine either in cultured flower buds or in extruded microsporocytes. For bud culture, flower buds of appropriate size were excised by cutting well below the floral receptacle and were surface sterilized by brief immersion in 70% ethanol. The entire perianth was removed and the stem kept in sterile medium while being cut about 2—3 mm below the receptacle. The bud was then transferred to a test tube containing 0.2 ml of basic medium (Ito and Stern, 1967). Tubes containing colchicine were kept dark with aluminum foil. The method of culturing extruded microsporocytes has been previously described (Ito and Stern, 1967). All cultures were incubated at 19° C. In the case of bud cultures, sampling was done by removing an entire anther at the desired time and extruding the microsporocytes for analysis. No more than six sequential samples could be obtained from any particular bud. Microsporocyte cultures were sampled by periodically removing a "string" of cells (Ito and Stern, 1967).

For light microscopy, cells were fixed in 3:1 alcohol:acetic and stained with aceto-orcein. For electron microscopy, samples were fixed in 2% gluteraldehyde for 5 hours, washed in cold phosphate buffer (0.1 M, pH 7.4), immersed in cold 2% osmium tetroxide, dehydrated through an alcohol series and propylene oxide, and embedded in Epon. Sections were stained with uranyl acetate and lead hydroxide.

Results

1. General Description of Colchicine Effect

Buds of *L. speciosum* were excised at late premeiotic interphase and cultured in the presence of 1×10^{-3} M colchicine. Microsporocytes sampled 17–19 days later were largely achiasmatic (Fig. 2). The result is the same as that reported for other organisms (Levan, 1939; Barber, 1942;

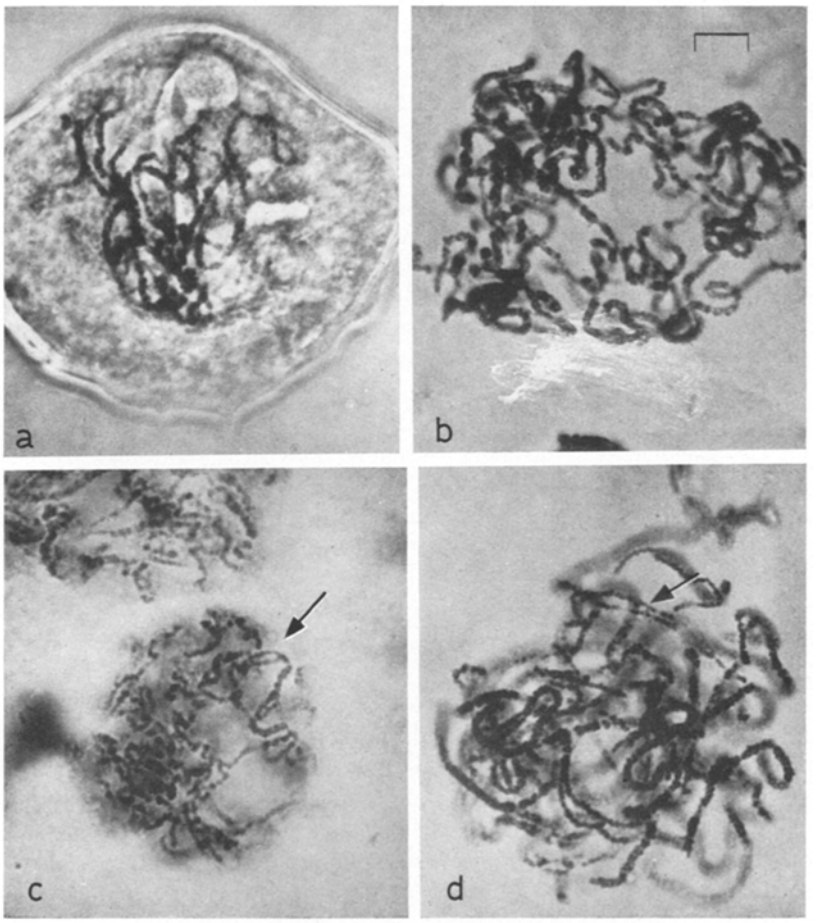

Fig. 1a—d. Chromosome pairing in microsporocytes of lily buds (*L. speciosum*) cultured in absence (a, b) or in presence (c, d) of 1×10^{-3} M colchicine. The scale in (b) corresponds to 10 microns. (a, c) After 11 days of culture. Cells are in late zygotene-early pachytene stage. Unpaired regions are evident in treated cells but not in controls. (b, d) After 13 days of culture. Cells in pachynema. Unpaired regions persist in treated cells

Driscoll *et al.*, 1967). Periodic sampling of anthers during the 19 day interval provided information about the sequence of cytological changes leading to the achiasmatic condition. Leptotene and zygotene cells appeared to be unaffected by colchicine. However, the abnormal presence of unpaired regions became increasingly evident as cells progressed into late zygotene and pachytene (Fig. 1). The variability in pairing from cell to cell was appreciable, but since pachytene bivalents could not be

traced over significant distances in squashed preparations, the ratio of paired to unpaired lengths could not even be approximated with the light microscope. Nevertheless, pairing discontinuities were consistently present in treated cells and were as consistently absent in controls.

To exclude the possibility that the discontinuities were apparent rather than real and that they resulted from a colchicine-induced precocious separation of sister chromatids, the triploid cultivar, Sonata, was examined. Pachytene smears of Sonata microsporocytes normally contain a mixture of thin and thick strands representing unpaired and paired chromosome segments respectively. Smears of pachytene cells from colchicine-treated buds showed the same two sizes of strands as did the controls. Since sister chromatid separation would have given rise to three sizes of strands, the absence of a third and thinner size in cells destined to become achiasmatic indicates that such separation is not a consequence of colchicine treatment. The earliest cytological effect of treatment in Sonata as in Lucie Wilson is a reduction in synapsis which is accompanied in both varieties by an inhibition of nucleolar fusion. Normally, the fusion occurs at late leptotene and, occasionally, in early zygotene (Studies of M. Westergaard, personal communication) but pachytene cells affected by colchicine consistently show multiple nucleoli. The relationship between synapsis and nucleolar fusion remains conjectural.

Very few of the cells which had been exposed to colchicine from the onset of meiosis showed normal chromosome associations at diplotene. In a typical experiment with *L. speciosum*, none of 22 buds which were cultured from late interphase to diplotene in the presence of 10^{-3} M colchicine, showed normal chiasma formation. There was considerable variation in the response of individual microsporocytes within the same anther. In one scoring of 100 cells, for example, there were 67 with no bivalents, 32 with 1—3 bivalents, and 1 with 11—12 bivalents. The heterogeneity may have arisen from the slight developmental gradient which is present within the anther, or from the uneven diffusion of colchicine among the microsporocytes. Whatever the mechanism, all anthers within a single bud showed the same kind and spread of colchicine effect. This identity of response between anthers of the same bud made sequential observations meaningful and led to the tentative conclusion that the occurrence of univalents at diplotene was a consequence of the pairing discontinuities seen at pachytene. A tighter conclusion could not be drawn from the cytological data because a quantitative comparison of unpaired to paired chromosomal stretches in pachytene cells with univalents to bivalents in 1st division cells could not be made. The difficulties in estimating the proportion of unpaired chromosome stretches should be evident from inspection of Figs. 1 and 2.

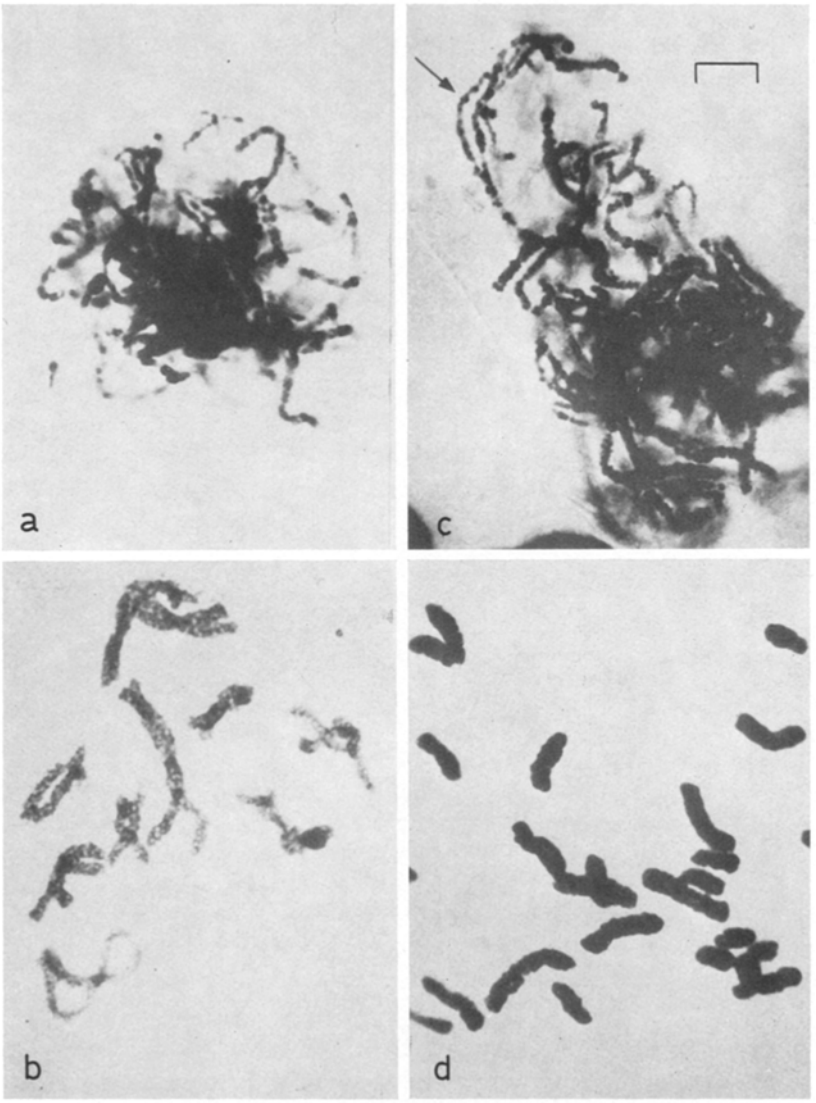

Fig. 2a—d. Chromosome pairing and chiasma formation in microsporocytes of lily buds (*L. speciosum*) cultured in absence (a, b) or in presence (c, d) of 1×10^{-3} M colchicine. The scale in (c) corresponds to 10 microns. (a, c) After 15 days of culture. Cells are in late pachytene. Unpaired regions in treated cells persist. (b, d) After 17 days of culture. Cells are in diakinesis. Univalents are abundant in the treated cells

2. The Time of Colchicine Action

Buds of different length were explanted into colchicine-containing media in order to determine the time at which microsporocytes were sensitive to the achiasmatic action of the drug. The results obtained are summarized in Fig. 3. Buds explanted when microsporocytes were in late interphase to early leptotene (16 mm lengths) all gave rise to normal chiasmatic cells. Buds measuring 15 mm were borderline in behavior. Some, probably the more advanced, gave rise to chiasmatic meioses while the others became achiasmatic. Buds of shorter lengths consistently gave rise to achiasmatic meioses or to cells with a mixture of univalents and bivalents. However, the interval of sensitivity to colchicine cannot be deduced directly from the data in Fig. 3. A lag exists between the time of bud exposure to colchicine and its uptake by the microsporocytes. The duration of the lag was determined by explanting buds into media containing ^{3}H-colchicine and sampling microsporocytes at different times. Virtually no radioactivity was found after

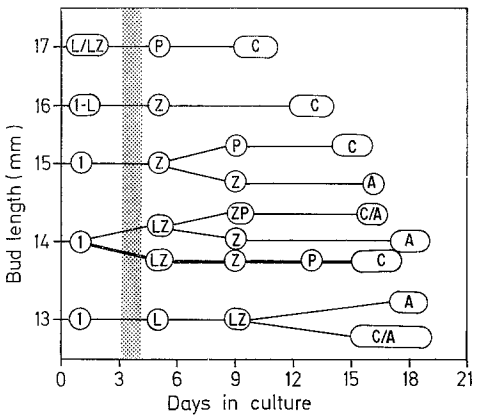

Fig. 3. The effect of colchicine on chiasma frequency in microsporocytes of *L. speciosum*. The semi-diagrammatic representation of results is based on at least 15 buds for each of the sizes in the ordinate. The first column of circles indicates the cytological stage of the microsporocytes at the time of bud explantation into medium containing 1×10^{-3} M colchicine. Buds explanted into standard medium at any of the stages indicated, completed chiasmatic meioses. The course of one control group (heavy line) is included in the diagram for rate comparison. The ellipses at the end of each row roughly mark the interval of time over which the cells reached diplotene-diakinesis and were scored for chiasma formation. In some cases, buds of the same length followed either of two courses and these are so indicated. *I* interphase; *L* leptotene; *Z* zygotene; *P* pachytene; *C* chiasmatic; *A* achiasmatic; *C/A* chiasmatic and achiasmatic in the same anther. The shaded portion indicates the time at which the concentration of colchicine in the microsporocytes reached about 1/4 the ultimate plateau level

36—48 hours of culture even though radioactivity was present in somatic tissues of the bud. Uptake of colchicine by the microsporocytes became manifest after 2 days and usually reached a plateau level at about the 5th—6th day of culture. In one bud, for example, the approximate plateau level in microsporocytes from 4 anthers excised successively at days 5—9 was 1100 cpm whereas the level was 200 cpm at day 3. Using different concentrations of colchicine, we found 2.5×10^{-4} M to be the lower limit of effectiveness. Since the concentration used in Fig. 3 was 1×10^{-3} M we may suppose that the colchicine became effective in the microsporocytes when it reached 25% of the plateau level, which would require about 3—4 days. Microsporocytes in 14—15 mm buds are at or near the end of the S-phase and would be in or near leptotene after 3 days of culture. Since such buds give rise to achiasmatics when exposed to colchicine (Fig. 3) it would appear that the drug can still interfere with chiasma formation after cells enter meiosis.

The forward limit of the colchicine-sensitive period was more directly determined with cultured microsporocytes. Although such meiocytes progressively lose synchrony with time, heterogeneity in meiotic stage is not extensive during the first 4—5 days of culture, and colchicine is absorbed to 1/2 maximum concentration within 24 hours or less. Results of experiments with cultured microsporocytes are summarized in Table 1. At least seven cultures from Lucie Wilson and Sonata varieties were run for each of the meiotic stages listed. Moreover, in order to identify the early prophase stages unambiguously, samples were taken at the time of explantation and fixed for electron microscopy. Early, mid, and late zygotene were identified by the frequency of synaptonemal complexes seen in sample sections. The results thus obtained indicate that cells may be rendered achiasmatic by exposure to colchicine during leptotene and, to some extent, during early zygotene. The response of cells at earlier stages (late interphase or early leptotene) is difficult to assess since those which are at or near the termination of S-phase frequently become achiasmatic due to explantation alone (Stern and Hotta, 1967).

In the course of this particular experiment we observed yet another effect of the drug on meiosis. Cells which are still in the S-phase at the time of explantation into colchicine medium become arrested later at meiotic prophase. A similar arrest was observed in the microsporocytes of cultured 12 mm buds which were exposed to colchicine during S-phase. The presence of colchicine during premeiotic S-phase results in a delayed inhibitory effect on meiosis regardless of whether it is applied to cultured buds or microsporocytes. The relationship of such meiotic arrest to asynapsis is not understood, but it is apparent that cells which have progressed beyond the stage of susceptibility to meiotic arrest become susceptible to the achiasmatic action of colchicine. From the results

Table 1. Effect of colchicine on chiasma formation in cultured microsporocytes of Lucie Wilson and Sonata cultivars

Stage of explantation	Presence of synaptonemal complexes	Concentration of colchicine		
		0	1×10^{-4} M	2×10^{-4} M
Interphase-leptotene	—	Mitotic and achiasmatic	Mitotic and achiasmatic	—
Leptotene	—	Chiasmatic	Achiasmatic	Achiasmatic
Early zygotene	Few	Chiasmatic	Bivalents and univalents	Bivalents and univalents
Mid zygotene	+	Chiasmatic	Chiasmatic	Bivalents and univalents
Late zygotene-pachytene	++	Chiasmatic	Chiasmatic	Chiasmatic

Data presented in this table represent the results of at least 7 cultures from the two varieties for each of the stages listed. Samples were taken for electron microscopy at the time of explantation to determine the frequency of synaptonemal complexes in each preparation at the beginning of colchicine exposure. This procedure was more precise than staging by light microscopy alone. Cells which had not yet formed synaptonemal complexes but were in meiosis (leptotene) became achiasmatic in the presence of colchicine. Earlier stages could not be analyzed effectively because of developmental artifacts. Cells in premeiotic interphase usually revert to mitosis on explantation if they are at, or just beyond the S-phase and, if near leptotene, enter meiosis but become achiasmatic.

obtained with cultured meiocytes we conclude that the period of susceptibility extends through leptotene Lily thus differs from wheat with respect to the time during which colchicine is effective in reducing chiasma frequency.

3. The Nature of the Colchicine Effect

Since *Lilium* microsporocytes can be monitored cytologically as they progress through meiosis, we sought to identify as clearly as possible the specific changes in chromosome behavior that led to univalent formation. Such monitoring is more difficult in other organisms and has usually been by-passed in studies of colchicine action. Our observations virtually eliminate the possibility that univalents are products of spindle inactivation. Cells destined to become entirely achiasmatic have distinctively thickened chromosomes at diplotene thus signalling a colchicine effect well before spindle formation. Moreover, when spindle function was disrupted by exposing pachytene cells to colchicine, only bivalents were observed during the Division I interval. As in wheat, chloral hydrate (3×10^{-3} M) had no effect on chiasma frequency even though it dis-

rupted spindle function (Dover and Riley, 1973). Vinblastine (10^{-4} M) was without effect on chiasma formation. The suggestion has been made that chiasma formation depends upon a function of centromeric microtubules and that these are affected by colchicine, but not by chloral hydrate (Dover and Riley, 1973). Our biochemical studies have shown that colchicine binds to a component in the nuclear membrane which can be differentiated chemically from cytoplasmic microtubular material (Hotta and Shepard, 1973). Both types of evidence thus point to a difference in colchicine target sites for affecting chiasma frequency and spindle function.

One other possible source of univalents is precocious terminalization. However, cytological studies of *Lilium* rule out terminalization as a significant process between early diplotene and metaphase (Mather, 1935). Our own observations have confirmed this conclusion and since colchicine slightly slows the rate of meiotic development (Table 3) the opportunity to detect terminalization would be better than in the controls unless the process were a cryptic one occurring prior to diplotene. However, this untestable possibility seems to be a less likely source of achiasmatics than defective pairing, especially in view of the fact that bivalents present in otherwise affected cells show no evidence of terminalization. It is unfortunate that the very high DNA content of lily nuclei prohibits good enough spreading of pachytene chromosomes to permit quantitation of the extent of pairing failure. Despite this limitation it is noteworthy that all colchicine-treated cells showed pairing gaps during prophase (Figs. 1 and 2). By contrast, pairing gaps were not observed in untreated pachytene cells of the pure species, *L. speciosum*. The fact that colchicine does induce defective synapsis suggests that it is an immediate cause of decreased chiasma formation.

4. Characteristics of Defective Synapsis

Analyses of chiasma frequency and distribution in colchicine-treated cells indicated that the effect is not always all-or-none. Such an effect would be expected if colchicine acted only on premeiotic positioning of homologs and if that positioning were a precondition of synapsis. Cultured 15 mm buds provided particularly useful information on this question because they were at the borderline of susceptibility to colchicine and most of the microsporocytes were only partly affected by the treatment. A comparison of chiasma and bivalent frequencies in controls and colchicine-treated cells is shown in Table 2. All of the 20 control cells had 12 bivalents each, and although the number of chiasmata per bivalent ranged from 1 to 5, only 1/3 of the cells had 2 or more bivalents with 1 chiasma. By contrast, only one of the 20 treated cells analyzed had the normal complement of 12 bivalents and despite the large number

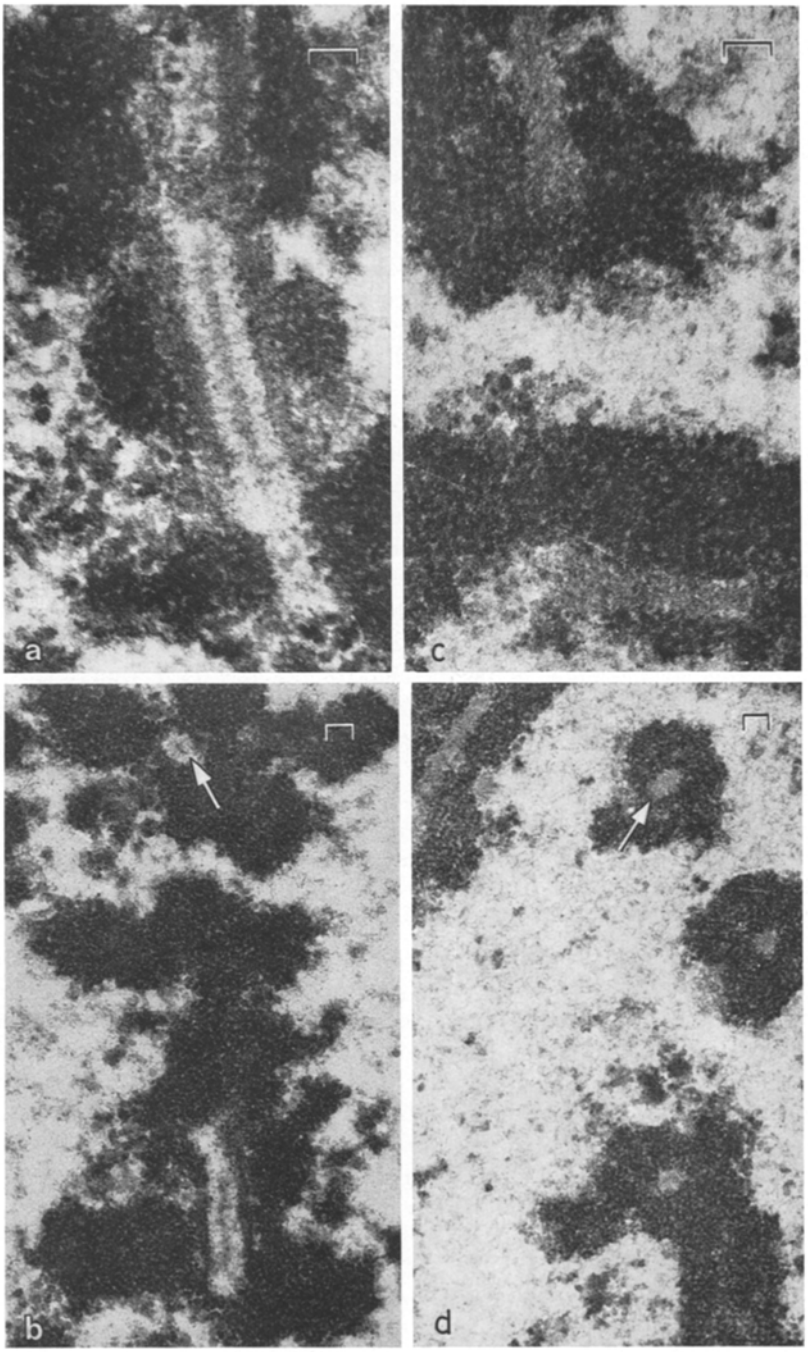

Fig. 4a—d

Table 2. Chiasma frequencies in microsporocytes of *L. speciosum* flower buds (15 mm) cultured with and without colchicine

	Chromosome number as		Chiasmata per bivalent				
	Univalents	Bivalents	1	2	3	>3	?
Control	0	480	26	83	81	38	12
+colchicine	270	210	44	39	17	5	—

The data are based on analyses of 20 cells in each of two 15 mm buds. As expected, control cells showed 12 bivalents (*i.e.* 24 chromosomes as bivalents) since univalents are rarely found in the pure species. In this analysis only those cells were scored that were adequately spread for counting chiasmata in a maximum number of bivalents. The cases in which a bivalent could not be scored are listed under ?.

of univalents among the rest, 2/3 of the cells had 2 or more bivalents with 1 chiasma. It is apparent that within an individual cell, colchicine may abolish some bivalents and reduce the number of chiasmata in others. It may therefore be concluded that colchicine partly or entirely disrupts synapsis in such a way as to render it ineffective for chiasma formation. The ineffectiveness might in all cases be due to a total absence of synapsis in the regions affected or it might be due to abnormalities in the pairing association. The latter possibility must be taken into account because appreciable pairing has been observed in pachytene cells destined to become achiasmatic.

All chromosomes in treated meiotic cells from leptotene through pachytene showed lateral elements when examined with the electron microscope regardless of the interval of colchicine treatment (Fig. 4). Since some cultures were treated prior to and during leptotene, it may be concluded that colchicine has little or no effect on lateral element formation. Moreover, the synaptonemal complex was unaffected in cells exposed to colchicine during mid-zygotene to late pachytene. Thus, neither the presynaptic nor synaptic morphology of the chromosomes are affected by colchicine presence. This leaves only the process of synapsis itself as the responsive target of colchicine action. This is borne

Fig. 4a—d. Fine structure of pachytene chromosomes in microsporocytes of *L. speciosum* obtained from buds cultured in the presence of 1×10^{-3} M colchicine. Normal (a, b) and colchicine affected (c, d) chromosomes are illustrated. These photographs are taken from a group of cells which showed both bivalents and univalents at division I. The normals shown here are identical in appearance with those observed in untreated cells. Arrows point to cross-sections of a synaptonemal complex (b) and of a lateral element (d). Magnification: (a) × 62500; (b) × 36000; (c) × 62500; (d) × 32000. The bars correspond to 0.1 micron

Table 3. Ratio of synaptonemal to lateral element lengths during zygotene-pachytene stages in control and colchicine-treated cells of *L. speciosum*

Time in culture	Control	Colchicine treated
6 days	6.9	0.2
9 days	4.5	0.6
12 days	only synaptonemal complex	0.4
15 days	early diplotene	0.3
18 days	—	early diplotene

Two 14 mm buds were used in this experiment, one of which was explanted into standard culture medium and the other into standard medium containing 1×10^{-3}M colchicine. Single anthers were removed from the buds on the days indicated. Half the anther was kept for light microscope observation and the other half was prepared for electron microscopy. Cells of the treated bud were achiasmatic when examined at diplotene. Ten sections, each from a different cell, were scored for each anther. The total lengths of synaptonemal complex and uncomplexed lateral elements were used in computing the ratios listed in columns 2 and 3. Although ratios varied from cell to cell, these variations were negligible compared with the overall difference between the treated and untreated cells.

out by the cytological consequences of exposing cells to colchicine during the sensitive interval.

A major reduction in the ratio of synaptonemal complex to lateral element was found in prophase cells which had been exposed to colchicine prior to or during the synaptic interval. Data were obtained by fixing portions of anthers sampled from the cultured buds and preparing thin sections for electron microscopy. Sections were scored for total lengths of fully formed synaptonemal complex and of uncomplexed lateral elements respectively. The results of a comparison between a treated and untreated 14 mm bud are summarized in Table 3. It may be seen that whereas no uncomplexed lateral elements were found in untreated pachytene cells, only a small fraction of chromosomes had complete synaptonemal complexes in the treated sample. Colchicine would appear to interfere with some phase of the process in which leptotene chromosomes are aligned to form bivalents. Our observations were neither complete nor thorough enough to permit conclusions about changes, if any, in the morphology of the synaptonemal complex. Although some of the micrographs are suggestive of abnormalities, serial sections would be required to establish their presence and these were not prepared. The electron micrographs shown in Fig. 4 illustrate what we have identified as either normal synaptonemal complex or as lateral element alone. In some cases, lateral elements were present in what were very probably paired homologs lacking a central element but in most cases they were associated with univalents.

Discussion

The results described in this paper confirm Levan's interpretation of colchicine action on meiotic cells, at least to the extent that they demonstrate univalent formation to be a consequence of reduced and/or ineffective synapsis. The long duration of meiosis in *Lilium* has made possible a monitoring of sequential meiotic stages and such monitoring has consistently revealed that a greater or lesser degree of asynapsis precedes the reduction in chiasma frequencies. Although it has been difficult to identify precisely the times at which meiotic cells lose their susceptibility to the achiasmatic action of colchicine the data are fairly clear in indicating that the period of susceptibility is present after the premeiotic S-phase and that it extends through leptotene, possibly into early zygotene. The interval of colchicine sensitivity is different from that occurring in wheat where colchicine is already ineffective at the premeiotic S-phase (Dover and Riley, 1973; Bennett *et al.*, 1973).

The basis for this difference is not obvious but there are other differences between the two genera which may bear on the particular one in question. Deposition of the distinctive callose wall occurs during the premeiotic G_1 phase in wheat but during the zygotene-pachytene stages in lily (Bennett *et al.*, 1973; Heslop-Harrison, 1966). In wheat, nucleolar fusion occurs during the late G_1 of premeiotic interphase (M. D. Bennett, personal communication) whereas in lily it occurs during the leptotene stage. The relationship of the fusion to meiosis is unknown but it may indicate a premeiotic association of chromosomes in the one group of plants and a later association in the other. In wheat, chiasmata show a marked terminalization by Metaphase I, whereas, as already mentioned, terminalization does not occur in lily except at the time of disjunction. Although the causes of terminalization have not been identified it is tempting to speculate that its early occurrence in wheat may be related to an early initiation of pairing. The four instances of relatively precocious events in wheat meiosis represent a coherent physiological pattern but, unfortunately, they do not reveal the basis of colchicine action. Nevertheless, these differences in pattern point to the probability that the primary requirements of synapsis may be met either before or after the premeiotic S-phase.

The fact that chiasma frequency in lily meiocytes can be affected by colchicine after the cells have entered meiosis and that in cells thus affected individual bivalents may suffer a reduction rather than an elimination of chiasmata leads to the conclusion that the drug interferes with some step in the synaptic process. This might be in the mechanism of initiation, as Driscoll and Darvey (1970) have suggested for wheat. If so, each lily homolog would be expected to have several sites at which synapsis is initiated since colchicine can reduce the number of chiasmata

in a bivalent. However, the weight of cytological evidence in *Liliaceae* is for synapsis to begin near the centromere (Darlington and La Cour, 1940; Frankel, 1940; Smith and Boothroyd, 1942) which makes the idea of multiple initiation points unlikely, at least at the level of light microscope localization. It is reasonable to suppose that the 2 homologous arms of wheat isochromosomes, being prealigned by a common centromere, are not susceptible to colchicine because it interferes with the mechanism which aligns homologous regions as a first step in synapsis. In lily this alignment occurs after the initiation of meiosis whereas in wheat it appears to occur prior to its initiation. These explanations, however, are entirely speculative, and we have yet to identify in specific terms the process affected by colchicine. To some extent, a beginning has been made in the biochemical analysis of the colchicine effect (Hotta and Shepard, 1973).

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