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In vivo effects of ortho-vanadate on spindle structure and dynamics of locust spermatocytes I

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Abstract. After external application of vanadate, a potent inhibitor of several ATPases including dynein, the following effects on living spermatocytes I are detectable: spherical metaphase cells change to a lemon shape due to a concentration-dependent elongation of the spindle, apparently achieved by pulling the plasma membrane-inserted poles apart, presumably through the assistance of cytoskeletal filaments. The observed dismembering of the spindle seems to be due to the separation of the half-spindle fibres, composed of usually interdigitating kinetochore microtubules (kMTs), free MTs (fMTs) and polar MTs (pMTs). As revealed by microcinematographic recordings, the lengthening of the half-spindles is accompanied by counter-clockwise twisting movements of the polar regions which, after prolonged vanadate treatment, lead to the formation of filiform appendices. Bundles of 5 nm microfilaments, which could be identified by indirect immunofluorescence microscopy (IIF) as actin, are concentrated within these appendices. In spite of a certain derangement of spindle architecture, half of the metaphases in 1 mM vanadate are capable of entering anaphase, but the rates of chromosome-to-pole movement have changed depending on the incubation time and the cell shape developed, respectively. Thus, chromosomes move with the highest speed in lemon-shaped cells but lag in cells with filiform appendices. However, it remains an open question whether the acceleration of chromosome migration is the result of spindle dismemberment or whether the slowing of anaphase motion is the consequence of a far-reaching displacement of the filamentous component from the spindle framework.

Introduction

In contrast to ciliary beat or actomyosin-dependent cellular movements we know relatively little about the molecular mechanisms involved in spindle dynamics. This may be because the mitotic spindles are highly labile ephemeral structures which do not share the structural uniformity found in muscular systems or cilia, and the probability that more than one mechanochemical system is involved in spindle dynamics. In any case, there is evidence that both dynein and actomyosin at least are present in the spindle apparatus (for review see McIntosh 1979; Pickett-Heaps et al. 1982).

The anionic substance ortho-vanadate, VO_4^{3-}, which has been shown to inhibit the Mg-ATPase of ciliary dynein (Gibbons et al. 1978; Nagata and Flavin 1978) and myosin (Goodno 1979) in a highly concentration-dependent manner, seemed to be an ideal tool to distinguish between both systems as possible force-transducers engaged in microtubule-bound transport phenomena. It was thus used by Cande and Wolniak (1978) and Cande (1982a) to analyse anaphase motion, by Yamin and Tamm (1982) to examine the rotating axostyle in certain termite flagellates, and by Buckley (1982) and Forman (1982) in their studies on saltatory particle movement. In their experiments with permeabilized mitotic PtK_1 cells, Cande and Wolniak (1978) found strong evidence for the presence of dynein as a functional component of the spindle. By the reduction of vanadate with norepinephrine, it could be shown that spindle function is fully restored (Cande 1982b).

Undeniably, lysed mitotic cells may approach unlysed cells in complexity, but they lack intact membranes as structurally and presumably functionally important constituents of the spindle. Although the existence of spindle membranes, especially in the polar region, has been known for several years (Harris 1975; Hepler 1980), they have been found only recently throughout the whole spindle in many plant and animal cells (e.g. Hepler et al. 1981; Paweletz 1981; Motzko and Ruthmann 1984). Evidence is now accumulating that the spindle ER, like the SR of muscle, regulates Ca^{2+} fluxes in the mitotic apparatus and may control microtubule (MT) assembly/disassembly and/or other force-generating elements (e.g. Silver et al. 1980; Kiehart 1981). Since vanadate may also affect several membrane-bound ion-translocating ATPases (reviewed by Ramarsarma and Crane 1981; Kustin and Macara 1982) as well as Ca-ATPases (O'Neal et al. 1979; O'Neill and Spanswick 1984), it appeared necessary to us to investigate its influence on living spindles with intact membrane systems, aiming to affect both the regulation and the dynamics of anaphase.

However, since vanadate seems to be readily reduced to vanadyl within living cells (Cantley et al. 1977; Willsky et al. 1984), rather high extracellular vanadate concentrations ($>10^{-4}$ M) are required to inhibit even the most sensitive ATPases (Nechay 1984; Willsky et al. 1984). The effects presented in this paper can thus be ascribed to low intracellular levels of vanadate, but may additionally be achieved by vanadyl in the +4 oxidation state of vanadium, which is a much weaker inhibitor of several ATPases (Willsky et al. 1984).

Materials and methods

Testes of fourth instar larvae of *Locusta migratoria* were placed on a slide in a drop of Ringer's solution (Bělař 1929)

immediately after dissection and disrupted to obtain a suspension of spermatocytes. Before sealing the coverslip with an inert and highly viscous silicon paste (Baysilon, Bayer AG), tissue remnants were carefully removed from the preparation. Control cells survived under these incubation conditions (chamber volume 20 µl) for approximately 8 h. Sodium (ortho)-vanadate (obtained from Fisher Scientific Co.) was applied in concentrations from 1 mM to 8 mM in Ringer. The osmolarity of the Ringer's solution (290 mosmole) is only slightly increased by the addition of vanadate (1 mM vanadate, 295 mosmole; 5 mM vanadate, 320 mosmole). When comparing cells in vanadate-containing solutions with controls we did not perceive any sign of cell shrinkage due to hypertonicity of the medium.

Microcinematographic recordings from meta- and anaphase I cells were made with a 16 mm Bolex camera in a cine-timelapse apparatus (Wild, Variotimer), placed on a ZEISS "Photomikroskop II" equipped with a planapochromatic phase contrast objective ((40 ×). Cinematographic runs were made with 1 frame/6 s and exposure times of 2 s.

Cryosections for the intracellular localization of vanadium by X-ray microanalysis were made on a Reichert Ultracut FC 4 at −110° C from suspensions of control (Ringer) and 4 mM vanadate-treated spermatocytes (incubation time 30 min) after shock-freezing in liquid propane. After freeze drying, sections were analysed at −135° C and 100 kV with a spot size of 30 to 50 nm in the Siemens ST 100 F scanning EM, equipped with a SiLi-detector (USC-Nuclear) and a multichannel analyser (Link-system).

For EM studies cells were incubated in plastic capsules (0.45 ml) and fixed after appropriate incubation times by addition of 0.05 ml 25% glutardialdehyde. Further treatments were performed according to the OsO_4Fe CN procedure of Hepler (1980). Ultrathin sections cut with an LKB Ultrotome II were stained with lead citrate and examined with a Philips EM 300 G, operated at 80 kV.

For indirect immunofluorescence microscopy (IIF), cells were prefixed for 1 min in the incubation medium by adding an equal volume of 9% formaldehyde (freshly prepared from paraformaldehyde) in PBS with 0.5% glutardialdehyde (grade 1, Sigma) in order to preserve vanadate-induced cell shape changes. After spinning down on poly-L-lysine coated slides in a Shandon cytocentrifuge at 1,600 rpm for 3 min, postfixation was carried out in 4.5% formaldehyde in PBS (Dulbecco) for 10 min. After that, slides were immersed in PBS with 0.5% Nonidet P40 (5 min) and after several rinses (3–10 min) in PBS treated with 1% borohydride in 50% ethanol during 15 min. Incubation with affinity column-purified antibodies against native porcine brain tubulin and smooth muscle actin (chicken) raised in rabbits was done for 45 min at 37° C in a moist chamber. Nativity and specifity of the antibodies were tested by indirect immunofluorescence microscopy, at concentrations of 20–50 µg/ml. Tubulin antibodies stained cytoplasmic, axonal and flagellar MTs of mammalian cells and anti-actin stained the I-bands of myofibrils and stress fibres of fibroblasts. As a second antibody, FITC-labelled goat IgG against rabbit (obtained from Miles) was applied and after several rinses (3 × 10 min), cells were immersed in a PBS-buffered glycerol medium containing 10% paraphenylendiamine to avoid bleaching.

Samples were examined in a ZEISS "Photomikroskop II" equipped with phase contrast optics and an epifluorescence condenser. Photomicrographs were made on Kodak Tri X or Ilford HP5 (400 ASA) at 436 nm with 40 × planapo or 63 × planapo objectives.

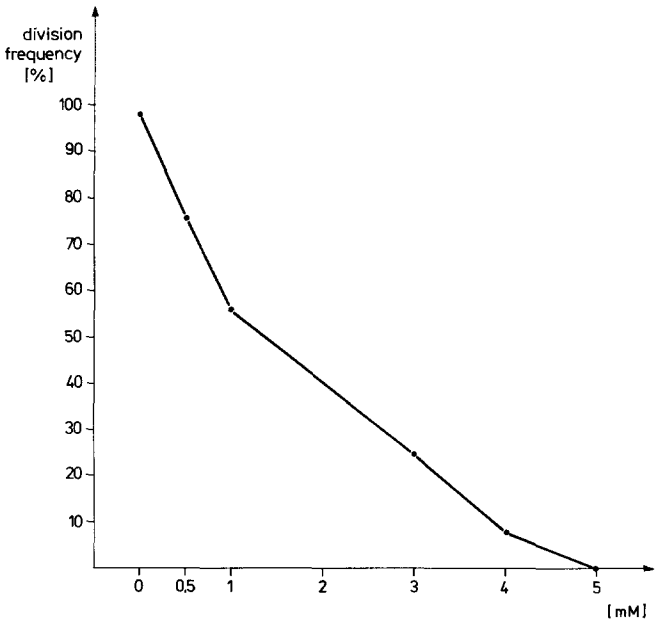

Fig. 1. "Division frequency" (percentage of cells performing regular karyo- and cytokinesis) after incubation in Ringer's solution, with vanadate concentrations from 1 to 5 mM

Results

In contrast to lysed mitotic cells (Cande and Wolniak 1978) various concentrations of vanadate in Ringer's solution in the micromolar range did not have any visible effect on the spindle structure and function of living locust spermatocytes. After raising the concentration of the externally applied vanadate to the millimolar range (1 to 8 mM Na_3VO_4), however, significant alterations were detectable. Concomitantly, it could be shown by X-ray microanalysis in ultrathin cryosections of shock-frozen spermatocytes that, after incubation of the cell suspension in 4 mM vanadate, intracellular vanadate was present in a concentration of 100 to 200 µM depending upon the region measured. Figure 1 illustrates a drastic decrease in the division frequency of spermatocytes I with increasing vanadate concentrations. Above 5 mM vanadate, divisions were totally arrested, but cells remained intact even for hours, whereas concentrations above 8 mM seemed to be cytotoxic. Shortly after incubation in 1 mM vanadate, metaphase stages, which are usually more or less spherical, begin to elongate, reaching different final lengths depending upon the concentration applied. EM- and IIF-investigations demonstrate an extreme elongation of the spindle with the centrosomes located in the farthest tip (Figs. 5, 6), leading to the pointed appearance of the enlarged half-spindles. Counter-clockwise rotational movements of these lobes lead to the pronounced lemon-shape of the vanadate-treated metaphases. For further investigations we used only two vanadate concentrations: 5 mM because of the cell's maximal lengthening, and 1 mM because of its clear lobe formation and the relatively high division rate of more than 50% (Figs. 2, 3).

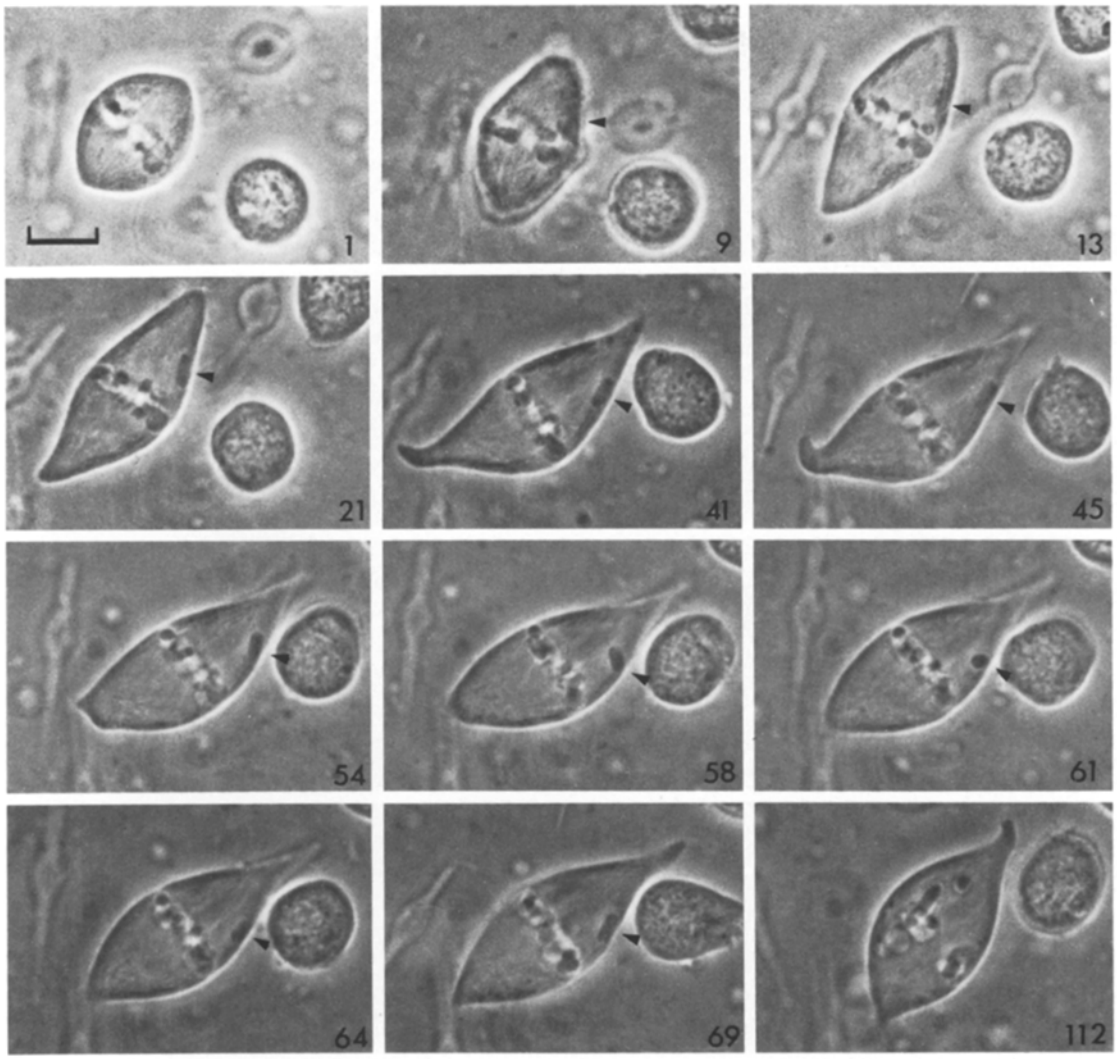

Fig. 2. Single frames from a 16 mm cinefilm, showing the shape changes of a spermatocyte at metaphase/anaphase I transition and the movement of the X-chromosome (*arrowheads*) after incubation in 5 mM vanadate. At the lower right, the incubation time in minutes is indicated. Bar represents 10 μm

In 1 mM vanadate, spindle length increases by about 50% as measured on photomicrographs showing the tubulin fluorescence pattern. Whereas controls show an average spindle length of 20.2 (±3.3) μm ($n=6$), vanadate-treated cells reach a pole-to-pole distance of 31.1 (±3.6) μm ($n=7$). Metaphase cells in 5 mM vanadate elongate even more, so that one lobe alone may become as long as an entire control cell. These giant cells formed in 5 mM vanadate (Fig. 2) are usually arrested at the metaphase stage. Nevertheless, microcinematographic analysis reveals that the unpaired X-chromosome, which, in control cells, reaches the polar region before the onset of autosomal movement, is still able to migrate as far as the length of the normal half-spindle. There the migration stops, the X-chromosome seems to loose contact with the spindle and glides back to the equatorial region. Autosomal movement, however, is blocked and, later on, the metaphase plate disaggregates. After prolonged incubation in 1 mM vanadate, the pointed lobes of the lemon-shaped cells become twisted due to their rotation, so that long, slender and filiform appendices are formed at each pole (Fig. 3). After approximately a 2 h retardation, the chromosomes start anaphase movement. Some of these dividing cells are still lemon shaped, others carry the filiform appendices.

Analysis of the microcinematographic recordings reveals large differences in the anaphase dynamics of the 1 mM vanadate cells depending upon their morphological state at the end of metaphase. Therefore it seems reasonable to compare them with control cells (group III in Fig. 4) by groups according to their length. Group I includes those that start anaphase when they are still lemon shaped and enlarged. Group II is somewhat non-homogeneous, including cells that have regained normal pole-to-pole distance either by forming filiform appendices (group IIb) or by recovering from the lemon shape by some sort of contraction as seen in cinemicrographs (group IIa). After microcinematographic recording of the divisions, we analysed them by marking graphs of each division, plotting the pole-to-pole distance and the separation of the chromosomes against time. Chromosome-to-pole movement was calculated as half the difference between both values. The diagram in Figure 4 gives the averages, for each group, of the increase

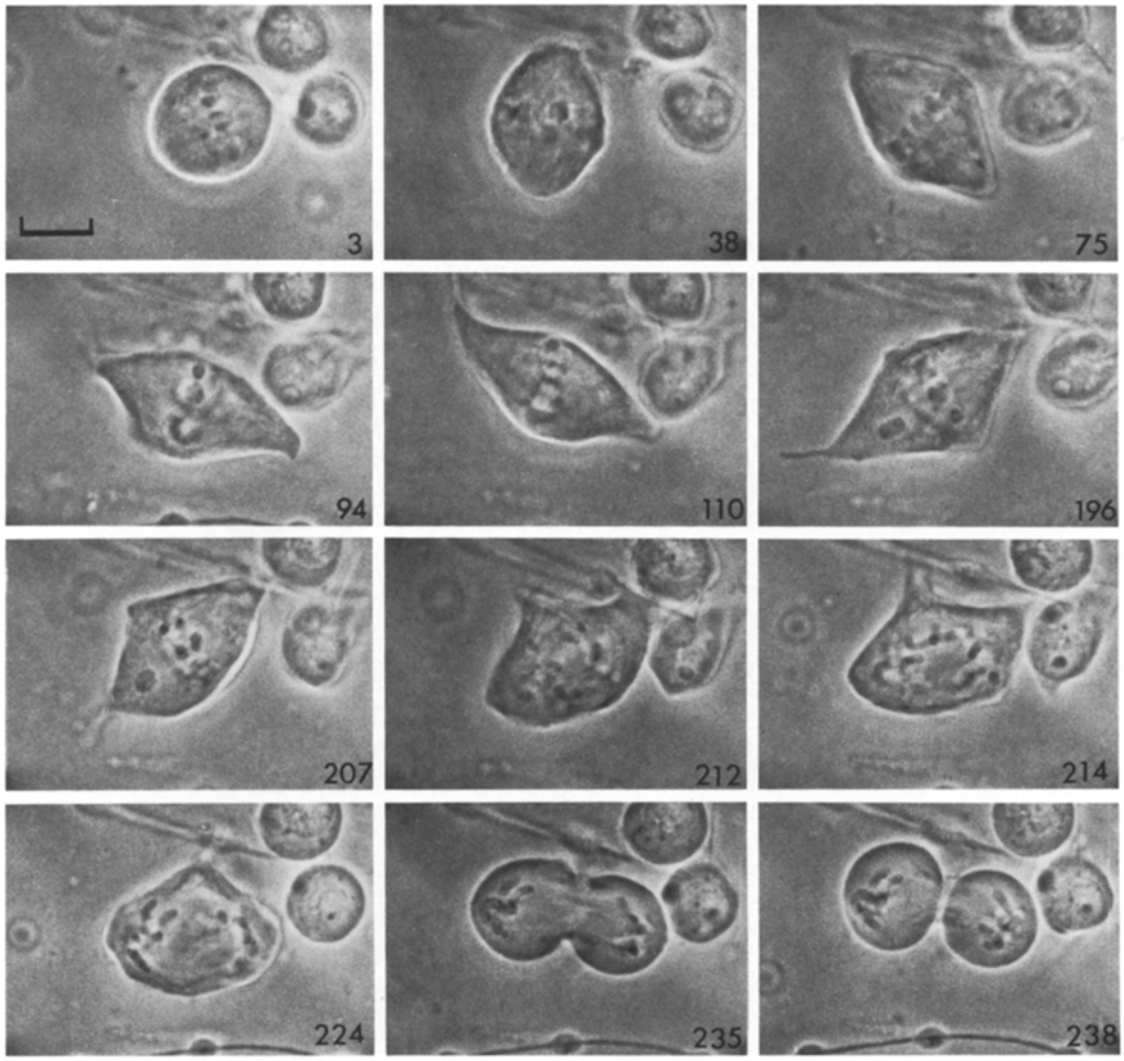

Fig. 3. Single-frame sequence from a 16 mm film of a spermatocyte I showing the outgrowth of pointed lobes and their conversion into filiform appendices in 1 mM vanadate. After an incubation time of 196 min, the separation of the autosomes is followed by regular fission. Bar represents 10 μm

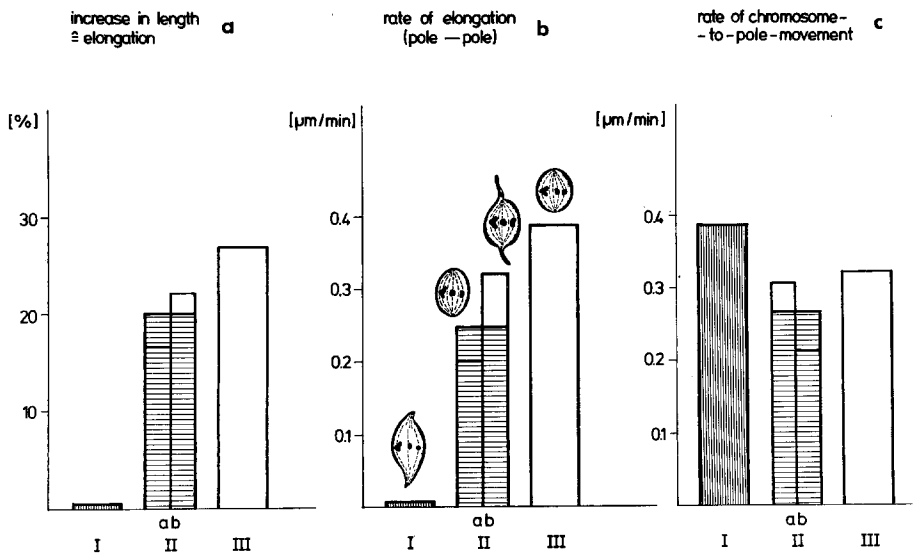

Fig. 4a–c. The diagram illustrates the differences in the maximal length increase (**a**), the rates of elongation (**b**) and chromosome-to-pole movement (**c**) in 1 mM vanadate-treated cells and controls. Group I represents cells starting anaphase in the lemon-shaped stage. Group II includes cells that have regained normal size by recovery from the lemon-shaped state (IIa) or by forming filiform appendices (IIb). Controls are represented by group III

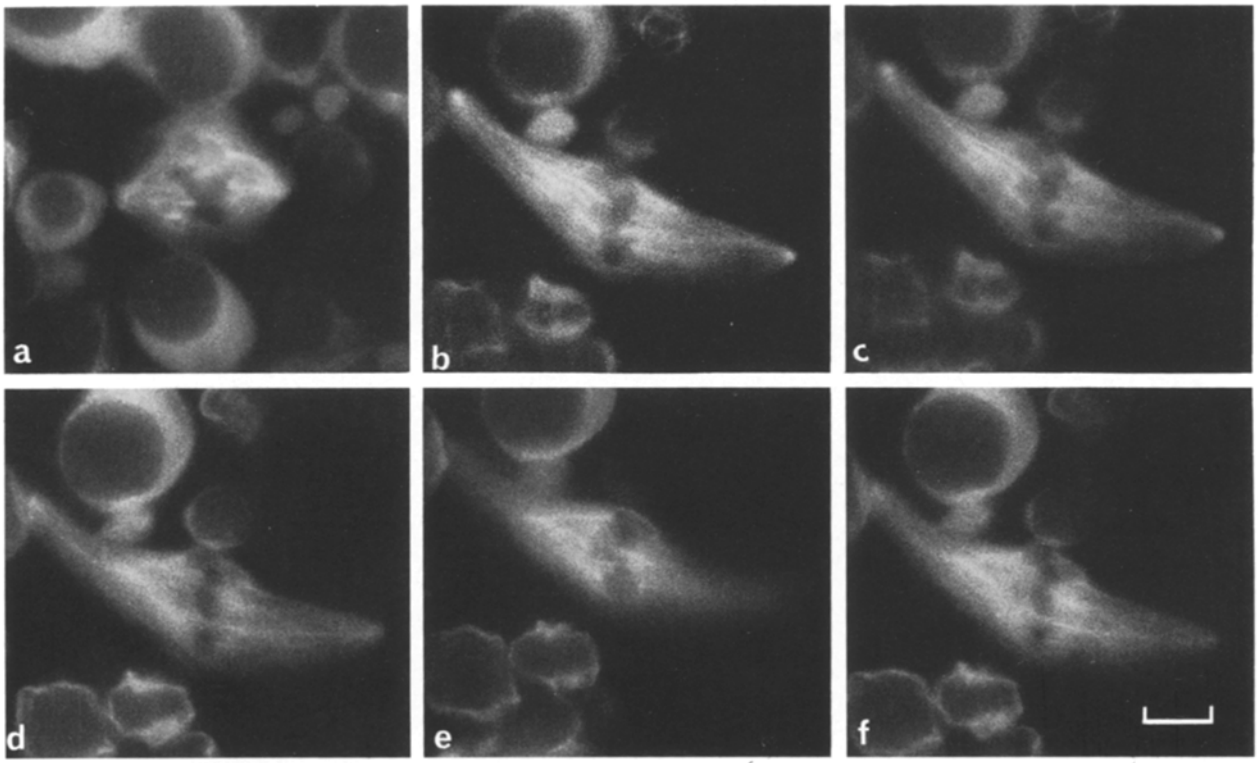

Fig. 5a–f. Anti-tubulin fluorescence pattern of control spindles (**a**) and a 5 mM vanadate-treated spindle (**b–f**). **b–f** represent a through-focus series of a lemon-shaped cell showing a metaphase I spindle which is pulled apart longitudinally. In **b** and **c** the centrioles are clearly visible. Some faintly stained fibres are not continuous through the half-spindles. Bar represents 10 μm

in length during elongation as a percentage of the initial length (Fig. 4a), for the rate of elongation (Fig. 4b), and for the rate of chromosome-to-pole movement (Fig. 4c).

As the lemon-shaped cells (group I) do not show any increase in length during anaphase, there is no value for elongation or elongation rate. For cells of group II, the values for elongation and elongation rate are lower than that of control cells, but statistically (5% error) not significantly distinct. This may, however, be the consequence of a rather small number of plots due to the low division frequency. As for the differences within group II, it is remarkable that the cells with filiform appendices come closer to the values of controls (group III) than those of group IIa. Perhaps group IIa could be regarded as a transitional state. In the diagram illustrating the rates of chromosome-to-pole movement, a similar role can be seen for the cells of group IIa. In contrast to the results for elongation, now the rates for group I and III, the controls, are statistically equal, although the movements of chromosomes in lemon-shaped cells tend to be even faster. Chromosomal movement in cells with filiform appendices (IIb) is much slower as in both controls and lemon-shaped cells, reflecting a restriction of anaphase A. Cleavage is never affected. Cells which were, at the onset of incubation, already in anaphase generally complete a normal divison even in vanadate concentrations above 5 mM.

Ultrastructurally, the lemon-shaped giant cells formed in 5 mM vanadate (Fig. 6) show a considerable derangement of the metaphase spindles, which now extend from the equatorial plane to the centrosomes located at the farthest tip of the lobes. Each centrosome is anchored to the plasma membrane via a primary cilium as is the case in all control cells from prophase to metaphase (Daub and Hauser, in preparation) and is embedded in pericentriolar material to which polar microtubules, directed towards the equatorial plane, adhere (Fig. 6b, c; Fig. 7b). Slim, poleward-oriented and highly parallel MT bundles with reduced MT numbers extend from the kinetochore region while controls usually reveal bundles whose MTs diverge somewhat in the poleward direction. In cross-sectioned bundles single MTs appear connected by cross-bridges (cbr, Fig. 6d). Serial sections of vanadate-treated metaphases cut parallel to the spindle axis showed a region in the middle of each elongated half-spindle where only very few MTs were present. These EM findings of a certain dismemberment of the spindle fibres were confirmed by the IIF technique. Through-focus series (Fig. 5a–f) of anti-tubulin stained vanadate-treated spindles show only some very thin fibres reaching all the way from the equatorial plate to the polar regions. The majority of spindle fibres or fibre relicts is discontinuous within the half-spindles, suggesting that they are pulled apart by poleward-directed forces. Thus, polar (pMT), free (fMT) and kinetochore MTs (kMT), usually interdigitating within the insect spindle fibres (see also Fuge 1984), now appear longitudinally separated. Apparently, most fibres respresent fragments and do not reach the polar or equatorial area, respectively.

The cisternae of smooth ER, normally emerging from vesicular centres (V in Fig. 6a) located near the spindle equator (Daub and Hauser, in preparation) and accompanying the spindle fibres to the poles, are now likewise dislocated into the polar part of the disarranged half-spindles (Fig. 6a). Another remarkable difference from the controls is the formation of numerous polysomes in the distal part

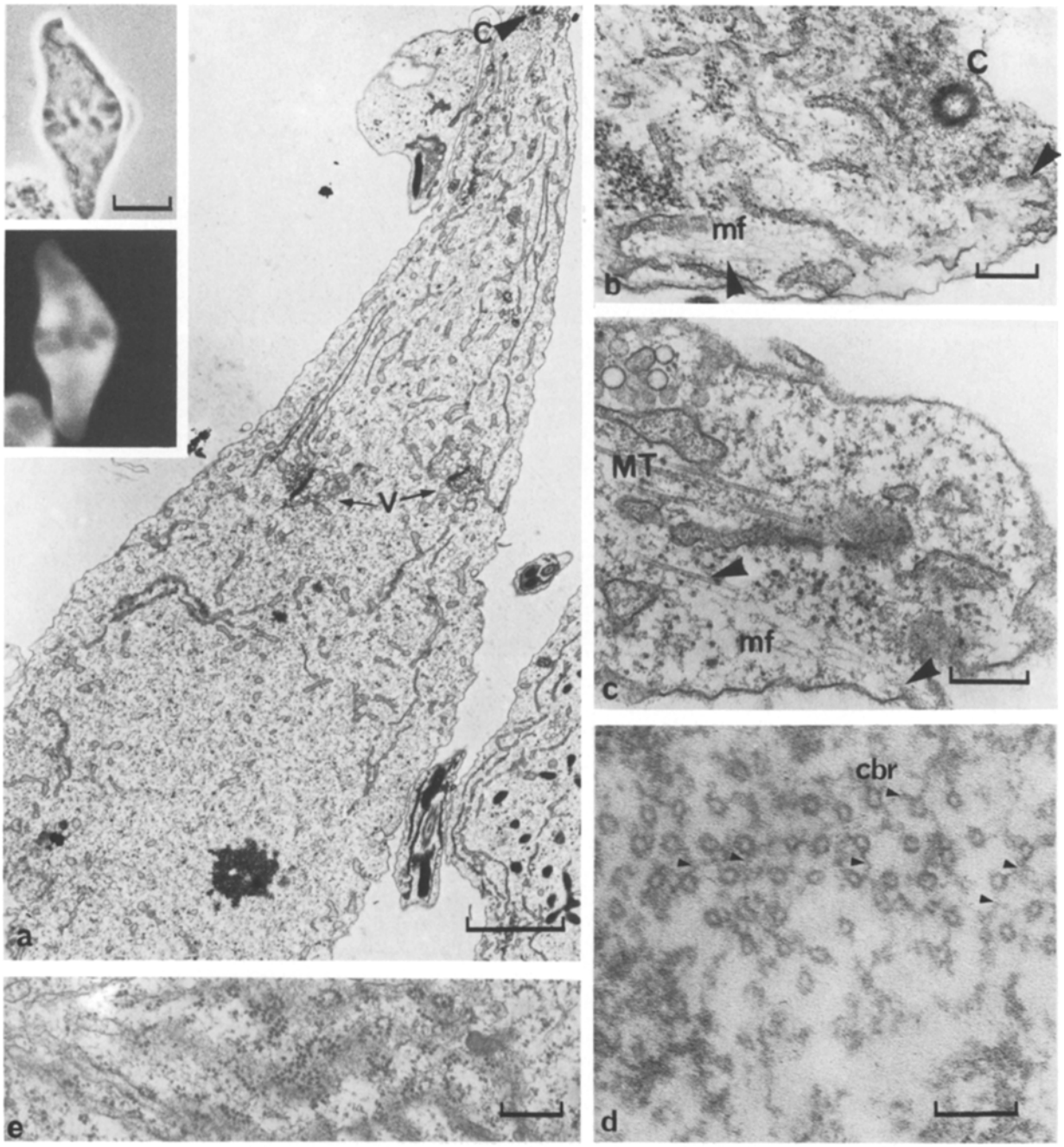

Fig. 6a–e. Ultrastructure of a lemon-shaped cell in 5 mM vanadate. **a** Longitudinally sectioned elongated half-spindle. Note poleward-directed dislocation of spindle ER. Due to the low magnification the microtubular spindle elements are not visible. *V*, vesicular centres; *C*, centriole; Bar represents 2 μm. *Inset*. Phase contrast photomicrograph and anti-actin fluorescence pattern of a 5 mM vanadate-treated cell. Note the anti-actin labelling throughout the cell excepting the polar regions which show fluorescence only underneath the plasma membrane. Bar represents 10 μm. **b, c** Two sections selected from serial sections through the tip of a vanadate lobe. Note microfilaments (*mf*) in close association with the plasma membrane and with some microtubules (*MT*; *arrowhead*). Bar represents 300 μm. **d** Cross-sectioned spindle fibre of a metaphase (control) spindle, composed of kinetochore MT (kMT), free MT (fMT) and polar MT (pMT) showing side projections (*cbr*, cross bridges) bridging single MTs. Bar represents 100 nm. **e** Whereas in control spindles only smooth ER is present, vanadate-treated spindles reveal a polysome decoration at the ER cisternae. Bar represents 300 nm

of each half-spindle (Fig. 6e). Most startling, however, is the EM observation of numerous 5 nm microfilaments in the vanadate-induced lobes which come into close contact with the plasma membrane and the spindle MTs as well (arrowhead in Fig. 6c). Apparently, their distribution is limited to the peripheral regions of the lobes, suggesting membrane adherence. After immunolabelling with anti-actin, in contrast to controls, the polar areas of lemon-shaped cells remained unstained except for a layer of fluorescent material underneath the plasma membrane, thus confirming the EM findings (see inset in Fig. 6a). As for the cells subjected to prolonged exposure to 1 mM vanadate, most inter-

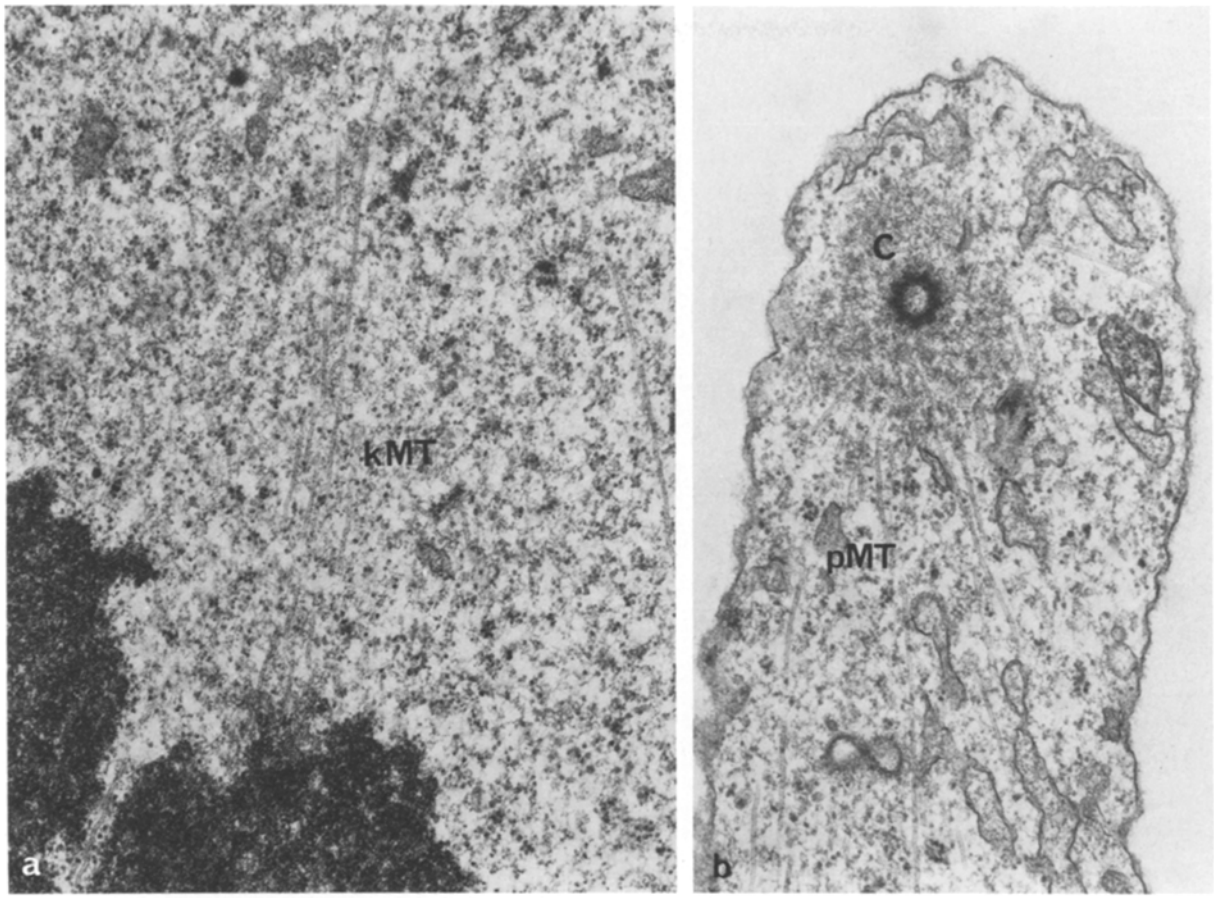

Fig. 7a, b. Spindle structure after longitudinal displacement in a lemon-shaped metaphase I cell (incubation with 1 mM vanadate). **a** Highly parallel kinetochore MT (*kMT*) are relicts of normally rather massive chromosomal fibres, composed also of free MT, which are often arranged obliquely to the fibre long axis and also of pole-inserted MT (*pMT*). **b** Longitudinal section of the polar region showing the centrosome (*C*) in the farthest tip of the vanadate-induced lobe with adhering polar MT (*pMT*). Bar represents 300 nm

est was focussed on the filiform appendices (Fig. 8). In longitudinally sectioned appendices (Fig. 8a) we found a solid core of bundled microfilaments (0.2 to 0.5 μm in diameter), accompanied by only a few MTs. Cross sections clearly demonstrate an asymmetric membrane attachment of these filamentous bundles (asterisk in Fig. 8b). As shown in Fig. 8c, in the IIF with anti-actin these solid bundles appear brightly fluorescent. It is of interest that, in contrast to the lemon-shaped cells, which show actin labelling almost throughout the cell, the actin fluorescence in cells after prolonged vanadate incubation is restricted to the plasma membrane area and concentrated in the filament-bearing appendices, indicating a considerable shift of the actin framework. The centrosomes of these cells, however, are located at the bases of the filiform appendices and are still closely connected to the plasma membrane via the primary cilium. Thus, cells possessing filiform appendices with a filamentous core at the poles, which are also slowed down in their anaphase movement as shown by microcinematographic analysis, appear to have a quite normal spindle structure.

In contrast to these findings, microfilaments of 5 nm diameter are rather scarce in control cells. Although the OsFeCN staining procedure proved to be somewhat filament-protecting, we could demonstrate only a few filaments paralleling the MTs of chromosomal fibres and free MTs in the vicinity of the pole.

Discussion

Orthovanadate, externally applied to living spermatocytes, affects anaphase dynamics in a way that is quite different from the effect on cell models since it alters cell shape and spindle architecture. It is interesting that the rates of the anaphase movement vary significantly depending upon the cellular shape developed.

First of all, it seems necessary to consider how the immediate shape change, elongation, may be achieved. Evidence exists that dehydration of metaphase or anaphase stages leads to spindle elongation. This effect was published long ago by Bělař (1929) who used two to five fold concentrated Ringer's solution for incubation and only recently by Snyder et al. (1984) who added 0.5 M sucrose. However, vanadate neither increases the osmolarity of the incubation medium to a considerable extent nor could the inhibition of Na/K-ATPase (Cantley et al. 1977) lead to dehydration of the cell. On the contrary, this inhibition would rather favour the opposite effect, that is swelling of the cell, as discussed by Sweadner and Goldin (1980). Indeed, an increase of cell volume was observed at least for the inhibition of the Na/K-ATPase of sheep red blood cells by cardiac glycosides (Tosteson and Hoffmann 1960). As already demonstrated in several other cellular systems, e.g. yeast cells, by NMR and ESR (Willsky et al. 1984), we were able to

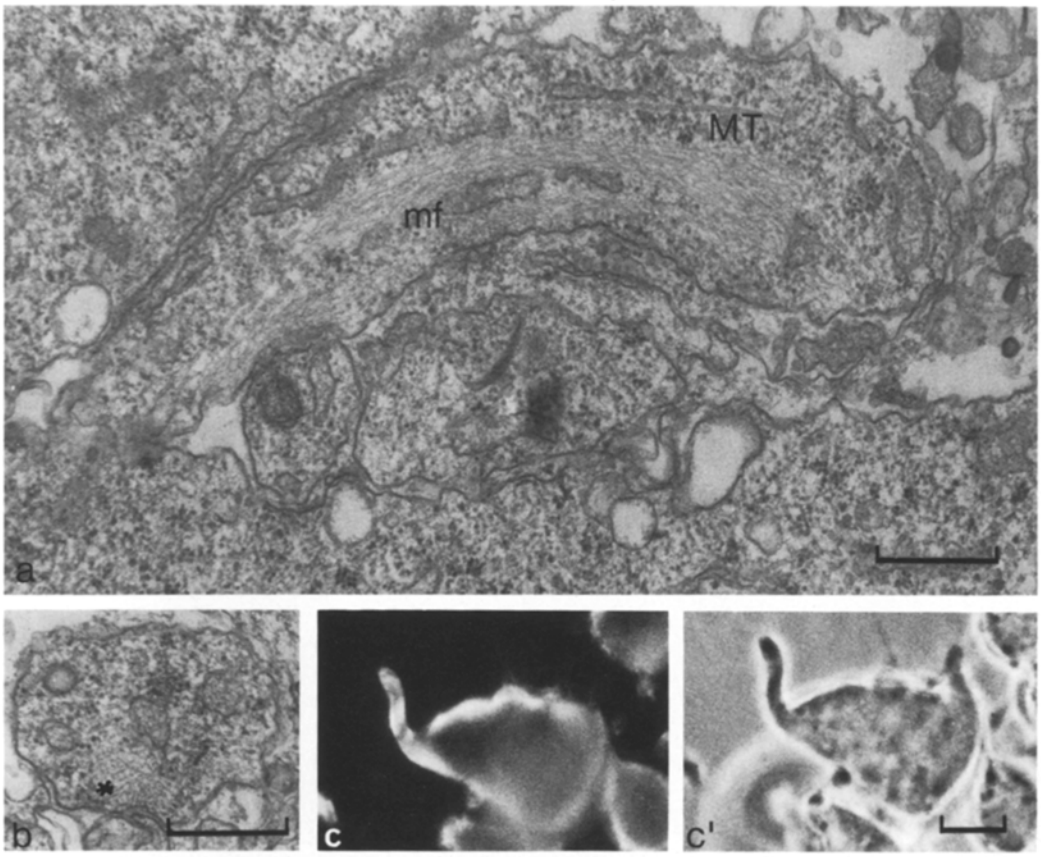

Fig. 8a–c. Microfilament (*mf*) bundle in a polar filiform appendix after prolonged incubation in 1 mM vanadate. **a** In a longitudinal section the filament bundle is paralleled by only a few *MT*. Bar represents 500 nm. **b** In the cross section the close proximity of the solid filament bundle to the plasma membrane is obvious (*asterisk*). Bar represents 500 nm. **c** Anti-actin fluorescence and phase contrast photomicrograph of a cell after prolonged incubation in 1 mM vanadate. One of the two appendices shown in **c'** is out of focus in **c**. Compare with Figure 6a (inset). In contrast to Figure 6a (inset) the cell body appears unlabelled, indicating a shift of actin to the appendices and to the cell surface. Bar represents 6 μm

show that vanadate penetrates into spermatocytes. Therefore, it seems unlikely that elongation instead of rounding up could be achieved solely by the inhibition of the Na/K-ATPase although a certain impairment of the enzyme might amplify elongation which has been caused primarily by other reasons. Another serious argument against regarding spindle elongation as a result of osmotic effects, due to abnormal internal ion concentrations similar to those described originally by Bělař (1929) and recently by Snyder et al. (1984), comes from phase contrast observations of living spermatocytes and from EM observations of vanadate-treated metaphases. We found no clumping of chromosomes, nor a dense cytoplasmic matrix or assortment of MTs to densely packed bundles as reported to occur shortly after exposure to 0.5 M sucrose (Snyder et al. 1984) and observed in cells treated with highly concentrated Ringer's solutions (unpublished results).

As pointed out by Nechay (1984) and Willsky et al. (1984), even at relatively high extracellular concentrations of vanadate, the intracellular concentrations will be low, firstly affecting the highly susceptible enzymes Mg-ATPase of dynein and the Na/K-ATPase. Since the rate of inhibition by vanadate is quite different for both enzymes, early effects may be solely ascribed to vanadate-induced inhibition of dynein or perhaps to the action of rapidly forming vanadyl, which represents a much weaker inhibitor (Willsky et al. 1984). It appears to us that the apparent dismembering of the spindle fibres observed in the enlarged vanadate-treated metaphase spindles may be due to inhibition of dynein-like cross-bridges.

Cross-bridges have been described in many spindle types, e.g. in the well examined diatom spindle (Tippit et al. 1978), in the dividing micronucleus of ciliates (Hauser 1972) and even in isolated spindles of sea urchin eggs (Pratt et al. 1980), but it is not clear whether these bridges produce sliding forces or play a merely static role by stabilizing the MT framework of the spindle. Since we also verified such bridges in Locusta spindles, irrespective of their role, inhibition by vanadate could lead to the obviously loosened state of the spindle. In the case of flagellar or ciliary dynein, vanadate does not lead to a state of rigor but to an isolation and subsequent falling apart of the doublets (Satir et al. 1981). The barrel-shaped spindle after vanadate treatment of cell models in the work of Cande and Wolniak (1978) may also have arisen in this way.

However, mere disfunction of such bridges cannot explain the observed separation of the poles. On the contrary, they must either by pulled in the poleward direction or migrate apart on their own accord, thus anticipating one part of anaphase. Since elongation mediated by sliding should not apply here, motive force production might be considered to be due to the observed 5 nm microfilaments

localised in the polar regions of vanadate-treated cells. They seem to be involved in the protrusion of the poles, first leading to the lemon shape, later giving rise to the formation of the appendices, presumably by bundling and attachment to the plasma membrane. The anti-actin fluorescence of the vanadate metaphases supports this hypothesis since it demonstrates a shift of the usually equally distributed actin from the cell body into the polar appendices. The failure to demonstrate actin-like microfilaments in controls by EM methods in spite of numerous positive proofs for actin by IIF (e.g. Beier and Hauser 1981), may be due to its prevailing monomeric state during most stages of mitosis, respectively to its delicate net-like organisation throughout the cytoplasm. There is probably no justification, however, for equating the observed elongation process with the physiological events during anaphase. It is merely an example of the potential of motile forces of this type of spindle.

The massive occurrence of 5 nm filaments in the apices of vanadate-treated metaphases could perhaps argue for an interference of vanadate with the conditions for polymerisation or even stabilization of actin filaments. It also seems possible that the observed occurrence of numerous polysomes is an indication of an enhanced production of proteins which become assembled into filaments. This could depend on a stimulatory effect of the reduced cationic substance vanadyl (VO^{2+}, with the vanadium in the +4 oxidation state) as shown in the yeast *Saccharomyces cerevisiae*, where vanadate is inhibitory, while the intracellularly formed vanadyl stimulates cell growth (Willsky et al. 1984).

If 5 nm filaments, although in relatively small numbers, are functional components of the mitotic spindle, they might exert tension between the centrosomes and the polar regions of the plasma membrane and pull the poles apart. Presumably, this process is assisted by simultaneous anaphasic surface growth. It is of interest in this respect, that Kronebusch and Borisy (1982), who studied the movement of the poles during spindle elongation and ruled out pushing via microtubule sliding, favoured independent migration of the poles. They concluded that the distance between the centrosomes and the plasma membrane shortens by some kind of aster-induced motion, mediated through contractile components attached to the membrane. A similar proposal has also been made by Aist and Berns (1981) for chromosome separation in Fusarium.

The results of the microcinematographic analysis of vanadate-treated spermatocytes are also in accord with a possible involvement of this filamentous material, most likely actin, in regular chromosomal movement. As already pointed out, cells which were incubated in 1 mM vanadate retain 50% of the normal division frequency and, interestingly, show varying rates of both anaphase motions (A and B), depending on cellular shape. Their half-spindles first increase in length so that those which start anaphase in the lemon-shaped state lack further elongation, whereas the anaphase poleward motion of their chromosomes even appears to be speeded up. Referring to the hypothesis of Forer (1974), who proposed contractile proteins as the force-producing elements of the spindle with MTs as velocity controlling factors, the acceleration of anaphase motion could be explicable as a result of the spindle dismembering which leads to thinned-out spindle fibres with reduced MT numbers. Secondly, the delay in the onset of anaphase could have a close temporal correlation with the time the cells need to substitute for the amount of microfilamentous proteins dislocated to the cell's apex.

Our cinemicrographic results show clearly that cells which start anaphase motion after the longer delay (group IIb in Fig. 4) have regained normal size and reveal themselves as only partly hindered in the elongation process. This can be explained by the only limited loss of membrane material during the formation of the thin and filiform appendices. The rate of chromosome-to-pole movement has remarkably slowed down in comparison to both control and lemon-shaped cells, possibly due to the extensive loss of proteins becoming assembled into 5 nm filaments within the appendices. The fact that the rate of chromosome-to-pole movement is less reduced in cells which have regained normal size by some kind of cellular contraction (as recognized in the cinemicrographs) appears especially interesting in this respect. Obviously, this slowing down of the chromosome-to-pole movement seems indeed to be correlated with a more or less extensive separation of MTs and Mfs. We consider therefore the filamentous component as at least one of the motile forces residing in the spindle.

In a similar report concerning the effect of vanadate on the cytoskeleton of living baby hamster kidney cells, Wang and Choppin (1981) described a separation of MTs and 10 nm filaments, also leading to a loss of organization of cell architecture and subsequent difficulties regarding the movement of cell organelles.

Even in the 5 mM vanadate-induced lemon-shaped cells the force-producing mechanism of anaphase movement is not really affected because in these cells also the migration of the univalent X-chromosome can be traced over a distance corresponding roughly to the length of the normal half-spindle. Since the poles are pulled apart and the spindle framework is disturbed the X-chromosome apparently loses spindle contact not far from the former polar region. The failure of the autosomes to leave the metaphase plate is possibly due only to the missing anaphase trigger which seems to be released by the univalent's arrival at the pole as indicated by cinematography of dividing control cells. Assuming Ca^{2+} fluxes between the ER cisternae and the metaphase plate as an anaphase trigger (discussed by Hepler et al. 1981), misregulation would also easily be explicable since our EM studies revealed considerable disorder and loss of endomembranes in the vicinity of the equatorial plane.

Irrespective of a possible influence on their Ca^{2+}-ATPases (O'Neal et al. 1979; O'Neill and Spanswick 1984) the disorganisation of spindle ER cisternae would sufficiently explain regulatory disorders. At all events, Petzelt and Wülfroth (1984), studying the inhibitory capacities of vanadate on the "mitotic" ATPase, did not find any change in the enzyme's activity, so that a direct influence on Ca^{2+} regulation can be excluded.

We conclude from our results that spindle elongation after vanadate treatment is achieved by breaking up the spindle framework under the assistance of cytoskeletal elements pulling the poles apart which are connected to the plasma membrane. The different rates of chromosome-to-pole movement in cells with dismembered spindle fibres (fast movement) or with extensive separation of MTs and Mfs (slow movement) support the hypothesis of Forer (1974) who characterised spindle MTs as the rate limiting factor of a motor that drives chromosomal movement. Further investigations, especially of the filamentous components under regular conditions, are needed to decide whether contractile elements have any relevance in anaphase movement, since there is also some physiological evi-

dence (Aubin et al. 1979; Cande 1982b; McIntosh 1979) which argues against their fundamental role in anaphase.

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