chromosomes as well as for the detection of translocations, and thus can form a helpful tool for the studies on genetic control methods of spider mites[18].

The results further demonstrate for the first time that bands can be induced in holokinetic chromosomes. According to the procedures employed the bands are G-bands. C-band patterns, however, could not be seen. In monocentric mammalian chromosomes C-bands are typically found adjacent to the centromeres and may represent constitutive heterochromatin with highly repetitive DNA[4,5]. In holokinetic chromosomes centromeres do not occur and the spindle attachment sites (kinetochores) extend the entire length or almost the entire length of the chromosomes[19–21]. Repeated DNA sequences, as found in centromeric constitutive heterochromatin, were found to be scattered throughout the holokinetic chromosomes of the milkweed bug *Oncopeltus fasciatus*[22]. The absence of C-bands in the chromosomes of *T. urticae* may thus be related to the holokinetic condition of the chromosomes. The centromeric type of constitutive heterochromatin may then be either not present at all or scattered throughout the genome in units too small to form visible bands.

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Contraction and Volume Reduction of the Glycerolated *Carchesium* Spasmoneme: Effects of Alkali Earth Cations

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Summary. The series Ca>Sr>Ba>Mg represents the relative activities of the alkali earth cations causing contraction of glycerolated *Carchesium* stalks. During contraction, spasmonemal volume is reduced by 37%.

Mature colonies of *Carchesium*, a sessile colonial peritrich ciliate, may consist of more than 100 zooids each with an individual spasmoneme. There is no structural continuity between spasmonemes of different zooids and individual stalks may retract independently. Stalk coiling in the peritrich ciliates results from spasmonemal shortening. Studies of the *Zoothamnium* spasmoneme have revealed mechanical, stress-optical, chemical and ultrastructural characteristics incompatible with either actomyosin or microtubule-based cell motile systems[2]. Specifically, contraction of the glycerolated spasmonemes in *Vorticella*[3–5], *Carchesium*[6] and *Zoothamnium*[2] can be induced simply by increasing the ambient free calcium ion concentration from 10^{-8} to 10^{-6} g ion/l. Neither ATP[7] nor magnesium ion is required. Spasmoneme re-extension follows the removal of free calcium ion. This is distinct from primitive actomyosin systems which require ATP and both calcium and magnesium ions, and from interacting microtubule mechanisms in which ATP and magnesium are essential and calcium inhibitory[8,9].

Materials and methods. Carchesium sp. were cultured at 20 °C in C solution (10^{-3} *M* $CaCl_2$, 10^{-4} *M* $MgCl_2$, 10^{-4} *M* KCl, 10^{-3} *M* $NaHCO_3$, in distilled water) to which was added 15% v/v 0.03% lettuce infusion. Glass microscope slides provided a convenient substrate. Cultures of young carchesia were obtained by treating mature colonies with 0.6 *M* urea in C solution. This treatment induced shedding from the colony of most zooids within 30 min, and shed zooids, when returned to C solution, developed new contractile stalks within 24 h[10]. In this report, young colonies are those with 1–2 zooids, mature colonies those with more than 20 zooids. The youngest stalks in mature colonies originate farthest from the pedal end.

The same glycerolation technique was used for both young and mature colonies. Slides bearing suitable colonies were washed and left overnight in C solution prior to extraction. The slides with attached carchesia were incubated for 24 h at 0 °C in relaxing solution (10^{-1} *M* KCl, 2×10^{-2} *M* *tris*-maleate buffer pH 6.8, 4×10^{-3} *M* EGTA[7], in distilled water) to which was added 50% v/v glycerol, then passed through 2×30 min washes in relaxing solution at 0 °C, and finally allowed to warm to room temperature. Cells remained anchored to the culture slide throughout extraction.

The threshold divalent cation concentration for spasmonemal contraction was determined by titration, using the spasmoneme itself as the indicator of end point. Individual glycerolated colonies or single cells were dislodged from the substrate and transferred to a glass, flat-bottomed, 50 mm petri dish containing 10.0 ml of relaxing solution. A coverslip shard resting on the main stalk pinioned the colony within the dish. Stalk coiling was prevented as required by similarly restraining both ends of the colony. Contraction was observed with a Zeiss Universal microscope equipped with phase contrast and Nomarski differential interference contrast optics with the aid of a water-immersion cap[11]. Threshold was measured by adding from a burette drop by drop a solution of divalent cation (4×10^{-3} *M* $MeCl_2$[7], 10^{-1} *M* KCl, 2×10^{-2} *M* *tris*-maleate buffer pH 6.8, in distilled water)

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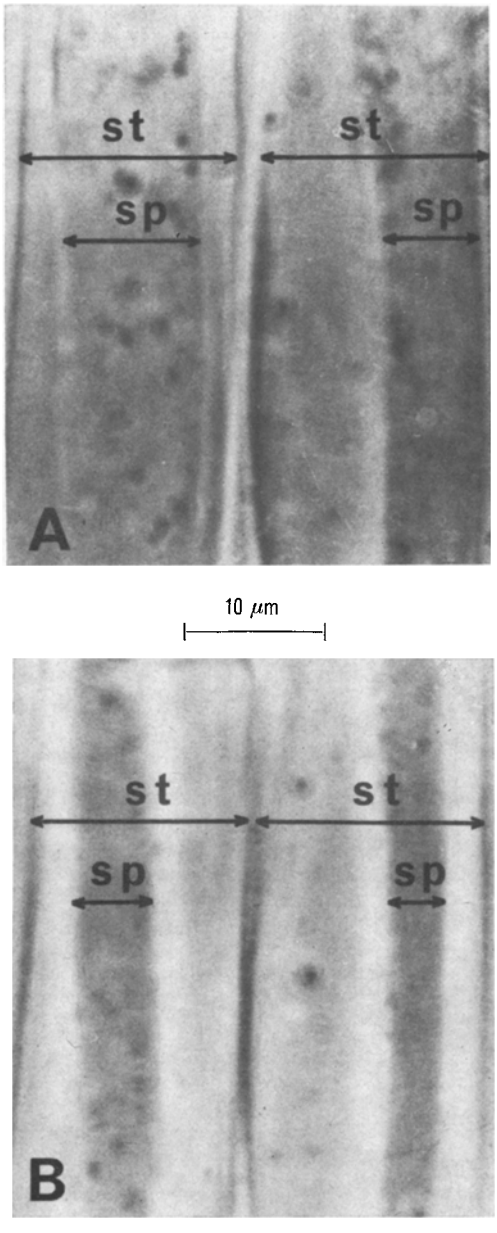
Threshold concentrations of alkali earth cations necessary to induce contraction in the glycerolated spasmoneme, expressed as the mean and standard deviation of sample number (n)

	pH	Calcium	Threshold Cation Concentrations (g ion/l) Strontium	Barium	Magnesium
Carchesium sp.					
1. Young colony	6.8	$2.04 \pm 0.12 \times 10^{-7}$ ($n = 17$)	$3.16 \pm 0.18 \times 10^{-5}$ ($n = 18$)	$6.01 \pm 0.28 \times 10^{-5}$ ($n = 15$)	Ineffective at 10^{-2}
2. Mature colony	6.0	$1.77 \pm 0.21 \times 10^{-7}$ ($n = 18$)			
	6.8	$1.95 \pm 0.16 \times 10^{-7}$ ($n = 24$)			Ineffective at 10^{-2}
	7.5	$1.61 \pm 0.27 \times 10^{-7}$ ($n = 24$)			
Vorticella[a]	6.86	$4.21 \pm 0.1 \times 10^{-7}$ ($n = 20$)			Ineffective at 2.5×10^{-2}

[a] The data for *Vorticella* are taken from Amos[5] and Weis-Fogh and Amos[2].



Two parallel stalks from a glycerolated mature colony of *Carchesium sp.*, A showing the spasmonemes (sp) extended in 10^{-8} g ion/l calcium ion, B the same stalks isometrically contracted (10^{-6} g ion/l calcium ion). The spasmonemes have narrowed during contraction but stalk diameters (st) are unchanged. The calibration bar is 10 μm.

while the contents of the dish were continually stirred. No change in pH accompanied titration. The threshold cation concentration was calculated from

$$K' = [\text{MeEGTA}] / [\text{Me}^{2+}]\,[\text{EGTA}^{2-}]$$

where square brackets denote concentration and K' is the apparent equilibrium constant, related to the true equilibrium constant K(MeEGTA) by

$$K(\text{MeEGTA})/K' = 1 + [\text{H}^+]K_1 + [\text{H}^+]^2K_1K_2 + [\text{H}^+]^3K_1K_2K_3 + + [\text{H}^+]^4K_1K_2K_3K_4$$

where $K_1 = \log 9.46$, $K_2 = \log 8.85$, $K_3 = \log 2.65$, $K_4 = \log 2.0$, and K(CaEGTA) = log 10.97, K(SrEGTA) = log 8.38, K(BaEGTA) = log 8.34, K(MgEGTA) = log 5.2[12].

Results and discussion. When the divalent cation concentration ambient to a glycerolated colony was raised beyond the contraction threshold, the younger stalks slowly coiled, reminiscent of but much slower than coiling in vivo. The older stalks have typically lost the ability to coil although the spasmoneme may still contract. For this reason threshold determinations were made on stalks prevented mechanically from coiling, thereby ensuring a more strict comparison between young and mature spasmonemes. Two simultaneous changes were observed in the isometrically contracted spasmoneme, a darkening when viewed by positive phase contrast and a significant reduction in diameter (Figure). It is plausible to consider phase darkening to be a consequence of that spasmonemal condensation which narrowing at constant length implies.

Measurements from relaxed and isometrically contracted stalks allow a more accurate determination than hitherto of any change in spasmonemal volume accompanying cation binding[13,14]. The relaxed spasmoneme (at 10^{-8} g ion/l calcium ion) is ellipsoidal in cross-section with an axial ratio close to two, becoming almost circular in cross-section when contracted (at 10^{-6} g ion/l). Volume changes can therefore be calculated from the measured change in either axis. Measurements from 14 spasmonemes gave a 44% mean reduction in major axis during isometric contraction, which corresponds to a 37% mean volume reduction. It is by no means clear that a similar volume change accompanies contraction in vivo: Sugi[13] reported a 24–38% volume reduction after coiling, but Amos[14] considered the measurement error from coiled

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stalks to exceed 40% and was therefore unable to confirm this finding. The contracted spasmoneme displays the mechanical properties of a swollen rubber[2], therefore its volume may vary with stretching, increasing roughly as the square root of the extension ratio[15]. By restraining the contracted spasmoneme at the relaxed stalk length, it is held at an extension ratio close to three, and therefore the decrease in spasmonemal volume may be even greater during isotonic contraction.

Threshold divalent cation concentrations for glycerolated *Carchesium* spasmonemal contraction are listed in the Table. The threshold calcium ion concentration is similar to that reported for *Vorticella*[5] (included in the Table for comparison). Threshold is independent of both 10^{-2} g ion/l magnesium ion and 2×10^{-3} *M* ATP, and is only slightly sensitive to pH between 6.0 and 7.5. Within a mature colony, no difference in threshold was ever observed between the spasmonemes of older and of younger stalks, nor was any difference in threshold revealed between young and mature colonies. In mature colonies, the fragments of spasmoneme in the main stalk contracted at the same threshold as for the intact spasmoneme. The same was found in young colonies in which the spasmoneme had been disrupted by prolonged incubation in 10^{-6} g ion/l calcium ion. The spasmoneme was less sensitive to strontium and barium ions than to calcium, and much higher free ion concentrations were required to induce contraction. In other respects, stalk coiling and isometric spasmonemal contraction induced by strontium or barium ions was indistinguishable from those induced by calcium. Contraction was not induced by magnesium ion buffers with a free ion concentration of 10^{-2} g ion/l, even in the presence of ATP. Spasmoneme re-extension followed promptly on cation chelation by EGTA. In all cases, contraction re-extension cycles could be repeated many times.

The effectiveness relative to calcium of alkali earth metals in inducing contraction is

$$Ca(1.0) > Sr(0.006) > Ba(0.003) > Mg(0).$$

Similar sequences are typical of the equilibrium binding constants to acidic sites on proteins, for example the skeletal muscle troponin C calcium binding site[16], and of binding to organic polyacids such as α poly-L-glutamic acid[17]. It has been suggested that spasmonemal extension results from repulsion between fixed negative charges, contraction following charge neutralization through calcium binding[2,4]. It is interesting that large dimensional changes in crosslinked polyacid fibres may result from a simple change in the degree of fibre ionization. The high relative sensitivity to calcium, together with the pH and magnesium insensitivity, indicate some ion-binding specificity. It may be that spasmonemal mechanochemistry resembles those polyacid fibre contractions that accompany ion exchange between sodium and calcium, where shortening occurs abruptly at a critical monovalent/divalent cation ratio[18].

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Dense Particles in Subcutaneous Collagen Fibrils

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Summary. Electron microscopy of unstained, fresh air-dried spreads of the subcutaneous connective tissue disclosed dense particles (6–20 nm in diameter) at the dark bands of collagen fibrils with 67 nm periodicity. The particles appear to consist of chlorides from the X-ray microanalysis of collagen fibres.

Electron microscopy of fresh frozen dried ultrathin sections and fresh air-dried tissue spreads has disclosed, without any treatment (i.e. fixation, embedding or staining), various fine structures[2–4]. Escape from any contact with liquid throughout the specimen preparation is essential to study the distribution of diffusible substances in tissues at the electron microscopic level. The specimens thus prepared are applicable to electron probe X-ray microanalysis and dry mount autoradiography.

Energy dispersive X-ray microanalysis affords a good qualitative survey of elements, though not quantitative, in small areas of the electron microscopic level. It gives information on the distribution of mineral elements, with an atomic number of more than 11 (Na), which is not available with the microincineration procedure. In this technique, it is very difficult to overcome the loss and dislocation of the elements during the specimen preparation, and to identify the elements of the ash after the incineration[5,6].

Dense structures in fresh air-dried tissue spreads, which were revealed by a conventional electron microscope, were observed first with a scanning transmission attachment and then analyzed for their elemental content with an energy dispersive X-ray microanalyzer. The platelet dense bodies[7], zymogen granules of the pancreatic acinar cell[8], neurosecretory granules of the mouse pituitary[9], the nucleus of a subcutaneous connective tissue cell[10], and mitochondrial granules[10] were examined

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