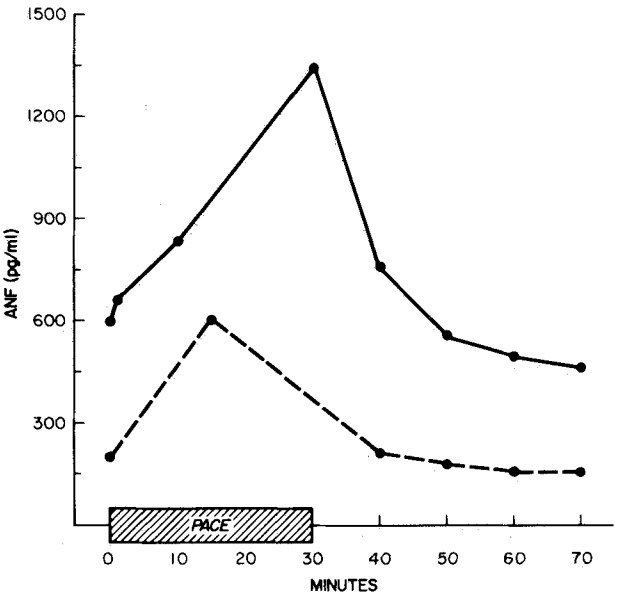

Figure 2. ANF levels of 2 pigs electrically paced at 150% of their basal heart rate for 30 min (pace). ANF levels returned to baseline over the following 40 min.

Atrial distention increases ANF levels. The many pharmacological and pathological stimuli associated with high ANF levels all increase right atrial pressure. During hemorrhage, however, right atrial pressure showed a significant and left atrial pressure, a non-significant decrease while ANF levels rose. These animals were capable of decreasing ANF levels from control values, and would be expected to do so following hemorrhage, based on their right atrial pressures. They instead increased ANF levels, so there is a physiologic mechanism for ANF release other than atrial stretch. Tachycardia seems the likely mediator of ANF release. Paroxysmal atrial tachycardia has been reported to increase ANF levels[5,6] and cardiac pacing markedly increased ANF levels (fig. 2). Hemorrhage and pacing both lower blood pressure, so they can both stimulate release of catecholamines, vasopressin and renin. Pacing is a lesser stimulus to release of these hormones but was associated with greater ANF levels, so stress hormones would not seem likely mediators of the ANF response. ANF levels can be increased by atrial distention and another mechanism, probably tachycardia.

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Detection of high concentration of Mg and Ca in the nematocysts of various cnidarians[1]

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Summary. An X-ray spectral analysis (EDAX) of isolated undischarged nematocysts of various cnidarians *(Hydrozoa, Scyphozoa, Anthozoa)* revealed the presence of extremely high concentrations of divalent cations. In *Hydra* nematocysts both Ca^{2+} (conc. 0.36 µmole/mg dry cysts) and Mg^{2+} (conc. 0.80 µmole/mg dry cysts) ions add up to a total in situ concentration of 0.5 to 1.0 M. More than 85% of the cations, which are believed to be involved in cyst discharge, are contained in the soluble fraction of the cysts, where they must be bound to high molecular weight molecules.
Key words. Calcium; magnesium; nematocysts; *Hydra; Cnidaria.*

The stinging cells (nematocytes, cnidocytes) of the *Cnidaria (Hydrozoa, Cubozoa, Scyphozoa, Anthozoa)* are undoubtedly the most complex cells found in the animal kingdom[3]. The prominent organelle of these cells is a spherical or oblong capsule (nematocyst, cnidocyst, fig. 1) which upon adequate triggering[4] ejects its tubular content by a process of evagination[5–7]. There are about 30 different types of cysts and, accordingly, of nematocytes[4,8,9]. Their function is associated mainly with prey capture and/or defense. The majority of cysts contain toxins and enzymes[10–13], which when introduced into the target by the exploding cyst paralyze and kill the prey or the potential aggressor[5,7]. Some of these toxins can even be fatal for man[12,14].

The violent discharge of the cyst is an extremely rapid event in the course of which the cyst's tubule and its associated structures, such as spines, stylets, barbs etc. are ejected by evagination from the interior of the capsule. In the stenoteles of *Hydra* it is completed within 3 ms[6].

Neither the magnitude nor the nature of the forces enacting and sustaining this process are known. However, various theories dealing with this phenomenon have been proposed[4,7]. There is increasing evidence that the intracapsular pressure required for the cyst's explosion is, at least partly, built up osmotically. The discharge of triggered anthozoan cysts is accompanied by a rapid uptake of water by the latter. Simultaneously the cysts release Ca-ions into the surrounding medium[15–17]. In fact, the undischarged nematocysts of *Anthozoa* have been found to contain large amounts of calcium[16,18,19]. The causal relationship between Ca release and water uptake, however, is still not understood.

In the course of investigations concerned with the morphodynamics and energetics of nematocyst discharge in *Hydra*[5–7]

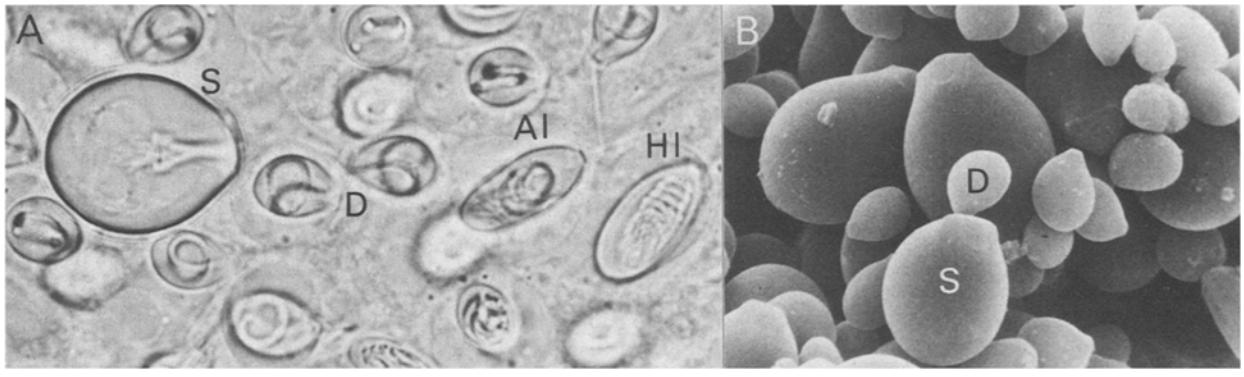

Figure 1. *A* Light microscopical picture of a squashed tentacle of *Hydra attenuata* Pall. featuring the 4 different types of nematocysts occurring in the genus *Hydra*. AI = atrichous isorhiza; D = desmoneme; HI = holotrichous isorhiza; S = stenotele × 1700. *B* SEM portrait of an isolated undischarged stenotele of *H. attenuata* × 1710.

we have analyzed the cysts of *Hydra* and various other cnidarians qualitatively and quantitatively as to their content of divalent cations.

Material and methods. Material. Nematocysts from several cnidarian species were isolated and analyzed. Various species of *Hydra* cultured in the laboratory under standard conditions (Loomis' medium[20], temperature: 14 °C, food: *Artemia)* as well as polyps of *Podocoryne carnea (Hydrozoa), Cassiopea andromeda, Aurelia aurita (Scyphozoa)* and the tentacle ectoderm of *Anemonia sulcata (Anthozoa)* (kept in artificial seawater[21] and also fed with nauplii of *Artemia*) were used as nematocyst sources. Nematocysts of *Physalia physalis (Hydrozoa, Siphonophora)* were kindly donated by Dr J. W. Burnett (Baltimore). Cysts of *Chrysaora quinquecirrha (Scyphozoa)* were purchased from Sigma. Before they were analyzed both preparations were purified by Percoll centrifugation as indicated below.

Methods. Pure, undischarged nematocysts were isolated as described elsewhere[30]. Briefly, whole polyps or polyp tentacles were frozen at −20 °C. After thawing the samples were gently homogenized with a Pasteur pipet in ice-cold water containing 50% Percoll (Pharmacia Fine Chemicals). After standing for 30 min on ice (which had proved to be advantageous) the homogenates were centrifuged for 10 min at 3000 g (Sorvall RC 5 B; SS-34 rotor). The supernatant containing the debris of the disrupted cells was discarded. The pellet, which consisted of clean undischarged cysts, was resuspended and washed several times in cold water. Examination of this material with the SEM revealed that the individual cysts were absolutely clean of adhering cellular residues.

For the X-ray spectral analysis (EDAX) the isolated cysts were dried on teflon stubs, sputtered with carbon and individually analyzed with a Cambridge S-4 stereoscan equipped with EDAX. The X-ray spectral analysis allows the detection of elements of an atomic weight above 16.

Complete oxidation of purified nematocysts was performed in ∅0.6 × 8 cm pyrex tubes. Samples of nematocysts (circa 0.5 mg) were transferred into the tubes, dried over $CaCl_2$ and, after the addition of 25 µl conc. HNO_3, refluxed in a sand-bath for 30 min and for another hour after addition of 15 µl $HClO_4$[24]. Following the addition of water the clear solutions were neutralized by NaOH and finally brought to a volume of 3.0 ml.

Quantitative determinations of Ca^{2+} and Mg^{2+} contained in the oxidized samples were carried out directly by atomic absorption on an Instrumentation Laboratory AA/AE spectrophotometer 451 or by spectrophotometric titration of Me^{2+} indicator complexes. For this purpose increasing concentrations of EDTA were added to aliquots of the samples in 50 mM glycine-NH_3 (pH 10.2) and either 0.02% erio T[22] or 0.1 mM phthalein purple[23]; the absorbances of the resulting metal-dye complexes were recorded at 520 nm and 580 nm respectively. Since the absorption coefficients of the Mg- and Ca-dye complexes differ, the concentrations of the two divalent cations in the samples could be deduced after plotting absorbance vs EDTA concentration as demonstrated in figure 3.

Results. The X-ray spectrum (fig. 2A) of a single isolated and purified stenotele (fig. 1B) of *Hydra* reveals that besides sulfur, which has been reported to be predominantly located in the wall of the capsule[25,26], magnesium and calcium are by far the most abundant atoms. The small F peak stems from the teflon support.

Surprisingly enough and in contrast to other reports[17,19,27] the cyst contains not only S and considerable quantities of Ca^{2+} but even more Mg^{2+}. This particular spectral pattern was found to be identical in the desmonemes and isorhizas of *Hydra* (fig. 1A).

In order to check whether or not the relative amounts of cations present in the cysts are dependent upon the feeding regime two batches of *Hydra attenuata* were fed differently for a period of 3 months. One of them was fed 3 times a week with brine-shrimp larvae hatched in artificial seawater[21] but washed in freshwater immediately before being given to the polyps; the other group received freshwater plankton consisting mainly of copepods and cladocerans at identical intervals. The 3-month period of time is sufficiently long to allow the complete replacement of the preexisting nematocyst population by cnidogenesis.

The X-ray spectra obtained from the purified nematocysts from the two experimental groups were absolutely identical as to the relative amounts of Ca^{2+} and Mg^{2+}, indicating that feeding freshwater polyps only with brine shrimps has no effect upon the concentrations of magnesium and calcium within the nematocysts.

The nematocysts of all marine species examined so far (*Podocoryne carnea,* fig. 2B; *Physalia physalis; Cassiopea andromeda; Aurelia aurita; Chrysaora quinquecirrha* and *Anemonia sulcata,* fig. 2C) yielded spectra that were slightly different from those of *Hydra,* but confirmed without exception the predominance of Ca^{2+}, Mg^{2+} and S, which indicates that these three elements are not a peculiarity of the nematocysts of the freshwater representatives of the *Cnidaria,* but also seem to be a common property of those of marine species.

Since the EDAX data cannot easily be quantified for various reasons[28] we estimated the respective concentrations of Ca^{2+} and Mg^{2+} of completely oxidized nematocysts of *Hydra* by titration and, independently, also by means of atomic absorption measurements (see Methods).

The average concentrations of Ca^{2+} and Mg^{2+} per mg dried

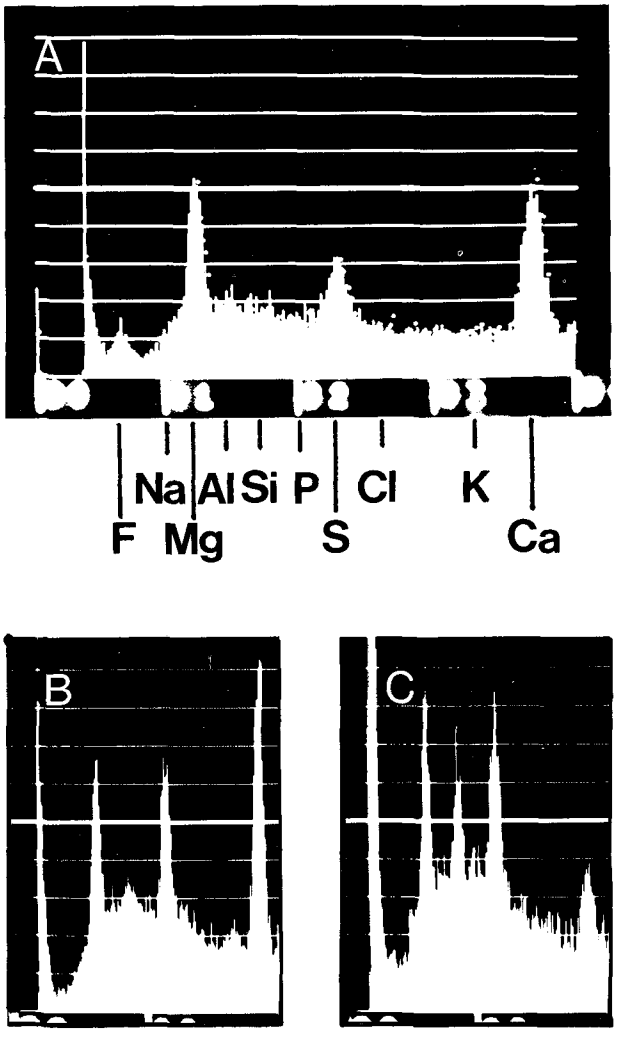

Figure 2. EDAX spectra of single isolated nematocysts. *A* Stenotele of *H. attenuata* (cfr. fig. 1B). *B* Nematocyst of a *Podocoryne carnea* Sars polyp *(Hydrozoa, Athecata)*. As compared to figure 2A the abscissa is reduced by a factor of two (the Si-peak stems from residual Percoll, while the small K-peak is due to residues of other cellular components). *C* B-Rhabdoid of *Anemonia sulcata (Anthozoa, Actiniaria)*. The small F-peak originates from the teflon support, the Na-peak most probably from contamination by seawater.

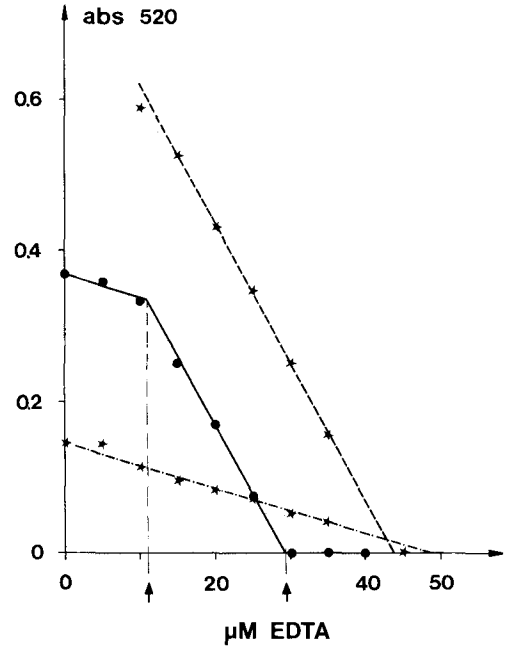

Figure 3. Example for a spectrophotometric titration of Mg^{2+} and Ca^{2+} of *Hydra* nematocyst extract. 100 µl nematocysts (dry wt 365 µg) were ashed, oxidized and titrated by EDTA in the presence of erio T as described under methods. ●——● titration of the sample (final dilution 150-fold), ×----× titration of a Mg standard; ×·–·–·× titration of a Ca standard. The sample curve was fitted by parallel shifting of the respective regression lines of the standards. The arrows indicate the approximate equivalent points for Ca and Ca plus Mg in the sample.

cysts including stenoteles, desmonemes and isorhizas, were found to be 0.36 µmole and 0.80 µmole respectively. When referring these values jointly to a minimal density of 1.3 g/ml of dried nematocysts the two divalent cations add up to a total concentration of little more than 1.5 µmole per ml dried cysts. In native fully hydrated cysts the concentration of the two cations ranges between 0.5 and 1.0 M. These values are in agreement with those recorded in the nematocysts of 2 anthozoan species[16], namely *Rhodactis rhodostoma* and *Anthopleura elegantissima*, although no mention was made by the authors[16] of the presence of Mg^{2+}. After disruption of the *Hydra* nematocysts in distilled water (i.e. without any potential soluble counter-ions for Mg^{2+} and Ca^{2+} present), more than 85% of the divalent cations are found in the soluble extract, and are thus not bound to insoluble structural components such as the cyst's wall or the tubule. The remaining 15% may either be trapped in cysts which had escaped disruption or bound to yet unidentified insoluble molecules[30].

Discussion. Since all nematocysts of members of three different classes *(Hydrozoa, Scyphozoa, Anthozoa)*, which we have examined so far, have extremely high concentrations of calcium and magnesium, it seems that this is a characteristic of all cnidarian nematocysts, although the ratio between the two cations may vary slightly from one species to another. No information in this respect is available yet about the anthozoan spirocysts, which are generally considered to represent a cellular organelle quite different from the nematocysts[4].

During cnidogenesis, i.e. the synthesis and assembly of the cysts, considerable amounts of energy have to be invested by the organisms in order to reach such high concentrations of divalent cations within a secretory vacuole. Since these alkaline earth ions do not seem to contribute directly to the toxic action of the discharging nematocysts[29] we agree with the views of Lubbock's group[15–17] according to which calcium, at least, is involved in the process of cyst discharge.

The presence of equal and even higher concentrations of magnesium is a new discovery and the question arises whether Ca^{2+} and Mg^{2+} act as agonists or antagonists and what their actual function is in this extremely rapid process of cyst discharge.

The question of how Ca^{2+} and Mg^{2+} are bound within the resting undischarged cyst and how their release is controlled when the cyst is triggered remains open.

Although the wall of the cyst is permeable to molecules up to the size of 600 daltons, as staining experiments have revealed repeated washing of isolated stenoteles of *Hydra* has not led to a significant loss of Ca^{2+} and Mg^{2+} from the interior of the capsules. From this finding we conclude that the majority of the divalent cations must be bound to large molecules, probably highly anionic peptides or proteins which are prevented by the cyst's wall from leaking out[30]. Experiments aimed at the identification and characterization of these binding sites are in progress.

1 The work has been generously supported by the Swiss National Science Foundation (Grant No 3.072-84) and the Antoine Claraz-Foundation. We are indebted to Dr J. Hertz and Mr U. Jauch for

their advice and help, to Mr P. Flückiger and Ms Y. Trixl for their assistance and to Dr R. Stidwill for reading the manuscript.

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0014-4754/87/091022-04$1.50 + 0.20/0

Biological effects of lithium: Experimental analysis in plant cytokinesis

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Summary. The biological effects of lithium ions have been studied, using plant cytokinesis in onion root meristems as the experimental model. Lithium induces binucleate cells by inhibiting cell plate formation. Moreover, lithium and caffeine have additive effects on the induction of binucleate cells. Na^+, K^+, Ca^{++} and Mg^{++} antagonize lithium-induced inhibition of cytokinesis.
Key words. Plant cytokinesis; lithium; caffeine, calcium; magnesium; sodium and potassium.

The effects of lithium on biological systems have become a subject of some importance following its initial use in psychiatry[1]. In both plant and animal physiology, the regulation of many inter-cellular or inter-organ metabolic pathways depends on gradients of specific alkali ions, maintained by ionic 'pumps'. Several authors have studied the transport mechanisms which move lithium across biological membranes from different sources[2-4]. The fact that lithium is a magnesium competitor[5,6] for certain Mg-ADP dependent enzymes could explain some of the observed biological effects.

In plant cytokinesis the cell plate dividing the mother cell is formed from small vesicles produced by the Golgi bodies which coalesce along the equatorial plane during telophase[7,8]. The inhibition of the process by caffeine has contributed in part to elucidating the molecular mechanisms involved[9]. Most probably, calcium, magnesium and the adenine nucleotides, are intimately implicated in this process[10-13]. It is possible that caffeine inhibits a Ca^{++}-Mg^{++}-ATPase activity required for membrane fusion[14].

The fact that plant cytokinesis depends on the presence of several essential ions and adenine nucleotides, suggests that lithium might behave similarly to the divalent cations and might interfere with them. The present work was designed to test this hypothesis.

Material and methods. Root meristems of *Allium cepa* L. bulbs (var. Flat Violet) were used according to the method explained in Becerra and López-Sáez[10]. The treatment solutions were prepared with double-distilled water, caffeine, and one of the following salts, as required: LiCl, NaCl, KCl, $CaNO_3$ and $MgNO_3$ (Merck). The roots were not separated from the bulbs during the experimental period. In every case, the pH of the solution was adjusted to 6.5. Except when otherwise specified, the roots were incubated in the treatment solution for 4 h and returned to tap water for 1 h before harvesting. Binucleate cells indicate blocked cytokinesis, and mononucleate cells, normal, uninhibited cytokinesis. The roots were fixed in 3:1 absolute ethanol: acetic acid mixture, they were then stained with acetic orcein according to Tjio and Levan[15]. Finally, meristem squashes were prepared for cytological analysis. Every point on each curve represents the observations of at least eight roots from two bulbs. In total, some 8000 cells were scored, about 1000 for each root meristem. The vertical bars in the figures represent the standard error. Appropriate controls were used in all experiments.

Results. The efficiency of lithium as a cytokinesis inhibitor was tested by subjecting the roots to continuous treatment with several lithium concentrations (10, 20 and 30 mM). We chose 4 h of treatment as the appropriate time to obtain the