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

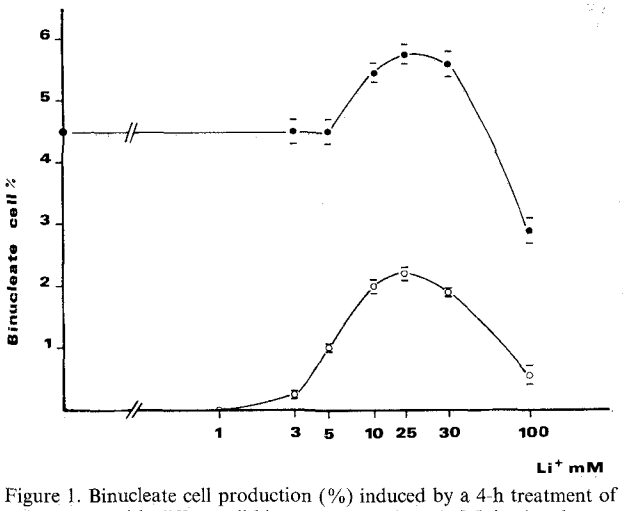

Figure 1. Binucleate cell production (%) induced by a 4-h treatment of onion roots with different lithium concentrations (mM) in the absence (○) and in the presence of 3 mM caffeine (●).

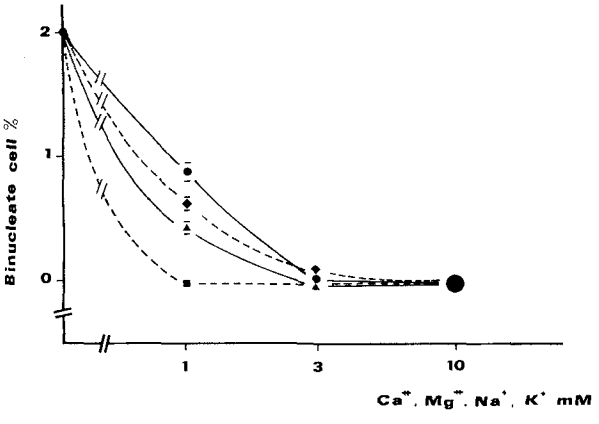

Figure 2. Na (●), K (▲), Ca (◆) and Mg (■) antagonism of lithium 10 mM induced binucleate cell production (%) after 4 h of treatment. At 10 mM of all cations the percentage of binucleate cells obtained is 0.

best efficiency (2% of binucleate cells). Figure 1 (lower curve) represents the efficiency of lithium at 4 h of treatment with concentrations ranging from 1 to 100 mM. The measure of cell flow showed that 100 mM lithium seriously affects the telophase rate.

To determine the existence of a possible cooperative inhibitory effect of lithium and caffeine on plant cytokinesis, the roots were treated for 4 h with lithium, at several concentrations, in the presence of 3 mM caffeine (fig. 1, upper curve). The results show that the induction of binucleate cells by 3 mM caffeine is increased by lithium concentrations ranging between 10 and 30 mM. In addition, the effect of 10 mM lithium in combination with caffeine at increasing concentrations were also studied. In the table, the induction of binucleate cells by caffeine (1–5 mM) with and without 10 mM lithium, is summarized.

The effect of other cations on lithium inhibition of plant cytokinesis was explored in a new set of experiments. Figure 2 shows the results obtained when roots were incubated with 10 mM Li^+ in the presence of different cations (Na^+, K^+, Ca^{++} and Mg^{++}) at several concentrations, from 1 to 10 mM. In every case, these cations counteracted the inhibition normally produced by 10 mM lithium. The most spectacular antagonism occurred in the presence of Mg^{++}; lithium inhibition was completely antagonized by Mg^{++} concentrations

Binucleate cell production (%) after a 4-h treatment of onion roots cells with different caffeine concentrations in the absence (control) and in the presence of 10 mM of lithium. Standard errors are also presented.

Caffeine (mM)	Control	Lithium (10 mM)
0	0.0	2.05 ± 0.15
1	0.3 ± 0.07	2.23 ± 0.16
2	2.41 ± 0.14	4.15 ± 0.22
3	4.72 ± 0.28	5.74 ± 0.25
4	5.53 ± 0.20	5.77 ± 0.25
5	5.80 ± 0.16	5.80 ± 0.18

as small as 1 mM. Quantifications of telophase rate and mitotic index in the presence of these cations, under the experimental conditions of figure 2, showed no significant modifications of these parameters.

Discussion. There are several ways by which ionic movement across biological membranes may occur. In red blood cells, lithium may be substituted for sodium and sometimes also for potassium in all these metabolic pathways[3,16,17]. The present observed effects of sodium and potassium may be related to lithium transport across the cell membranes. It is known that many magnesium-dependent biological processes may suffer from lithium interference[6]. Birch and coworkers investigated many different magnesium-dependent enzymes; the majority were inhibited by lithium. Furthermore, these same enzymes are also ADP-dependent. Becerra and López-Sáez[14] have postulated that caffeine blocks plant cytokinesis by inhibiting a certain Ca^{++} and Mg^{++}-activated ATP-ase fundamental for the coalescence of the Golgi vesicles. It is a reasonable working hypothesis that lithium similarly affects the process, perhaps by competing with magnesium for certain binding-sites of the ATP-ase.

The effect of calcium is more difficult to explain. A search of the literature reveals that there are still no reports of calcium effects on lithium transport across biological membranes[3], nor does intracellular calcium ion prevent lithium binding[5].

In conclusion, we present here a simple living system which permits us to test several biological effects of lithium. If, as we proposed in the seventies[10], plant cytokinesis may be considered as a cell secretion process, lithium could interfere not only with plant cytokinesis, but with many other important processes in living organisms where membrane fusion occurs (synapses, secretion processes, etc.). Although the molecular mechanisms of plant cytokinesis and its inhibition need more investigation, the results presented in this paper suggest that this cation has an action similar to caffeine on plant cytokinesis. If so, lithium could interfere with some membrane recognition mechanisms and/or fusion factors for which calcium and magnesium are essential. This could be at the level of primary fusion of the vesicles which gives rise to the cell plate, or at the secondary fusion level when the cell plate establishes contact with the mother-cell membrane, as proposed by Bonsignore and Hepler[18].

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0014-4754/87/091025-03$1.50 + 0.20/0

Amino acid and allozyme frequency changes in overwintering *Chymomyza amoena* (Diptera: Drosophilidae) larvae[1]

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Summary: Proline is accumulated by overwintering *C. amonea* larvae. Mortality averages over 50% by spring. PGM^F in the population shifts from 70% in summer to less than 50% during winter, increasing again in summer. Product synthesis from stored glycogen may be mandated in dipteran larvae also using larval proteins for cold hardiness when completion of development is delayed until spring.
Key words. Drosophilid; *Chymomyza amoena;* overwintering; amino acids; proline; allozymes; phosphoglucomutase.

How drosophilids survive the winter has become of research interest[2–7]. To date, studies have been carried out principally in two groups: species in the genus *Chymomyza* which are larval overwinterers[3,4,6] and species in the genus *Drosophila*[2,5–7]. Species in neither group accumulate glycerol[8,9]. The role of sugars remains ambiguous[7]. The larval proteins have been implicated in coldhardiness in dipteran larval overwinterers[9–11]. Nevertheless, time-dependent changes affecting subzero survival have been found for both *C. amoena* and *D. auraria*[2–4]. Water loss does not appear to be a factor in overwintering success in *C. amoena*[1].

Amino acid accumulation has been implicated in successful overwintering in many insects[12–17]. Accumulation implies an active metabolic state at chilling to subzero temperatures under non-feeding conditions[14,15].

Chymomyza amoena is a low density, furtive species. Large numbers are not always available. In autumn 1984 there was a heavy invasion of black walnut husks, *Juglans nigra*. Studies were undertaken in winter 1984–85 to determine if amino acid changes occurred. Proline was accumulated. However, there was a 50% mortality by March despite the association of this amino acid with freeze tolerance[12,15–17].

The natural population was then monitored beginning summer 1985 to determine if allozyme polymorphisms might be implicated in the shift between warm and cold-adapted phenotypes. Significant seasonal changes in the frequencies of alleles at the *Pgm* locus were found. This locus plays a major role in the storage and retrieval of glycogen in the fat body[18]. Heat sensitive alleles have been found in other species[19]. None were detected.

Dipteran larval overwinterers delay metamorphosis to spring. Alanine increases in dead larvae; its suppression in living larvae suggests proline is actively synthesized. Proline increase also argues for conservatism in nature, possibly related to membrane stabilization at subzero temperatures[20].

Materials and methods. Black walnuts infested by *C. amoena* larvae were collected in November 1984 and stored in an unheated shelter. Apples with *C. amoena* larvae were kept outside at the same location. Supercooling point (SCP) changes were monitored to detect SCP declines in the walnut population following the warm autumn. Amino acid determinations were made in 10 larvae from walnut husks in January, after a significant SCP decline from the November level. Snow and ice blanketed the area from 1 January 1985 to 23 February 1985. Walnuts were again obtained at the original site, SCPs determined for 10 living and 11 dead larvae, then two groups of 20 larvae each were used for amino acid analysis for living and dead larvae. Controls were 10 larvae in January and 20 in February grown in the laboratory at 22 °C. Amino acid analysis was also made on a group of 20 *D. melanogaster* larvae in February, also grown in the laboratory. All work involved third instar size larvae only. The remaining walnuts (33) in the shelter were used to estimate the number of larvae successfully completing development per walnut husk.

SCPs were measured as before. Rapid cooling of 2–3 °C per min, is used in drosophilid work[3,4]. Freeze sensitive (FS) larvae cannot tolerate ice formation in the body fluids and die at the SCP; freeze tolerant (FT) organisms may recover. A second category of freeze tolerance has the physiological property of equivalence of SCP and freezing points (FP) and allows recognition in summer that the population will contain FT individuals in winter.

For amino acid analyses, samples were homogenized, deproteinized by filtration and amino acid content measured on a modified Dionex amino acid analyzer. Each third instar larva weighs approximately 1 mg. Samples were also coded, then decoded after analysis.

Pgm polymorphism. Enzymes phosphoglucomutase (PGM), phosphoglucose isomerase (PGI), malic enzyme (ME), malic dehydrogenase (MDH), α-glycerophosphate dehydrogenase (α-GPDH) and 6-phosphogluconate dehydrogenase (6PGHD), all associated with the glycolytic cycle, were screened for polymorphism via starch gel electrophoresis at Mt. Lake Biological Station in the Allegheny Mountains of Virginia in summer 1985. In June Michigan *C. amoena* from endemic crabapples, *Malus coronaria,* were allowed to oviposit on apples to provide the research material. PGM was found to be polymorphic in Michigan (MI) and Virginia (VA) populations. Alleles have the same migration rate in larvae and adults. Seasonal changes were monitored for MI *C. amoena* in natural substrates beginning in autumn 1985. Substrates sampled included black walnut husks (November), apples (January, March) from an MSU orchard and ornamental crabapples (March) from a residence. Adults from endemic crabapples supplied the *C. amoena* summer 1986 sample. Estimates of *Pgm* allele frequencies were also made for VA populations from apples obtained at four locations to eliminate possible effects of substrate: Pamplin (near Lynchburg), Danville, Blacksburg and a site along highway 700 to the Station.

Sample preparation followed established procedures. Electrophoresis[21] employed 12% starch gel (Sigma), tris maleate buffer pH 7.4 diluted 1:9 for the gel and was run at 120 volts