Since the cages exclude the passage of electric fields below the frequency of X-rays, the effective fields cannot be like those by which sharks locate prey[6] and fish communicate[7]. The cages are freely permeable to high-energy radiation and to magnetism, factors to which an organism has been shown to respond with great sensitivity[8–12], and to gravity to which organisms may possibly respond[13]. The weak field involved in the mutual bean interactions is postulated to be magnetic[14]. This hypothesis is being investigated further.

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Calcium and magnesium in plant cytokinesis and their antagonism with caffeine

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Summary. The efficiency of caffeine at different concentration on the induction of binucleate cells in onion root-tip was studied. The drug effect is strongly depressed in the Ca^{++} and/or Mg^{++} presence at half-rate of maximum efficiency (0.04%), about 2 mM). We therefore conclude that both cations must play a role in plant cytokinesis.

Xanthic bases, and especially their methyl derivatives, such as caffeine, theophylline and theobromine, are well-known as inhibitors of cytokinesis, and their cytological effects on plant cells have been studied by several authors[1–3]. Moreover, the blockage of cytokinesis by 8-ethoxycaffeine and caffeine has been employed to induce a binucleate cell population characterized by a synchronous development of the cell cycle[4–6] and these synchronous cells have proved a very successful tool for cell cycle dissectioning[7–9].

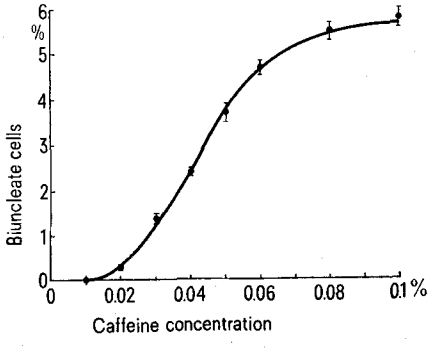
The experimental analysis of cytokinesis[10, 11] showed that, in the presence of these inhibitors, the Golgi vesicles, apparently present in similar number to those found in controls, do not form the cell plate, but disperse all over the cytoplasm. That is to say, the arrangement and fusion of Golgi vesicles does not take place. However, the first approach to the molecular mechanism of such an inhibition was made by Paul and Goff[12] when they studied the comparative effects of caffeine and calcium deficiency on cytokinesis. As a consequence, they proposed calcium requirement as a feature of plant cytokinesis and the calcium-caffeine antagonism as a molecular basis of the caffeine effect.

In order to test this hypothesis, and also to study the possible role played by magnesium, we decided to develop and use the 50% inhibited-cytokinesis as a test system: the first results are presented here.

Material and methods. The material used was the root meristem of Allium cepa L. bulbs. The onion bulbs (15–30 g) were grown in the dark at a constant temperature (15 °C ± 0.5) in cylindrical glass receptacles of about 80 ml capacity in tap water renewed every 24 h and aerated by continuous bubbling at the rate of 10–20 ml air/min. The bulbs were so placed that only their bases remained submerged in the water.

The treatment solutions were prepared with distilled water and Merck reagents. All the roots were submerged in the treatment solution without separating them from bulbs, and the environmental conditions already described were carefully maintained throughout the treatment period.

In every case, the roots were incubated for 4 h in the treatment solutions and returned for 1 h to water before harvest. This short recovery must permit all mitoses affected by caffeine to reach interphase in order to appear either as mononucleate cells, if not inhibited, or binucleate cells, when cytokinesis has been blocked[13].



Production of binucleate cells by treatments with different caffeine concentrations at 15 °C for 4 h. Abscissae: Caffeine concentration. Ordinate: Percentage of binucleate cells within the meristem population: Under these conditions, the threshold concentration appears to be 0.02% and caffeine about 0.1% the maximum efficiency of the drug is achieved.

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Calcium and magnesium antagonism with caffeine in cytokinesis inhibition

Treatments	Mononucleate cells			Binucleate cells			Binucleate (%)		
	0.04	0.06	0.10	0.04	0.06	0.10	0.04	0.06	0.10
Control	35.500	18.446	14.190	850	867	823	2.45 ± 0.08	4.71 ± 0.15	5.87 ± 0.19
10 mM Ca^{++}	15.833	8.028	4.385	190	289	228	1.31 ± 0.08	3.60 ± 0.20	5.21 ± 0.33
10 mM Mg^{++}	5.213	6.606	6.480	75	218	423	1.30 ± 0.15	3.21 ± 0.15	6.06 ± 0.29
Ca^{++} + Mg^{++} (10 mM)	20.500	4.454	3.379	106	98	196	0.52 ± 0.05	2.23 ± 0.21	5.86 ± 0.40

For cytological analysis, the roots were fixed in 3:1 ethanol-acetic mixture and the specimens prepared by staining the roots with acetic orcein according to the technique of Tjio and Levan[14]. Every treatment was studied in at least 2 bulbs and every root-tip was studied by scoring about 1000 meristem cells at random.

As binucleate cells were considered all cells with 2 nuclei sharing a common cytoplasm, in spite of the presence of partial cell plates.

Results and discussion. Caffeine efficiency in cytokinesis inhibition. With onion roots, a steady state of growth can be obtained under controlled conditions, characterized by a constant growth rate, a reliable cycle time and a mitotic index which remains steady over a long time period[9]. To estimate the caffeine efficiency, roots in such a steady state were incubated in different drug concentrations and the results are summarized in the figure. As a consequence, the concentration 0.04% (about 2 mM) of caffeine has been considered the 50%-inhibition-dose. Very low concentrations, below 0.02%, do not induced binucleate cells, while higher concentrations such as 0.08 or 0.1% show the maximum efficiency.

Caffeine-calcium antagonism. According to Paul and Goff's hypothesis, caffeine must inhibit cytokinesis by interfering with intracellular calcium, and we expected its competition to be detected easily by testing with 50%-inhibition-doses. Our results are recorded in the table, where the reduction in caffeine efficiency induced by calcium is remarkable at concentration 0.04% and negligible at 0.1% caffeine. It is logical to assume that the calcium concentration required to reverse the 0.1% caffeine solution must be too high to be compatible with in vivo assays.

Caffeine-magnesium antagonism. Similar results were obtained with magnesium nitrate, 10^{-2} M, and both cations appear to have a synergic effect (table), for the presence of both in the culture medium hardly depreses the caffeine efficiency at 0.04% to about 20% of control binucleate cell production.

On the other hand, the presence of any cation does not significantly modify the induction of binucleate cells when the roots are incubated in caffeine at 0.1%. This steady efficiency at this caffeine concentration strongly discounts the effects of cations as a general depression of the mitotic rate, for in this case the binucleate cell production at any caffeine concentration would also necessarily decline.

In short, we conclude that the effect of caffeine on cytokinesis may be modified by calcium and magnesium, especially at threshold concentrations of caffeine. We propose that both cations must play a role in plant cytokinesis, probably related with the fusion of Golgi vesicles to give rise to the new cell wall. The intracellular concentrations must be adequate under control conditions, but insufficient in the presence of caffeine at certain concentrations.

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An ammoniacal silver staining technique for mitotic chromosomes of *Triturus* (Urodela: Salamandridae)[1]

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Summary. An ammoniacal silver staining technique was applied to mitotic metaphase chromosomes of 2 species of Newts (Triturus). The method is useful for identifying nucleolar organizer regions. In addition, it reveals other sites of unknown significance.

Two procedures for a differential staining of certain chromosome regions have recently been proposed by Howell et al.[2] and Goodpasture and Bloom[3]. The first is an ammoniacal silver technique, designated AS-SAT, which stains the satellite regions of D and G group human chromosomes. The second one is a simpler ammoniacal silver staining reaction, designated Ag-AS, which stains differentially the nucleolus organizer regions (NORs) of some mammalian species. We tried both techniques on the mitotic chromosomes of Triturus to improve our knowledge of karyotypic events involved in evolution and speciation mechanisms within the genus[4].

Material and methods. 30 larvae and 5 males from Pisa and a couple from the River Sarno, near Pompeii, of Triturus vulgaris meridionalis (Boulenger, 1882) as well as 10 lar-

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