Results and discussion. During the early and middle pachytene of our amphibian, the bivalents exhibit distinct chromomeres with protruding loops. These loops are attached to axial fibres which run along the chromosome length (figure 1). The axial fibres presented globules about 80 Å in diameter. The globules' diameter in the fibres of the loops was greater, about 160 Å. Sometimes, these loop fibrils showed a double structure (figure 2). From the point of view of chromosome ultrastructure, snakes provide an important advantage: the small size of the microchromosomes, with few chromomeres, facilitates the morphological analysis (figure 3). In the snake bivalents, the globules also measured 80 Å and 160 Å, in the axial and loop fibres, respectively.

Metaphase chromosomes, somatic as well as of first meiotic division, also show loops with 170 Å globules (figure 4). The enzymatic treatments showed that the globules of the chromatin fibre were resistant to trypsin and DNase I, but the interglobule fibres were removed by DNase I. Some pachytene peripheral loops showed unfolded segments. These segments were thinner, tortuous and unstained, except for discrete globules about 80 Å, widely spaced (figure 5). During the middle pachytene, bushes of lateral fibrils attached to the loops were observed. These fibrils were sensitive to trypsin and RNase treatments (figure 6).

Our assumption that the PTA stained globules (80 Å) connected by thin fibres are similar to the 'nucleosomes' arrangement is supported by the morphology and the enzymatic results. The bigger diameter (160 Å) found in pachytene loops, as well as the double segments seen in some loops, may be explained by Henderson's model[15]. According to this investigator, during pachytene the arrangement of the double loops of each replicated homologue is one-sided. Later in diplotene, rotation occurs, displaying the single loops symmetrically at both sides. Therefore, replication during interphase would explain the double loops of pachytene. One question remains about the increased diameter (170 Å) found for single loops of mitotic and first meiotic metaphase. In these perhaps condensation by assembly of globules could play a role.

Regarding chromosome structure, the chromomeric loops we found in amphibian and snake spermatocytes are similar to the ones described for mammalian spermatocytes[11]. We could not elucidate how the loops protrude from the insertion point of the longitudinal fibril. The lateral fibrils attached to pachytene loops were presumed to be precursors of non-ribosomal RNA, as already known in other systems[10,12]. However, this eventual transcriptional activity of the loops needs further investigation. Concerning the degree of chromatin dispersion obtained by longer mild NaCl treatment, we believe it may be due to histone H1 extraction[3]. Perhaps this could explain the 15 Å of the connecting filaments in our preparations.

Interactions among beans in neighboring Faraday cages[1]

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Summary. Bean seeds, during their initial 4 h of absorption of water while in a Faraday cage, are able to interact mutually with similar absorbing beans in nearby Faraday cages. The interaction effects complementarity of response between adjacent cages to a common, fluctuating environmental factor affecting water uptake.

Day to day termite food intake[2,3] and bean-seed water uptake[4,5] reflect variation in unknown subtle atmospheric factors. The bean-seeds can adopt either of 2 complementary states, displaying either +- or --correlations with it. Placed in water in separate vessels in close proximity 2 groups of seeds can mutually induce the 2 complementary states, their concurrent rates of water uptake correlating negatively with one another. Such interaction can have great potential significance for many facets of biology. The present experiment was designed to determine whether the fields involved in these actions can pervade 0.41 mm copper Faraday cages.

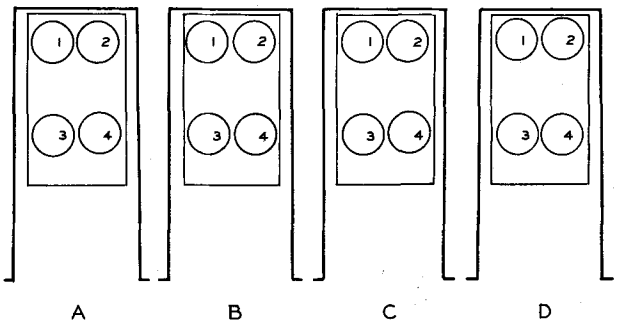

Fig. 1. The arrangement of 4 Faraday cages (*A–D*), and contained vessels (1–4) each with 20 bean-seeds in water.

4 cylindrical Faraday cages were lined up in a row with 35 cm between centers (figure 1). 16 20-bean (Phaseolus vulgaris) samples were weighed to the nearest centigram in flat (6×6 cm) aluminium-screen baskets[4]. 4 were submerged in vessels and placed in each of the successive cages at 4-min-intervals. Aluminium covers were clamped to the openings. After exactly 4 h the beans were rapidly blotted and wet-weighed, similarly at 4-min-intervals, and discarded. Water uptake, the weight difference between final wet weight and dry weight + 15 cg (= wetting) was expressed as percent weight increase of the original dry beans. Although cages A and B were grounded and C and D were not, no difference between them was noted. This experiment was performed on 73 days between 25 February and 7 June 1974 repeated using a new stock supply of beans on 49 days during 10 June through 16 August and repeated again on 42 days during 29 August through 28 October 1974.

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To eliminate day to day differences among the means in all 4 cages, the mean percentage water uptake in each group of 4 vessels was expressed as the difference from the mean in all vessels on that day. This resulted daily in 4 serially correlated values. Correlating any 2 values would be expected to yield, randomly, $r = -1/(N-1) = -0.333$. The coefficient of correlation, r, of beans in adjacent (A×B, B×C, C×D) cages was compared with that in alternate ones (A×C and B×D).

Adjacent cages correlated more negatively than random expectation, $r = -0.4086$, $N = 492$ (figure 2, on left). Alternate cages correlated less negatively, $r = -0.1379$, $N = 328$ (figure 2, on right). These experimentally obtained values were examined to learn whether they deviated significantly from random expectation. Converting the foregoing 3 r's to z's gave -0.3465 (randomness), -0.4246 ± 0.0452 (adjacents), and -0.1388 ± 0.0554 (alternates). The value of z for the alternate-cage correlation was highly significantly different from randomness, $p < 0.001$.

These findings indicate that beans in adjacent cages are mutually inducing opposite signs of response to a common atmospheric factor that influences the rate of water uptake. The consecutive order of signs within the 4 cages is + − + −, or, − + − +. While these orders account for all the foregoing results, they also predict that correlating A×D would yield a value more negative than chance. Calculating r for A×D gave -0.4314, $N = 164$. Combining the 3 adjacent-cage values with the A×D ones gave $r = -0.4148$, $N = 656$; $z = -0.4414 \pm 0.0391$, a significant deviation from randomness, $p < 0.02$.

The same experiment was also conducted at Woods Hole, Massachusetts, USA, on 50 days during 17 June through 23 August 1974. This gave for adjacents and A×D, $r = -0.3929$, $N = 200$; $z = -0.4152 \pm 0.0707$, and for alternates, $r = -0.2460$, $N = 100$; $z = -0.2511 \pm 0.1013$. The pooled results for the Evanston and Woods Hole experiments gave for the 3 adjacents and A×D, $z = -0.4353 \pm 0.0342$, $N = 856$ and for the alternates, $z = -0.1635 \pm 0.0485$, $N = 428$. These deviate significantly from randomness; $p < 0.003$ and $p < 0.001$, respectively. Correlating *all* combinations in the data, $r = -0.3318$, confirmed closely the theoretical expectation.

The results indicate that the beans in each cage are being influenced by fields generated by beans in neighboring cages. Such interactions among the seeds effect a pattern of alternating signs of response to a nonbiological field factor which, in turn, influences the rate of bean water uptake. The latter field also penetrates the cages. Through interactions the 4 bean groups have mutually organized a pattern in their water-uptake behavior.

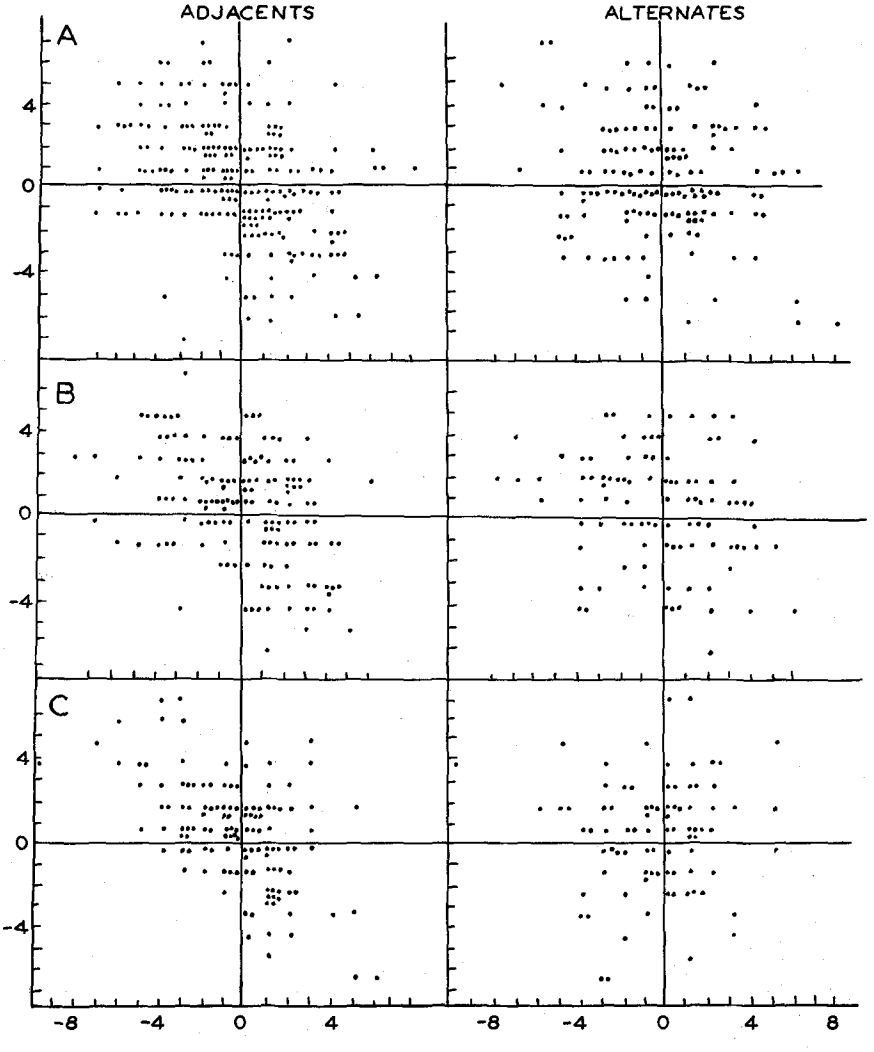

Fig. 2. Scatter-plots of the relationship in Evanston, Illinois, between daily water uptake in one cage and the concurrent rate in the next adjacent cage (on left), and the alternate cages (on right). *A* For 73 days from 25 February through 7 June 1974. *B* For 49 days from 10 June through 16 August 1974. *C* For 42 days from 29 August through 28 October 1974.

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].

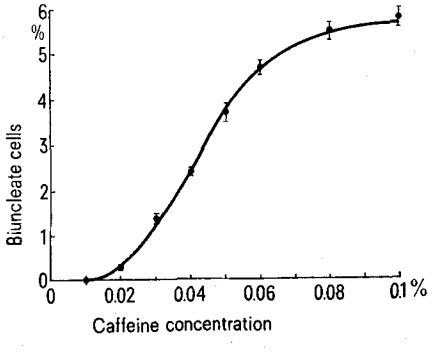
The experimental analysis of cytokinesis[10, 11] showed that, in the presence of these inhibitors, the Golgi vesicles, apparently present in similar number to those found in controls, do not form the cell plate, but disperse all over the cytoplasm. That is to say, the arrangement and fusion of Golgi 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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