(commercial hexametaphosphate) per 90 g of soil, and agitated for 8 hours in a large mechanical mixer (7 kg of soil and 36 liters of water per batch). A preliminary particle-size fractionation of the < 20-μ fraction was achieved by conventional sedimentation-decantation techniques (*6*) prior to removal of soil organic matter by hydrogen peroxide (H_2O_2) oxidation. Two 50-liter settling containers, sufficiently tall to permit a particle fall of 45 cm and a sedimentation time of about 27 minutes at 10°C, were used for this separation. The sedimentation-decantation procedure was repeated seven times. The > 20-μ sediment was then treated with 4.5 liters of 30 percent H_2O_2 and continuously mixed for 4 hours at about −6.6°C. The oxidation procedure was repeated twice prior to redispersion of the > 20-μ fraction in Calgon and final particle fractionation of the sediment as described above.

The > 20-μ total mineral fraction was dried at 110°C, and biogenetic opal was isolated from this fraction by a sink-float specific gravity technique. Approximately 500 g of this > 20-μ fraction was thoroughly mixed with 12 liters of a nitrobenzene-bromoform solution (specific gravity, 2.30). Sufficient time was allowed for opaline constituents to float to the surface of the heavy liquid from which they were removed and then purified by a centrifugation-decantation method similar to that described by Jones and Beavers (*5*). These procedures were repeated until the yield of opal reduced sharply. The opal isolate was washed thoroughly with acetone to remove the heavy liquid as an impurity.

Final preparation of opal for carbon dating consisted of treating the sample with boiling 1*N* chromic acid and cold 30 percent H_2O_2 to reduce the danger of contamination of occluded carbon with extraneous sources of soil carbon. Effectiveness of such procedures have been discussed previously (*2*). The sample was then pulverized to a fine powder by grinding it for 8 hours in a mortar with an automatic pestle. It was given a final 12-hour treatment with 6*N* HCl at room temperature to remove possible carbonate contamination. After removing excess acids from the sample with distilled water and then drying it for several days in an oven at 70 to 150 mm-Hg and 70°C, the absence of carbonates was verified by x-ray powder patterns of the treated specimen. Infrared spectra of opal samples treated in a similar manner (*2*) suggest no evidence that bromoform remained as a contaminant after this pretreatment.

A 60-g sample of opal that contained 1.30 percent carbon, or a total of about 0.75 g, was dated by the radiocarbon method by Isotopes, Inc., Westwood, New Jersey, and a carbon date (I-2277) of $13{,}300 \pm 450$ years before the present was obtained. This places the age of the opal considerably older than was anticipated and provides evidence that opal phytoliths are stable under these soil weathering conditions for at least 13,000 years. Apparently those opal phytoliths containing carbon occlusions that are resistant to the oxidizing pretreatment were deposited shortly after the close of the last glacial period in Ohio (*3*). Evidence that such opaline constituents are in fact authigenic lies in their marked decrease with depth in the profile. Similar depth distributions have been established for a number of other Ohio soils.

Additional work is underway to understand the apparent anomaly between the anticipated and obtained carbon dates. It is known that at least 50 percent of the occluded organic constituents are readily oxidizable (*2*). However, it is not known whether the oxidation reaction is of equal magnitude for all opal bodies or whether organic occlusions in some specimens (presumably more recently deposited open framework structures) are completely digested while others remain essentially inert. Upon oxidation, such a phenomenon would favor preservation of older carbon occlusions at the expense of younger ones, and thus may account in part for the older carbon date obtained. Preferential oxidation would not affect the validity of the date as an estimate of the minimum age of the valley train sediments. It would, however, preclude the use of such dates to reconstruct ecologically the major period of grass vegetation at a particular site.

At this time data are insufficient to speculate which of the above interpretations is valid. When additional information is obtained to evaluate the factors affecting radiocarbon dating of opal, this material may become a very useful C^{14} source.

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References and Notes

1. R. L. Jones and A. H. Beavers, *Soil Sci.* **96**, 375 (1963).
2. L. P. Wilding, R. E. Brown, N. Holowaychuk, *ibid.* **103**, 56 (1967).
3. R. P. Goldthwait, A. Dreimanis, J. L. Forsyth, P. F. Karrow, G. W. White, in *The Quaternary of the United States*, H. E. Wright, Jr., and D. G. Frey, Eds. (Princeton Univ. Press, Princeton, N.J., 1965), pp. 85–97.
4. R. B. Gordon, *Natural Vegetation of Ohio* (Ohio Biological Survey, Columbus 1966), map.
5. R. L. Jones and A. H. Beavers, *Soil Sci. Soc. Amer. Proc.* **28**, 413 (1964).
6. M. L. Jackson, *Soil Chemical Analysis—Advanced Course* (Univ. of Wisconsin Press, Madison, 1956), pp. 101–171.
7. Supported by State Project 371, Ohio Agr. Res. and Development Center and is journal paper No. 6-67. I thank R. E. Brown and L. R. Drees for technical assistance, and N. Holowaychuk and G. F. Hall for helpful suggestions and criticisms throughout the work.

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Osmotic Mechanism and Negative Pressure

Abstract. *When solute molecules are confined, they exert a positive pressure on the barrier. If this is simply the free solvent surface, balance of forces requires the solvent to attain an equal negative hydrostatic pressure. This offers a sufficient explanation for the reduction of the vapor pressure over a solution.*

As a result of work on mangroves and other vascular plants, osmotic relation between parenchyma cells and the xylem sap has been defined. As had been predicted, the osmotic pressure of the cells is indeed balanced by a hydrostatic tension (equivalent to negative pressure) in the nearly salt-free xylem sap. These studies have suggested a mechanism of osmosis different from what is generally postulated (*1*). In this study I neglect gravity, and denote an outward-directed force as positive and an inward-directed force as negative.

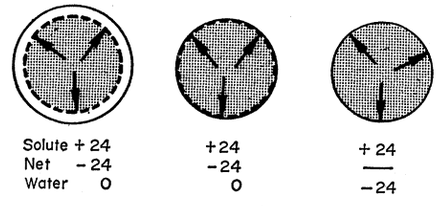

Fig. 1. (Left) Diagram of a 1 molal solution in a semipermeable net surrounded by water; (center) water removed to coincide with net; (right) net removed. Pressure in atmospheres. The arrows indicate the osmotic pressure of the solute molecules against the net or solvent boundary.

When a mole of gas fills a liter flask at 20°, it exerts a pressure of +24 atm on the bottle, which counteracts this by −24 atm. Similarly, when a mole of solute molecules is confined within a liter of water, the molecules exert a pressure of +24 atm on the retaining boundary, which becomes stressed by −24 atm. If this boundary is the free solvent surface, the stress is transmitted hydraulically from the surface throughout the solvent, giving a "partial hydrostatic solvent pressure" of −24 atm. The normal cohesiveness of the water, at least 10,000 atm (*2*), has been lowered by 24 atm.

An imaginary experiment may illustrate the point (Fig. 1). A 1 molal solution is contained in a semipermeable net which is surrounded by a shell of water (left). The net is expanded by an osmotic pressure of +24 atm which is due to the solute molecules alone, for the water slips right through the mesh. The net reacts by −24 atm. If the surrounding water is removed (center), the pressure is still on the net. Now if the net is peeled away (right), the question of where the expanding force goes arises. The solute pressure of +24 atm is now taken up by the solvent surface and hence lowers the solvent pressure from ambient value to −24 atm.

When water at 20°C is compressed to +24 atm, its vapor pressure of 17.5 mm increases by 0.31 mm (Fig. 2, A and B), according to the equation of Poynting (*3*). Similarly, when water is subjected to a tensile stress of −24 atm, for example, by an expanding noncavitating structure, the vapor pressure decreases by 0.31 mm (Fig. 2C).

A 1 molal solution also has a vapor-pressure lowering of 0.31 mm (Fig. 2D), and is empirically in osmotic equilibrium with water at −24 atm pressure (Fig. 2E).

One may therefore conclude that the solvent (water) in both compartments (Fig. 2E) is under the same hydrostatic pressure, that is, −24 atm. This negative pressure of the solvent is generated by the impinging force of the solute molecules on the solvent surface, and is hydraulically balanced by the stress on the glass wall (+24 atm) in the lower compartment. The solvent acts as a hydraulic fluid between the two compartments.

Accordingly, a multicomponent osmotic system at equilibrium has the same hydrostatic solvent pressure throughout, and this is the reason that the vapor pressure is also the same throughout. A good example is a non-transpiring mangrove where, indeed, all compartments have the same hydrostatic solvent pressure as the sea water, namely −24 atm (Fig. 2F). At osmotic imbalance, the solvent moves by a hydraulic as well as by a diffusive gradient; which of these dominates depends mainly upon permeability and convection.

When a molal solution is in osmotic equilibrium with −24 atm of water, the lowering of the vapor pressure is the same in both compartments (Fig. 2E). Yet, in the solute compartment, according to Raoult's law, the lowering is proportional to the mole fraction; whereas in the water compartment, according to Poynting's relation, it is proportional to the mole ratio (*4*). This is obviously an inconsistency.

The concept of "partial hydrostatic solvent pressure" gives a simple and sufficient explanation of the lowering of the vapor pressure over a solution. It allows for observed hydraulic flow through the membrane (*5*), and a greater movement of water through a living cell than can be afforded by diffusion alone. It does away with the contradictory postulate that osmotic equilibrium maintains a hydrostatic solvent gradient across the membrane without flow.

Osmotic pressure and matrix swelling (capillary or colloidal) are essentially produced in the same way. In either case, the water is subject to a dilative force, whether this is by a solute or by a compressed matrix, which is exerted against the surface. This is why the ensuing lowering of the vapor pressure fits (with reverse signs) both swelling pressure (*6*) and osmotic pressure, according to the relation of Poynting.

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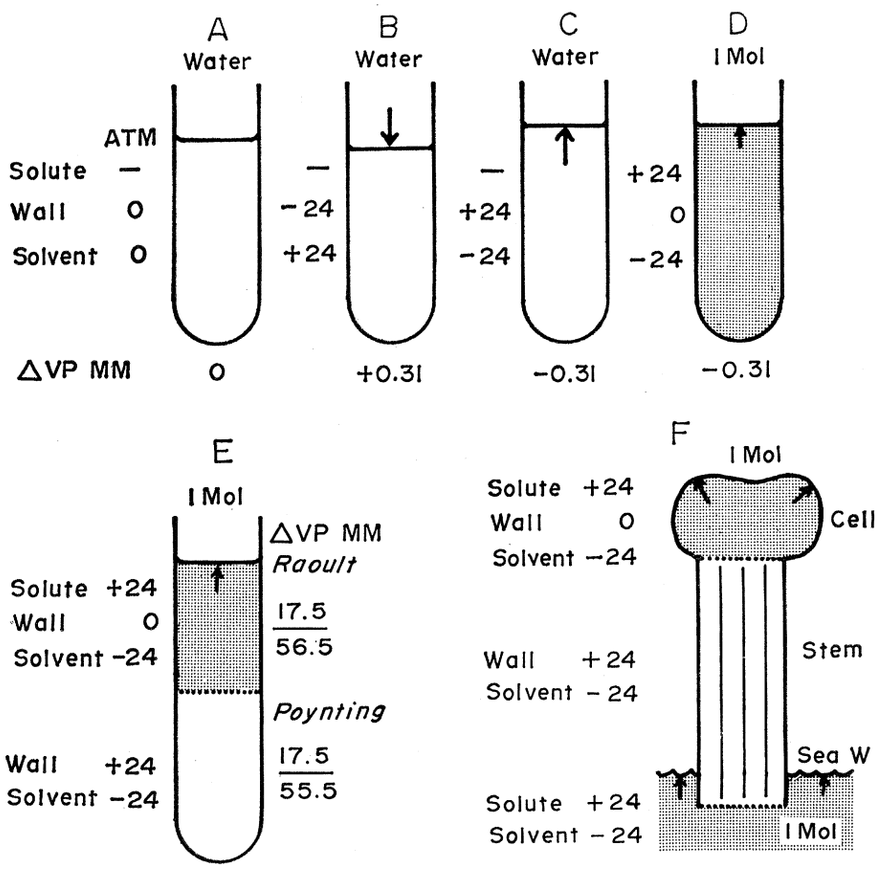

Fig. 2. Relation of solute, solvent, wall, and vapor pressure in various systems at equilibrium. (A) Water at zero gauge pressure; (B) water compressed by 24 atm; (C) water stretched by 24 atm; (D) a 1 molal solution; (E) rigid semipermeable membrane separating 1 molal solution from −24 atm of water; (F) a schematic mangrove plant separating solute-free stem water from sea water and cell solution, both of 1 molal strength. The arrows in solutions denote the osmotic pressures by the solute molecules.

References and Notes

1. P. F. Scholander, H. T. Hammel, E. D. Bradstreet. E. A. Hemmingsen, *Science* **148**, 339 (196[illegible] P. F. Scholander, *Proc. Nat. Acad. Sci. U.S.* **55**, 1407 (1966).
2. A. S. Crafts, H. B. Currier, C. R. Stocking, *Water in the Physiology of Plants* (Chronica Botanica, Waltham, Massachusetts, 1949).
3. J. H. Poynting, *Phil. Mag.* **12**, 32 (1881), quoted in G. N. Lewis and M. Randall, *Thermodynamics* (McGraw-Hill, New York, 1961).
4. The equation of Poynting reads: $dP_l \times \bar{V}_l = dP_g \times \bar{V}_g$, where P is pressure, l is liquid, g is gas phase, and $\bar{V}$ is molecular volume. At 24 atm, 20°C, we have: $24 \times 760 \times 18 = dP_g \times 24{,}000 \times 760/17.5$; $dP_g = 17.5/55.5 =$ 0.31 mm. At dP_l of 12 and 48 atm, dP_g would be, respectively, $17.5 \times 0.5/55.5$ and $17.5 \times 2/55.5$ mm. According to Raoult's law, dP_g is proportional to the mole fraction, and at 0.5, 1, and 2 moles would be $17.5 \times 0.5/56.0$, $17.5 \times 1/56.5$, and $17.5 \times 2/57.5$ mm.
5. G. Meschia and I. Stenikar, *J. Gen. Physiol.* **42**, 429 (1958).
6. H. B. Bull, *Physical Biochemistry* (Wiley, New York, 1951).

7. Supported by NSF grant GB-4259. I thank my colleagues on the "Billabong" expedition, which took the R.V. *Alpha Helix* to the Great Barrier Reefs in 1965, for stimulating discussions.

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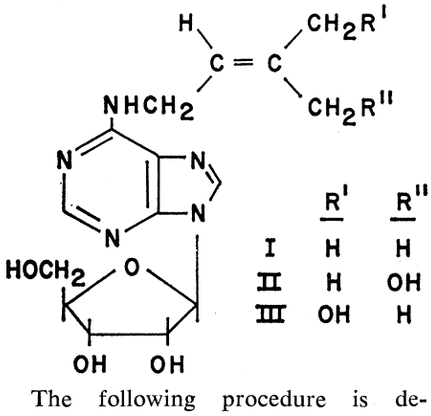
Cytokinins in the Soluble RNA of Plant Tissues

Abstract. *The cytokinin,* N^6-(Δ^2-*isopentenyl*)*adenosine occurs in the soluble RNA of yeast and mammalian tissue and has now been detected in plant soluble RNA. A hydroxylated derivative of this cytokinin 6-*(cis-*4-hydroxy-3-methylbut-2-enylamino)-9-*β*-D-*ribofuranosylpurine has also been identified as a constituent of plant soluble RNA.*

The sRNA (*1*) of yeast contains a nucleoside identified as N^6-(Δ^2-isopentenyl)adenosine (IPA) (*2–4*). This nucleoside, I, also occurs in the sRNA of several mammalian tissues (*2, 3*). It shows a selective toxicity toward various mammalian cell lines grown in culture (*5*) and possesses exceptionally high cytokinin activity (*2, 6*), that is, IPA promotes cell division, growth, and organ formation in cultured plant cells.

We have found IPA in the sRNA of spinach and garden peas. In addition, these sRNA samples and that of immature sweet corn kernels contain a hydroxylated derivative of IPA related to ribosylzeatin, III, identified as 6-(*cis*-4-hydroxy-3-methylbut-2-enylamino)-9-*β*-D-ribofuranosylpurine, II.



	R'	R''
I	H	H
II	H	OH
III	OH	H

The following procedure is described for the extraction of sRNA from corn. Frozen fresh sweet corn kernels (46 kg) were immersed in liquid nitrogen and, when thoroughly frozen at this temperature, were ground in a meat grinder. A constant stream of liquid nitrogen was poured over the corn during the grinding process. The frozen flour was warmed to 0°C and suspended in 100 liters of distilled water at 25°C, and the mixture was stirred with a high-speed stirrer until all the lumps of frozen material had disappeared (20 minutes). During this process, the solution was heated with a 1650-watt immersion heater to prevent the temperature from falling below 12°C. The suspension was allowed to stand 30 minutes at room temperature, and the supernatant was then siphoned off, filtered through muslin, and diluted with two volumes of 95 percent ethanol. The mixture was kept at 4°C for 24 hours and centrifuged at 10,000*g* for 10 minutes. The precipitate was dissolved immediately in 10 liters of 0.1*M* tris-HCl (*p*H 7.5), and the solution was centrifuged at 20,000*g* for 20 minutes. The following procedures were carried out at 4°C. The milky solution was run onto a DEAE-cellulose [Cl$^-$] column (10 by 30 cm). The column was washed with 0.1*M* tris-HCl (*p*H 7.5) until the effluent was clear and the OD at 260 mμ was less than 0.25. The material on the column was eluted with 1.0*M* sodium chloride in 0.1*M* tris-HCl (*p*H 7.5) as described by Holley (*7*). The fraction containing the RNA was diluted with three volumes of ethanol, and the mixture was kept for 24 hours at 4°C. The sRNA was recovered by centrifugation and dissolved in 500 ml of 0.1*M* tris-HCl (*p*H 7.5). The sRNA sample was chromatographed on a second column of DEAE-cellulose [Cl$^-$] (5.0 by 35 cm), with a sodium chloride gradient (0.0 → 1.0*M* NaCl) in 8 liters of 0.1*M* tris-HCl (*p*H 7.5). The fraction containing the sRNA was dialyzed for 24 hours against three changes of distilled water, and the sRNA was precipitated by addition of three volumes of cold ethanol. The recovered sRNA was washed with a mixture of ethanol and water (3:1), and then with ethanol; after being air-dried, it weighed 4.0 g. Spectrophotometric analysis (24 $OD_{260m\mu}$ units equals 1 mg) showed that the sample contained 2.0 g of RNA. This sample had a sedimentation constant $s_{20,w}$ of 2.9 (determined in the Spinco model E ultracentrifuge, schlieren optics).

The isolation of the nucleosides from sRNA was based on that devised for the isolation of IPA from yeast sRNA (*3*). In brief, the RNA was hydrolyzed by whole snake venom (*Crotalus adamanteus*) and bacterial alkaline phosphatase to its constituent nucleosides, and the nucleosides were separated by means of column partition chromatography.

The sRNA from 230 kg of corn kernels (10.05 g) was hydrolyzed, and the resultant nucleoside mixture was lyophilized and suspended in 75 ml of the lower phase of solvent E (*8*). The solution was stirred for 1 hour at room temperature, centrifuged, and mixed with 140 g of Celite 545. This mixture was packed into a glass column, 5.08 cm in diameter, containing 500 g of Celite 545 (Johns-Manville Co.) mixed with 230 ml of lower phase of solvent E (*9*). The elution pattern for this separation is shown in Fig. 1.

If any IPA were present in the sRNA hydrolyzate, it would be eluted in fraction 1 (*3*). Fraction 1 was concentrated in a vacuum to 0.5 ml, and the concentrate was chromatographed on Whatman No. 1 paper in solvent D. A sample of authentic IPA (*2*) was chromatographed on the same sheet. No IPA was detected even though as little as 10 to 15 μg was detectable. The eluate corresponding to fraction 2 was evaporated in a vacuum, and the residue was chromatographed on Whatman No. 3 MM paper in solvent D. Elution of the ultraviolet-absorbing band (R_F 0.73) with water yielded 305 $OD_{270m\mu}$ units. This material was chromatographed in solvent B, and two ultraviolet-absorbing bands were obtained, R_F 0.72 and 0.79. The slower-moving compound (182 $OD_{270m\mu}$ units) was identified as N^6,N^6-dimethyladenosine. The faster-moving compound