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*HYDROSTATIC PRESSURE AND OSMOTIC POTENTIAL IN LEAVES OF MANGROVES AND SOME OTHER PLANTS**

BY P. F. SCHOLANDER, H. T. HAMMEL,† E. A. HEMMINGSEN, AND E. D. BRADSTREET

SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA (SAN DIEGO), AND THE JOHN D. PIERCE FOUNDATION, NEW HAVEN, CONNECTICUT

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It has long been known that mangroves and other halophytes have a salt concentration in their cells which as a rule exceeds that of the sea water.[1–3] Only recently, however, has it been found that these plants run essentially fresh water in their xylem vessels.[4] They maintain, therefore, at all times a difference in osmotic potential between the sea water and xylem sap equivalent to 20–30 atm. It is generally held that the living protoplasm of plant cells is surrounded by a semipermeable membrane which separates it from the more or less rigid cellulose wall. The micellar structure of the wall is pervious to the solutes of the xylem sap, and forms a micropore "free space" extension of the vascular system.[5–9] These premises indicate equivalence between hydrostatic pressure and osmotic potential. The latter is always negative, and hence the relations can be expressed in the following simple equation:

$$HP_E + OP_E = HP_I + OP_I, \quad (1)$$

which states that the sum of hydrostatic pressure and osmotic potential of extra- and intracellular fluids is equal when equilibrium obtains. Therefore, if mangroves were to balance or exceed the osmotic potential of the sea water, one might expect a hydrostatic sap pressure of at least −20 to −30 atm. So we meet in these low plants an exaggerated version of the old problem of how sap rises in tall trees.

Various attempts at measuring high negative pressures in plants by manometric or filtration techniques have failed,[4] and when such staggering tensions as −100 atm or more are inferred, one might well ask for proof in direct hydrostatic terms. In the present study, such an approach has been applied and, indeed, revealed a negative sap pressure closely reflecting the osmotic potential of the cells.

Freezing-Point Depression and Salinity of Extra- and Intracellular Fluids.—Samples of xylem sap were obtained by standard vacuum technique,[4] and cell sap was obtained by crushing leaves held in plastic tubing between the jaws of a powerful vise. Freezing point was determined by observing microscopically the freeze-thaw equilibrium of a minute ice crystal within the stirred liquid. It was found that the pressure equivalent of the freezing point ranges from −30 to −60 atm in the cell sap, against mostly one or two atm in the xylem sap. In salt-secreting species like Avicennia the xylem sap concentration may be higher, 3–6 atm. These data confirm earlier measurements obtained by similar methods and by plasmolysis.

Experiments with External Gas Pressure.—Assume negative hydrostatic pressure in a vessel connected to a living cell (Figs. 1 and 2, left). (In the present connection only pressure deviations from the ambient are relevant and hence all hydrostatic values are expressed as gauge pressures.) Further assume that the ambient air cannot enter the system because of surface tension (indicated by tiny

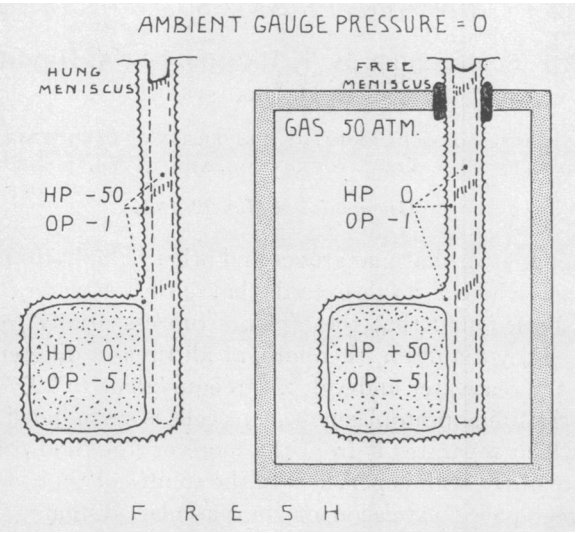

FIG. 1.—*Left:* Leaf cell with vessel. Air cannot enter the system because of surface tension (indicated as concave menisci). *HP* = hydrostatic pressure, *OP* = osmotic potential. *Right*: Balancing pressure on the same system, produced by compressed nitrogen. Notice the free meniscus at the cut end of the vessel.

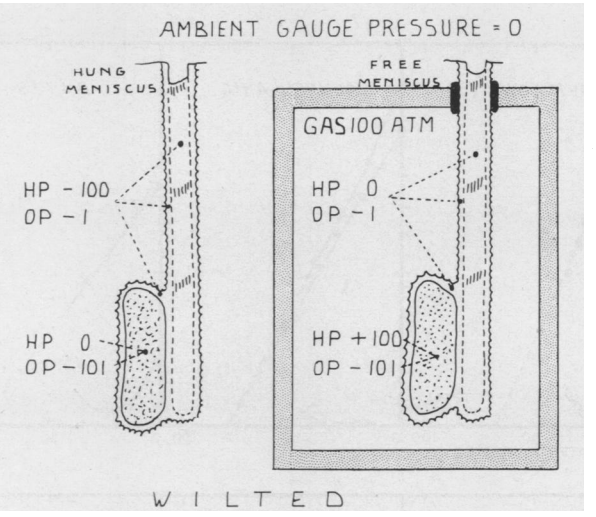

FIG. 2.—Same system as in Fig. 1, but wilted, with about half of the intracellular water extruded.

menisci), and that the entrance to the vessel is similarly closed by the pit membranes of a cross wall. The hydrostatic-osmotic equilibrium relationship will be seen from Figures 1 and 2, in which it obtains according to equation (1).

On these premises both the vessel and the micells of the cellulose walls are structurally strong enough to withstand collapse even at a very high pressure differential between the outside and the inside. The same pressure differential can be applied, however, by leaving the xylem sap at ambient pressure and raising the gas pressure around the system. Theoretically, therefore, one should be able to measure the hydrostatic pressure which existed in the vascular system prior to the cut simply by observing at which external gas pressure the system starts to yield liquid (Figs. 1 and 2, right).

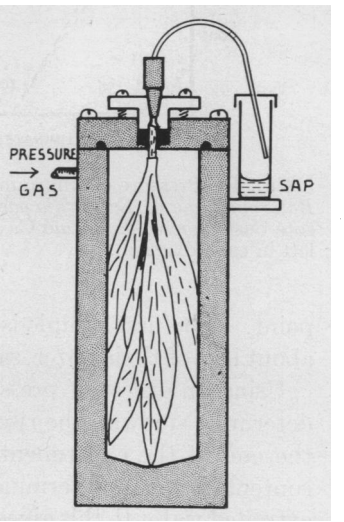

FIG. 3.—Pressure bomb with leafy twig, rubber compression gland, and plastic capillary for collection of sap.

The practical execution of such an experiment is simple (Fig. 3). A leafy shoot bared to the xylem at the cut end is fitted through a rubber compression gland in the lid of a pressure cylinder. The cut end of the twig may either protrude through the gland for microscopic examination or, as in the figure, a plastic capillary may be attached to collect the sap. When the gas (nitrogen) pressure is increased in the chamber, one usually observes a small and spotty exudation, but at a critical point the liquid comes gushing out with vigorous bubbling. In our mangroves this usually happened at 30–50 atm. If the pressure is lowered, sap does not reappear when wiped off.

It is of basic importance that the extruded fluid is close to pure water, having a salinity of only 0.1–0.01 per cent and a barely measurable depression of the freezing

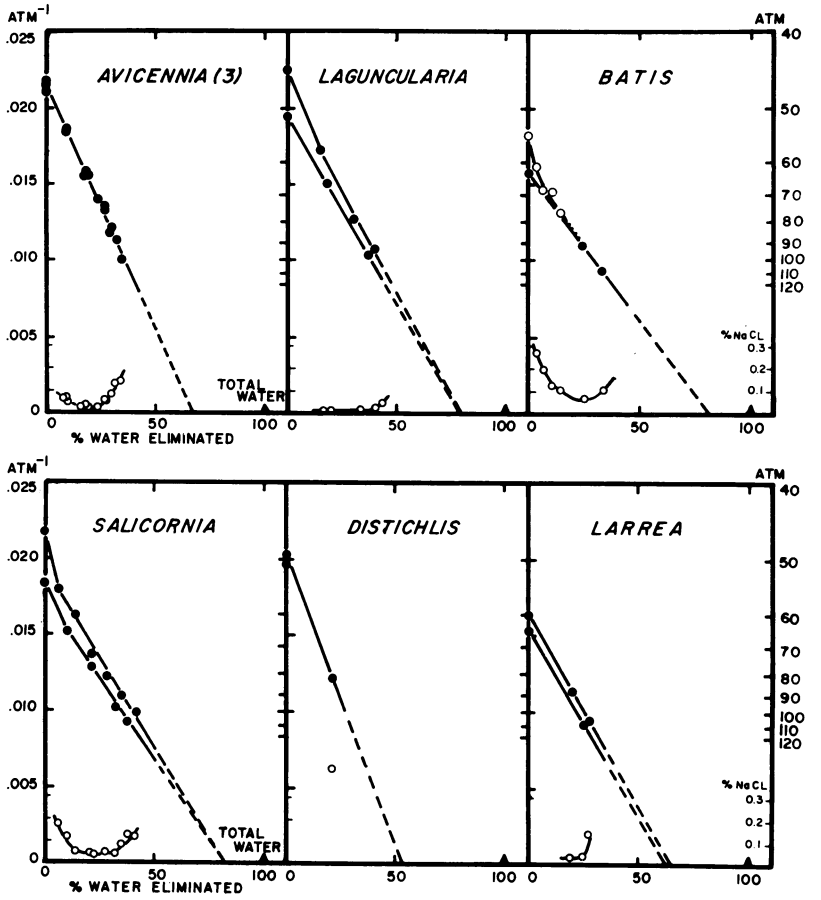

FIG. 4.—Pressure equilibrium curves in *Avicennia nitida* Jacq., *Laguncularia racemosa* Gaertn., *Batis maritima* L., *Salicornia pacifica Standley*, salt grass *Distichlis spicata* (L.) Greene, and the creosote bush, *Larrea divaricata* Cav. Salt concentration of the expressed sap is given below to the left in each diagram.

point. The first sample is usually slightly higher, and when the cells have yielded about half of their water, salinity increases and delivery fails (Fig. 4).

Using an excess of pressure (80–120 atm), sap is yielded rapidly, and one may determine stepwise the rise in equilibrium pressure as the liquid is eliminated. At the end of the experiment the twig has lost its turgor and is wilted. The water content is then determined by drying and weighing. Adding the amount of extruded water to this gives the total water content before the experiment.

The fact that water is yielded upon application of pressure means that the system either is elastic or that it suffers plastic collapse, or both. If we were dealing with fluid loss from a deforming cell through a semipermeable membrane, as in Figure 2, then (1) the extruded liquid should be plain water, and (2) the rise in intracellular solute concentration should be proportional to the rise in the equilibrium pressure, according to the following relation: $S(x - v)^{-1} = KP$; or $X - V = K_1P^{-1}$ where X is total water, V eliminated water, S solutes, and P hydrostatic pressure.

Thus, when we plot volume of eliminated water against the reciprocal of pressure, our data give a straight line where the intercept with the abscissa gives the volume of water which is being concentrated, that is, the intracellular water. As the total water is known, we may now replot the curves in terms of per cent water eliminated from the total (Fig. 4). It will be seen that the relation is linear until about half of the intracellular water is gone. Evidently, 20–30 per cent of the total water remains in a space which does not collapse. From the dry weight one may estimate that about 10 per cent of the total water is bound to cellulose and protein, leaving only 10–20 per cent free water for vessels and cell walls. Liquid extrusion by elastic deformation of vessel and cell wall is therefore at best a very small factor; in any case, it is irrelevant to the measurement of the sap pressure.

It will be seen from Figure 4 that the initial equilibrium pressure is frequently lower than that extrapolated from the rest of the curve. This, no doubt, reflects

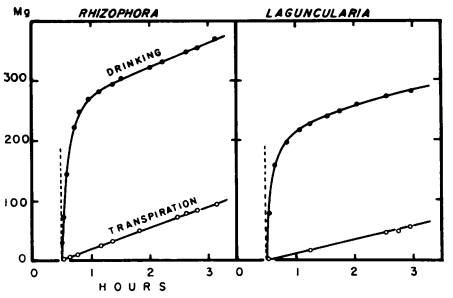

FIG. 5.—Effect of relieving negative pressure on leaves of *Rhizophora mangle* L. and *Laguncularia racemosa* Gaertn. The vertical distance between the curves is accumulation of water. Rhizophora increased its water content by 15%; Laguncularia by 20%.

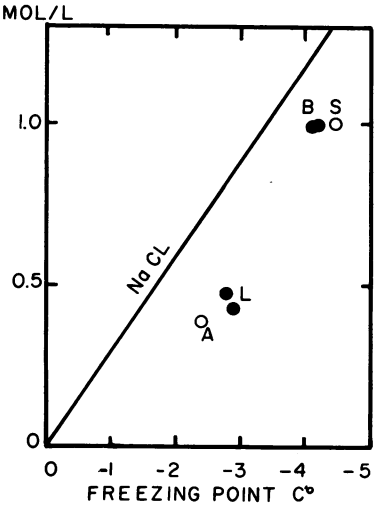

FIG. 6.—Freezing-point depression and chlorides (as NaCl) in juice pooled from several crushed leaves. $NaCl \times (NaCl + KCl)^{-1}$ = Batis 95%, Salicornia 95%.

a positive turgor pressure in the cells. The following experiment bears this out (Fig. 5). The petiole of an attached leaf is cut off under water and immediately joined with a graduated capillary filled with vacuum-extracted water. At no time is air admitted to the cut end. The assembly is placed on a balance, and the weight loss (transpiration) and drinking rates are followed. Within 15 sec one can thus follow the effect of a sudden release of the negative pressure. It will be seen that the drinking rate shoots up tenfold or more, revealing the high suction force in the leaf stalk. Within half an hour the rate slows down to match the transpiration. The leaves at this time have added 10–20 per cent water and yield sap upon the slightest pressure, reflecting a steep rise in the turgor pressure. We may hence conclude that linearity obtains only when turgor pressure is lost.

In Figure 6 is shown the relation between sodium chloride concentration and the freezing point in the juices from crushed leaves. It will be seen that solutes other

than salt account for a substantial fraction of the depression. It is necessary, therefore, to consider the freezing-point depression.

In Figure 7 the equilibration pressure at zero turgor is compared with the intracellular freezing point depression. The latter has been calculated from crush juices by referring the entire lowering back to the intracellular water volume which was revealed by the pressure curves. In the case of Batis and Salicornia, the initial sap yield is relatively concentrated with a freezing point of about −0.4°. When corrected for this also, the agreement between the no-turgor hydrostatic balancing pressure and the intracellular freezing point becomes quite close.

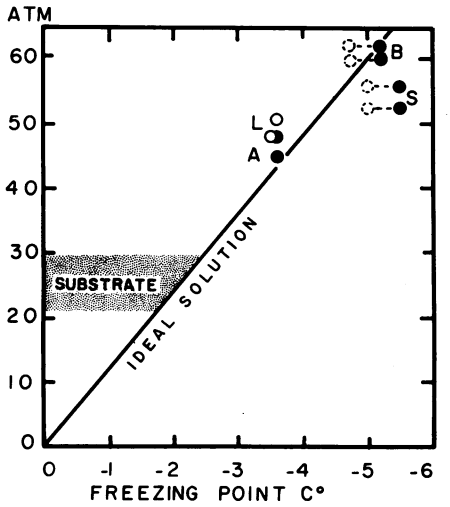

FIG. 7.—Freezing point of leaf crush juices in Avicennia (*A*), Laguncularia (*L*), Batis (*B*), and Salicornia (*S*) in relation to equilibrium pressure, extrapolated to zero turgor from Fig. 4. Freezing point corrected for dilution by extracellular space. Dotted points: additional correction because of high concentration of initial expressed fluid. Substrate concentration is based on salinity determination of sea water and of brine from habitats away from the shore line. Presumably higher salinities may be found.

Briefly, the pressure technique has revealed the following facts: (1) the expressed cell sap is almost pure water. The balancing pressure (2) measures directly the sap tension in the leaf and twig xylem; (3) it rises linearly with the intracellular solute concentration and determines the intracellular water volume; (4) it closely matches the osmotic equivalent of the intracellular freezing point when turgor pressure and xylem salt concentration are low; (5) it usually exceeds the osmotic potential of the sea water or substrate of other salinity. (6) The balancing pressure reflects a diurnal variation in the mangroves, with less severe tension at night, and can also vary quite markedly with the habitat. (7) The results were the same when performed aerobically or with 100 per cent nitrogen, and seemed not to be materially influenced by cyanide or dinitrophenol poisoning. (8) The measurements introduce the hydrostatic pressure variable directly and verify the classical concept of a semipermeable system with osmotic-hydrostatic balance in the leaves of these plants.

The approach seems valid for a large variety of plants including, for instance, the creosote bush of the desert. The measurements reveal an unexplained discordance with earlier data obtained with various filtration techniques.

This investigation was performed on an expedition to Baja, California, Feb.–March, 1964, with the R/VSpencer F. Baird from Scripps Institution of Oceanography, UCSD, sponsored by National Science Foundation grant GB 1205, and also supported in part by USPHS research grant GM 10521.

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* Contribution from the Scripps Institution of Oceanography, University of California (San Diego).

† John B. Pierce Foundation, New Haven, Connecticut.

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*ISOLATION OF THE GROWING POINT IN THE BACTERIAL CHROMOSOME**

BY PHILIP C. HANAWALT AND DAN S. RAY

BIOPHYSICS LABORATORY, STANFORD UNIVERSITY

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Current ideas and evidence concerning the replication of the bacterial chromosome can be summarized as follows: (1) The chromosome consists of one piece of *double-stranded* DNA[1] (mol wt 2×10^9) which may exist as a closed circle.[1, 2] (2) It replicates *sequentially* from a starting point[3, 4] by forming a single growing point at a fork which moves along the structure.[1, 5] (3) Protein and/or RNA synthesis is required to initiate replication of the chromosome but the cycle can then be completed under conditions of protein synthesis inhibition.[6–8] (4) Replication is *semiconservative* and involves the separation of parental DNA strands "near" the growing point as complementary daughter strands are formed.[9–12]

In the autoradiographic analysis by Cairns,[1] the entire replicating chromosome was observed, but the linear grain density in the photographic emulsion (approximately one exposed grain per micron) limited the resolution of the growing point region to that of a 2×10^6 molecular weight segment of DNA. Preparatory to molecular characterization of the growing point region we have developed a procedure for the isolation of chromosome fragments containing this region, and we have defined some of the necessary conditions for the observation of these partially replicated DNA units.

Materials and Methods.—The thymine-requiring *E. coli* strain TAU-bar[13] was cultured aerobically in a glucose-salts synthetic medium at 37°C with required supplements as previously described.[6, 13] Exponential growth (mean generation time, 40 min) was maintained by periodic dilution into prewarmed medium to keep the cell concentration between 5×10^7 and 2×10^8 cells/ml.