cant rise in the intracellular concentration of Ca^{2+} results from repetitive activity in the presynaptic terminal of the giant synapse even when bathed in normal seawater (*12*). However, the time course of that rise is probably several orders of magnitude slower than that of the events responsible for the release of transmitter. This suggests that if a rapid increase in $[Ca^{2+}]_i$ is responsible for transmitter release, it does not take place throughout the space occupied by the injected aequorin. If such a rise occurred in the bulk of the aequorin space, one might expect a rapid flash of greater intensity than the slow rise in luminescence actually recorded, since the increase in $[Ca^{2+}]_i$ which produced the slow change in luminescence did not result in or interfere with synaptic transmission. The possibility may be considered that a fast calcium transient did occur throughout the aequorin space, but that it was too rapid to be detected by the aequorin reaction, which does not precisely follow very rapid changes in calcium concentration (*13*). This seems unlikely, however, since some luminescence is detectable within the first millisecond after aequorin is exposed to calcium (*13*), and it is improbable that any calcium transient produced by the action potential would be substantially briefer than the action potential itself (Fig. 2).

Although we found no direct indication of rapid calcium transients synchronous with individual synaptic events, we believe that our results are consistent with the hypothesis that a transient increase in calcium concentration somewhere in the axoplasm of the presynaptic terminal is a significant step in the release phenomenon. This belief is based on the fact that our experiments did demonstrate a rise in $[Ca^{2+}]_i$ associated with repetitive activation of the presynaptic terminal, suggesting the flux of free calcium into the axoplasm. Our inability to detect rapid calcium transients associated with the individual action potentials might well result from the restriction of these transients to a small portion of the axoplasm of the presynaptic terminal. At least two likely mechanisms could produce such a restriction. First, the axoplasm might contain a calcium buffering system capable of confining large calcium transients to a very narrow zone of axoplasm in the immediate vicinity of the plasma membrane. (Such a system would ensure that the calcium transient generated by an action potential was terminated rapidly.) Mitochondria bind calcium in large amounts and might represent at least one component of such an intracellular calcium sequestering system (*5, 14*). In the presence of such a system the calcium concentration detected by the bulk of the intracellular aequorin would be that in equilibrium with the buffering system, and changes in that concentration would reflect changes in the degree of saturation of the buffer capacity. In a well-buffered system these changes would be slow and relatively slight except in the immediate vicinity of the site of calcium entry.

A second possible factor that might contribute to the difficulty in detecting rapid calcium transients is the small diameter of the terminal digits relative to their length (considerably smaller than indicated in the schematic diagram of Fig. 1). If rapid calcium transients were more pronounced in the digits than elsewhere (as they might well be, if for no other reason than the greater surface to volume ratio), the aequorin in the digits might be discharged relatively early. Unspent aequorin would diffuse down the long narrow digits relatively slowly, with the result that the rapid transients would go undetected. Further experiments are required to test these possibilities.

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9. In an ancillary study in the squid giant axon, we found this to be true also under our experimental conditions, which differed from those of Baker *et al.* (*5*) primarily in that a different species of squid was used, and the concentrations of active aequorin that we injected were two orders of magnitude higher.
10. The much more rapid consumption of aequorin in the presynaptic terminal is presumptive evidence that the resting $[Ca^{2+}]_i$ is considerably higher than in the giant axon. This conclusion requires the assumption that other conditions influencing the rate of the luminescent reaction (of which $[Mg^{2+}]$ is the most important one known) are similar in the two fibers.
11. When stimulation at 100 per second was maintained for much over 3 seconds, there was in most cases a large reduction and on occasions an apparent blockade of the excitatory postsynaptic potentials (EPSP's) (Fig. 2C), whether or not aequorin had been injected. The repetitive stimulation was interrupted for the periods described above in order to have effective EPSP's recorded throughout the period of stimulation.
12. The very unlikely possibility that the increase in luminescence is due to liberation of aequorin into the extracellular medium by the synaptic release process is ruled out by the slow decay of the luminescence change after the end of stimulation.
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Ascent of Sap in Trees

Abstract. *Experimental results concerning the ascent of sap in the xylem are usually interpreted in terms of gradients of hydrostatic pressure in the xylem conduits. In this report an alternative model is proposed that is equally consistent with the experimental results: under static conditions the water column is supported by a gradient in the chemical activity of the water, and the hydrostatic pressure is constant throughout. Observations that support the new model are cited, and experiments are suggested that would permit a choice between the two models.*

Many definitive experiments concerning the ascent of water through the xylem have been performed. Colloidal metal particles and large organic dye molecules (*1*, p. 182) are carried by water ascending the column, which shows that the water does not pass through semipermeable membranes. Samples of xylem water removed from the xylem conduits during the growing period have very low concentrations of inorganic and organic solutes although

some photosynthetic products are present. Xylem conduits that have been treated with cell-killing picric acid or copper sulfate solutions conduct water (*1*, p. 186) as well as untreated xylem conduits, so that fully differentiated xylem cells need not be alive to function. The observed rates of transport of water, the dimensions of xylem conduits, and the viscosities and diffusivities have been compared and establish that the transport is by hydrodynamic flow and is not diffusion controlled (*1*, pp. 190–199).

These facts lead one to a model of the xylem as simple channels through which hydrodynamic flow takes place; however, columns of water supported by atmospheric pressure in the earth's gravitational field have pressure gradients within them and can be only 10.3 m high if the pressure at the top is to stay above the vapor pressure of water. Since trees grow higher than this, it has been assumed that the water is under tension, that is, negative hydrostatic pressures. The gradient of pressure in a water column is about -0.1 atm/m, and a column of water in a redwood 100 m high would require a pressure at the top of about -9 atm. It has been demonstrated that carefully prepared water can sustain tensions well in excess of this, and it has been shown that transpiring leaves can support columns of specially prepared water and mercury under hydrostatic tension (*1*, p. 187). These experiments, of course, show only the plausibility of the negative hydrostatic pressure model of the xylem in trees.

It was not until the experiments by Scholander *et al.* (*2*) that the existence of negative pressures in trees was thought to have been demonstrated. In these experiments a specimen, such as a twig with leaves from a particular height on a tree, was placed in a gas-filled bomb with the twig and cut xylem conduits protruding through a gas-tight seal. The gas pressure in the bomb was increased, and the pressure at which water was observed to exude from the xylem was recorded. It was found that the pressure which caused water to appear at the end of the cut xylem increased as the specimens were cut from increasing heights on a tree, at the rate of about $+0.1$ atm/m. It has been assumed that the observed gradient of $+0.1$ atm/m is required to balance a gradient in hydrostatic pressure of -0.1 atm/m present in the growing plant. (In this assumption the relaxation that occurs at the time of cutting and the compression that occurs under gas pressure are not taken into consideration.)

We suggest an alternative interpretation based on the idea that Scholander *et al.* measured the gradient of water activity in the xylem conduits rather than the gradient of hydrostatic pressure, and we propose a mechanism by which the xylem could create that gradient.

The expression for the total chemical potential of water, in vertical columns such as those in the xylem, contains terms due to gravitational potential, pressure, and activity. If it is assumed that the water is incompressible

$$\mu_{H_2O(l)} = \mu^\circ_{H_2O(l)} + Mgz + \overline{V}_1[P(z) - 1] + RT\ln a(z)$$

where M is the molecular weight, g is the gravitational constant, z is the height above that chosen for standard conditions, $\overline{V}_1$ is the molar volume, P is the pressure in atmospheres, a is the activity, R is the gas constant, and T is the temperature. There are several interesting features of columns in which the functional dependence of $a(z)$ is such that $RT\ln a(z)$ exactly cancels Mgz (*3*). Under these conditions the hydrostatic pressure P is constant throughout the column, the transport of water is hydrodynamic and not diffusion controlled, and the column is supported by whatever mechanisms constrain the activity gradient to its particular form.

One way of constraining the activity gradient is by dividing the column into segments in which solutes of different concentrations are separated by semipermeable membranes. This mechanism is not in accord with experimental observations of the xylem. A second method of constraining the activity gradient is by having the xylem cells contain filamentary monomolecular chains attached to the walls; the chains serve the same function as solute but are held in a particular region of the xylem and resist displacement during hydrodynamic flow of solvent. We think it likely that this is the primary support mechanism of static water columns in the xylem. A third and a fourth method of constraining the activity gradient are applicable only under conditions of transport. Dynamic balance of the gravitational potential with an activity gradient while the pressure is kept relatively constant may be achieved in a flowing column by either injection of a solute-rich solvent or extraction of solvent through semipermeable membranes. The first of these may help to provide dynamic balance in rapidly transpiring trees. This seems likely because the solute-rich phloem adjoining the xylem can serve as a reservoir, and a pressure below atmospheric in the xylem might cause flow and injection into the xylem system.

We suggest that the xylem conduit contains a filamentous gel-like structure having a concentration gradient sufficient to support the column without pressure gradients. Flow through the column could produce pressures different from 1 atm, but these can be compensated for, at least to some extent, by injection of solute. The experimental facts concerning transport, the composition of xylem water, and the absence of membranes are all consistent with this hypothesis.

According to this hypothesis, the experiments of Scholander *et al.* would be interpreted as a syneresis effect. We suppose that their experiments measure the pressure required to squeeze water out of the gel structure of the xylem. In the measurement of the gel swelling pressure, the applied pressure π is a direct measure of the activity of water in the gel

$$\overline{V}_1\pi = RT\ln a$$

If the gradient of activity is just the correct value to balance the gravitational potential, then the gradient in applied pressure will be $+0.10$ atm/m, which Scholander *et al.* observed.

We can estimate the gradient in gel concentration in the liquid volume of the xylem cells required to produce the gradient in water activity (and hence a gradient in syneresis pressure) that will support the column. If ϕ_x represents the number of degrees of freedom of a segment in the gel chain and $[x]$ the concentration of these segments in moles per liter, the syneresis pressure is

$$\pi_s = \frac{[x]\phi_x}{3}RT$$

If the segments consist of β-1,4–linked glucose residues of molecular weight 160, and if each residue has 2 degrees of freedom, then the concentration of the gel in the solution is about 1 weight percent for each atmosphere of syneresis pressure. Thus, the gradient of concentration is about 0.1 percent per meter of height. At a height of 10 m only 1 percent of the cell contents would be gel. It should be noted that this gel would not be removable by expulsion of the sap,

rather it would be left behind as a mat or amorphous material on the cell walls. The xylem of a tree at 100 m of height would contain about 10 percent of this gel and would be more readily studied. This calculation is only a first approximation. It is likely that the freedom of motion of the segments is overestimated, which causes the weight percent to be underestimated. Neglect of the low-frequency lattice vibrations of the chains and of the lower-frequency vibrations within the individual segments causes the weight percent to be overestimated.

The gel structure which we propose may be a residue of the last stages of cellulose microfibril synthesis, at the cell walls, and a characteristic of fully differentiated tracheid or vessel cells in the xylem. It is not clear at this time whether the fibril synthesis in such cells occurs at the ends of fibrils, with individual glucose residues being attached to the ends, or whether it occurs by formation of chains that extend out into the solution and then condense into fibrils bonded together by hydrogen bonding and dispersion forces.

It is attractive to think in accord with our hypothesis that the growth is by chains and that the ends are left dangling in the fully differentiated tracheid cells when growth stops. There is some evidence that this may be the case. A number of electron microscopy studies have shown that there are amorphous residues—slimelike or matlike desposits on the cell walls (*4*). These can be eliminated by thoroughly washing the cells before examination.

A second bit of evidence for the existence of the gel is available. The gel-like structure would be expected to be very fragile and to break off after prolonged functioning of the tracheid conduits, much as straw breaks off and is carried downstream when a wheat field is flooded. It is found that in mature tracheids there are encrustations on the tori (*5*), which may result from the gradual erosion of a very delicate structure.

The hypothesis that a gel structure supports the water columns in the xylem is in accord with the existing experimental facts and is an attractive alternative to the hydrostatic tension hypothesis. Studies of the activity of water in the xylem and measurements of the concentration of gel in the xylem will determine if it is to be preferred.

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Peyote Alkaloids: Identification in the Mexican Cactus Pelecyphora aselliformis Ehrenberg

Abstract. *Hordenine, anhalidine, pellotine, 3-demethyltrichocereine, mescaline, 3,4-dimethoxy-β-phenethylamine, and the* N*-monomethyl derivatives of mescaline and 3,4-dimethoxy-β-phenethylamine have been isolated or identified, or both, in alkaloid extracts of a Mexican "peyote" cactus,* Pelecyphora aselliformis *Ehrenberg. This is the first report of the occurrence of some of these alkaloids, including mescaline, in a North American cactus other than* Lophophora.

Lophophora williamsii (Lemaire) Coulter, the peyote cactus, contains many alkaloids including mescaline (3,4,5 - trimethoxy - β - phenethylamine) and has a history of use by the Indians of North America as a medicine and a hallucinogen (*1*, *2*). Many other North American cacti have also been recorded as primitive medicines in anthropological and ethnobotanical reports (*3*, *4*). Of particular interest to us are several cactus species that are also called "peyote" by the Indians and have reputed uses as stimulants, inebriants, narcotics, or hallucinogens. Schultes has repeatedly suggested that these additional "peyote" cacti be examined phytochemically (*5*). In our previous work we have followed these leads, and our investigations have resulted in the identification of several new and several previously known cactus alkaloids (*6*).

Pelecyphora aselliformis Ehrenberg, from the state of San Luis Potosí, Mexico (*7*), is one of these "peyote" cacti. Its oddly flattened tubercles (see Fig. 1) have given rise to the common name "hatchet cactus"; the additional common names of "peyote" and "peyotillo" (little "peyote") may refer to some slight morphological similarity to *Lophophora* or perhaps to similar physiological effects (*8*). Several authors have referred to its sale and use as a drug among the Mexican Indians (*4*, *5*, *8*–*10*). Reko (*10*) has reported that the plant appears to contain toxic alkaloids, and recently, as our current investigation was being concluded, Agurell *et al.* (*11*) reported the identification of anhalidine (2-methyl-6,7-dimethoxy-8-hydroxy-1,2,3,4-tetrahydroisoquinoline) and hordenine (*N*,*N*-dimethyltyramine) and the detection of additional unknown alkaloids in the plant. In this report we present the results of further analyses of the alkaloid content of the plant in an attempt to explain its reputed physiological activity.

Approximately 300 fresh *P. aselliformis* (*12*) were sliced, dried (62 percent moisture), and pulverized in a Wiley mill to yield 5.5 kg of powdered material which was de-fatted with petroleum ether in large Soxhlet extractors. The de-fatted plant material was made alkaline and extracted by means of chloroformic percolation, and the extract was purified and resolved into phenolic and nonphenolic portions as previously described (*13*, *14*).

The nonphenolic extract was applied to four 1-mm plates of silica gel preparative F_{254} for preparative thin-layer chromatography (TLC) (*15*) and developed in a mixture of chloroform, ethanol, and concentrated ammonium hydroxide (15:20:1). Two of the nine bands observed under short-wavelength ultraviolet light, after removal, elution, and analytical TLC, showed components giving R_F values and color reactions (*14*) similar to those of mescaline and some of its analogs. The analytical TLC was carried out on 0.25-mm silica gel G plates in mixtures of either ethyl acetate, methanol, and ammonium hydroxide (17:2:1); chloroform, methanol, and concentrated ammonium hydroxide (80:20:1); or chloroform, acetone, and concentrated ammonium hydroxide (10:8:1). We could find no TLC system capable of separating mescaline from 3,4-dimethoxy-