

Chapter 4

Pressure Probe

The pressure probe is the only instrument that can measure the water status of single cells. It consists of a transducer that monitors the pressure in an oil-filled microcapillary whose microscopic tip is inserted into a cell (Fig. 4.1A). The pressure necessary to prevent cytoplasm from entering the microcapillary equals the turgor pressure of the cell.

The probe can be used with isolated cells and with cells in a tissue. It gives data not only on the pressure but also on the elasticity of the wall, the hydraulic conductivity of the plasmalemma, and the selectivity of the plasmalemma for solute and water. The microcapillary can be used to collect a small sample of cell solution for later analysis or to inject solutions into cells.

The probe was initially developed for large cells of algae such as *Valonia* or *Nitella* (Steudle and Zimmermann, 1971) and it had large dimensions to allow various characteristics of the cells to be explored. A miniaturized version subsequently was developed for small cells using the principles of the larger instrument and is used for cells in tissues of multicellular plants (Hüsken *et al.*, 1978). The probe is particularly suited for detailed studies of the pressure-related properties of cells. For measuring the overall water status of the tissue, the psychrometer and pressure chamber require fewer measurements and are more suitable (see Chaps. 2 and 3). There is no commercial manufacturer of pressure probes. To help you make your own, some detailed drawings and addresses for equipment suppliers are given in Appendix 4.1.

Principles of the Method

Plant cells generally contain cytoplasm with a solute concentration higher than in the external solution, and water enters osmotically and stretches the wall. The resistance to stretching causes the wall to press on the cell contents and to establish a positive

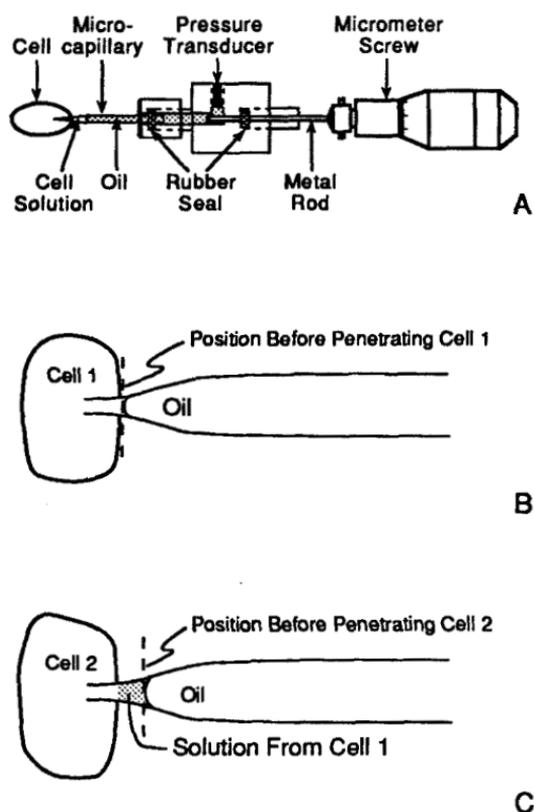


Figure 4.1. Diagram of a pressure probe (A) and position of cell solution during the measurement of turgor pressure (B and C). A) Pressure probe showing microcapillary, pressure transducer, metal rod, and oil-filled spaces (shaded). After inserting the microcapillary tip in a cell, a meniscus forms between the cell solution and the oil in the microcapillary and is observed with a microscope. The position of the meniscus is controlled by moving the metal rod in or out which raises or lowers the pressure in the oil. Turgor is measured by returning the meniscus to its position before entering the cell. B) Turgor measurement when the microcapillary is filled only with oil before entering cell. The meniscus is returned to the cell surface. C) Turgor measurement when the microcapillary already contains some solution from cell 1 (shaded) before entering cell 2. The meniscus is returned to its position before entering cell 2.

pressure, termed the turgor pressure or sometimes simply the turgor.¹ If the wall is highly resistant to stretching, a small amount of water entry causes a high pressure to develop. If the wall is easily stretched, the same amount of water entry causes only a small pressure. The wall thus controls the volume and pressure relations of the cell.

In an osmometer consisting of a rigid membrane separating a solution from pure water, a similar pressure develops that at its maximum is termed the osmotic pressure. However, the effect of solutes on water is most usefully expressed as an osmotic potential having pressure units. The pressure units indicate the maximum pressure that can be generated from the osmotic potential when a membrane is present but the osmotic potential is a fundamental property of the solution indicating the concentration, or more precisely, the activity of water in the solution. The osmotic potential exists whether or not a pressure is present.

It should be noted that pressures in cells are measured relative to the atmospheric pressure. They are thus differential or "gauge" pressures, and often are not at the maximum. Turgor pressures can vary from zero to pressures substantially above atmospheric, and tensions can develop that are below atmospheric. The developing pressures are determined to a large degree by membrane properties in the cell. In general, the membranes exclude solute and allow only water to pass through, creating a net water movement to the side with the lowest concentration of water (highest concentration of solute). If a membrane does not exclude solute, both solute and water move and there is essentially no net flow of molecules across the membrane. This lack of movement occurs despite the concentrated solution on one side of the membrane and pure water on the other. No pressure builds up. The membrane conductivity for water but selectivity against solute are thus the keys to the build up of cell pressure and volume generally.

¹Pfeffer (1900) originally defined the turgor to be the stretch-induced strain in cell walls, and the turgor pressure to be the resulting pressure. Turgor pressure continues to refer to the pressure but recent usage of the term turgor has broadened to include either wall strain (turgidity) or the resulting pressure.

The pressure probe measures the pressure in the cell by puncturing the cell wall and plasmalemma with a sharpened tip of a microcapillary and measuring the pressure inside the microcapillary (Fig. 4.1A). When the tip enters the cell, the pressure in the cell pushes cytoplasm into the oil-filled microcapillary. The cytoplasm is pushed back into the cell with a metal rod that is forced into the oil, raising its pressure. As the pressure in the microcapillary rises, the cytoplasm is completely returned to the cell and the cytoplasm/oil meniscus is at its original position before puncturing. The pressure in the microcapillary is then the same as the pressure in the cell and is indicated by a pressure transducer.

The method relies on pushing the cytoplasmic solution back into the cell because the entry of a significant volume of cell solution into the microcapillary changes the volume and pressure in the cell, and an accurate turgor pressure for an intact cell can be determined only when there is no volume change. For a microcapillary completely filled with oil before puncturing, the meniscus is returned to the cell surface (Fig. 4.1B). However, if solution from previously punctured cells is already in the microcapillary, the meniscus is returned only to its original position before puncture (Fig. 4.1C).

Note that the pressure in the cell is measured by creating an opposing pressure in the oil of the microcapillary. The opposing pressure is the measured variable. Therefore, the measurement should be made at equilibrium where there is no significant flow in or out of the microcapillary or the tip should be large enough to allow flow without a significant resistance to ensure that the pressures are the same in the cell and the microcapillary.

Pressure Probe Theory

Figure 4.1A shows that the volume of the oil-filled compartment is small in the pressure probe to keep volume changes small and to minimize effects of temperature variation and apparatus elasticity on the pressure measurement. Designs with remote controls needing large oil volumes or large pressure transducers should be avoided. Rubber seals prevent leakage but allow movement of a metal rod and replacement of the microcapillary. The metal rod is screw driven and moves inward to increase the pressure and outward to

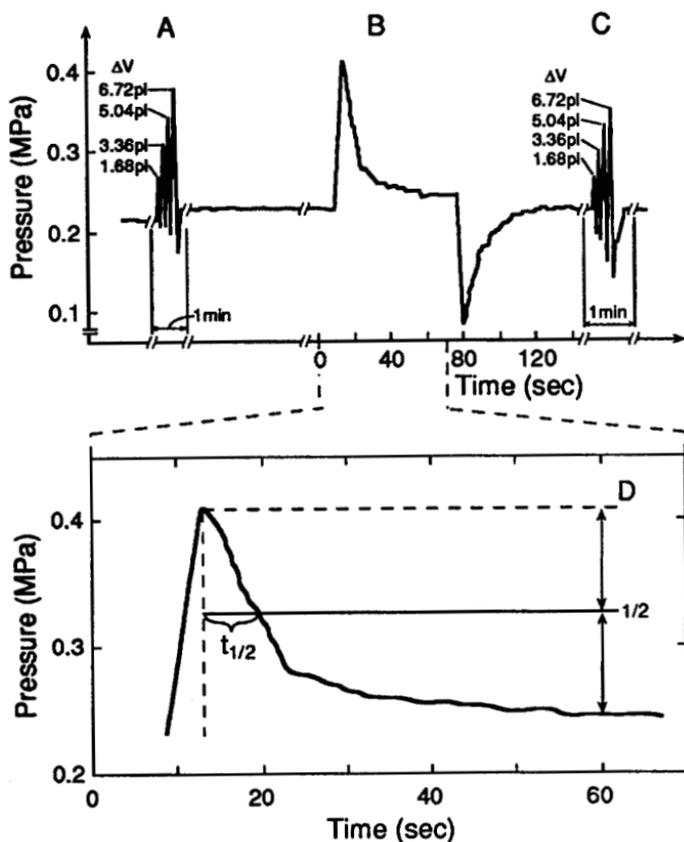


Figure 4.2. Turgor pressure when cell solution is injected into or removed from a *Tradescantia* subsidiary cell (next to guard cell in epidermis). A) Rapid injection and removal of cell solution to measure the turgor change for a known volume change ($d\Psi_{p(p)}/dV$). B) Rapid injection of cell solution and return of turgor toward original value as water flows out of the cell. Also shown is the reverse when cell solution is rapidly removed and water reenters the cell. C) Repeat of A. D) Expanded view of B showing the $t_{1/2}$ for water flow out of the cell. The $t_{1/2}$ is the time for half of the turgor to be lost and is used in calculating the hydraulic conductivity of the plasmalemma/cell wall (see Eq. 4.9). Adapted from Zimmermann *et al.* (1980).

decrease the pressure on the oil. The position of the oil/solution meniscus is observed under a stereomicroscope at a magnification of around 80X.

One of the most interesting aspects of osmosis is that water moves hydraulically through membranes and water diffusion plays only a negligible role. As a consequence, raising or lowering the cell pressure with a pressure probe causes an immediate hydraulically driven water flow out of or into the cell, which allows the probe to be used not only to measure pressure but also the nature of these flows. Figure 4.2B shows the immediate response of cell turgor pressure to pressure changes caused by the probe and the relaxation back toward the original pressure as water flows out of or into the cell in response (Zimmermann *et al.*, 1980). Such a behavior indicates that flow is hydraulically driven rather than diffusion driven because changing the pressure immediately changes the flow without altering the concentration of water. For this reason it is difficult to study water flow with isotopes of water or other molecular tracers that move by diffusion according to concentration differences. Hydraulically driven flow is also observed with pressure chambers.

As in Chap. 2, we will consider cells to have two compartments, the cell interior or protoplast and the cell exterior or apoplast (Fig. 2.3). In each compartment, particular components act on water and, for the protoplast, solute and pressure components are the main ones:

$$\Psi_{w(p)} = \Psi_{s(p)} + \Psi_{p(p)}. \quad (4.1)$$

For the apoplast, cell wall matrix and solute components are the main ones (the external pressure is atmospheric):

$$\Psi_{w(a)} = \Psi_{s(a)} + \Psi_{m(a)}. \quad (4.2)$$

The matric potential is mostly a negative pressure (tension) in the capillaries of the wall and associated vascular supply (xylem, see Fig. 2.3).

Under many conditions, the water potential is so similar on the inside and outside of the cell that they may be considered to be essentially equal:

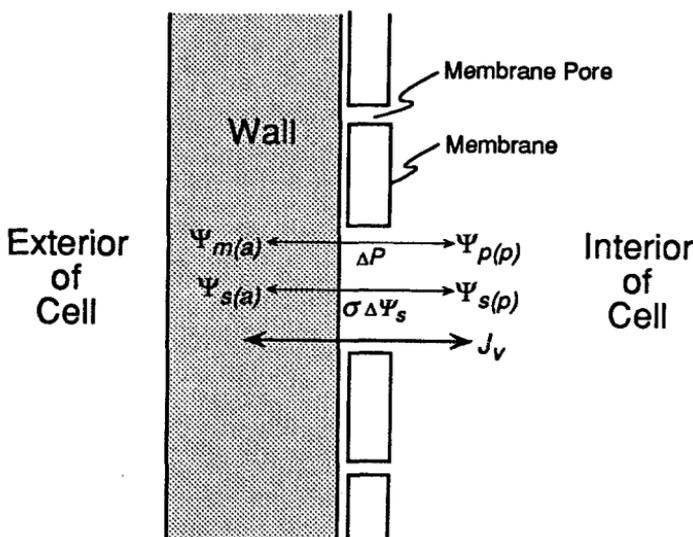


Figure 4.3. Diagram of the forces across the plasmalemma leading to water flow in cells in a tissue. The difference in pressure ΔP is determined by the turgor pressure inside and the matric potential (usually a tension) on the outside. The difference in osmotic potential $\Delta\Psi_s$ is expressed across the membrane according to the membrane reflectivity for solute shown as the reflection coefficient σ which can vary from 0 to 1. A membrane reflecting all solute and allowing only water to pass has a $\sigma = 1$ and allows all $\Delta\Psi_s$ to express itself. A membrane reflecting no solute has a $\sigma = 0$ and none of $\Delta\Psi_s$ will express itself. A cell with the latter membrane does not take up water despite a large concentration difference across the membrane. A net flow of molecules J_v results from $\sigma\Delta\Psi_s$ and ΔP .

$$\Psi_{w(a)} = \Psi_{w(p)} \quad (4.3)$$

and it becomes clear that the water potential inside the cell is balanced outside by any solute effects and matric forces.

The components outside differ according to where the cell is located. If a cell is in a tissue, solutes can be present outside (low $\Psi_{s(a)}$) together with a tension (low $\Psi_{m(a)}$) and the tensions extend to the water in the xylem and out into the soil. On the other hand, if a cell is not in a tissue and instead is directly surrounded by external solution, the wall

is saturated with water and no tension exists (the pressure is atmospheric) and only $\Psi_{s(a)}$ contributes. Equation 4.3 then simplifies to

$$\Psi_{w(a)} = \Psi_{s(a)}. \quad (4.4)$$

Caution needs to be used when interpreting changes in water potential or turgor measured with a pressure probe because of this distinction. The pressure probe is alone among the methods of this book in measuring the water status of the cell interior only. No information is given about conditions in the wall. Thus, when the turgor decreases inside, the cause cannot be attributed to external solutes unless the cell is surrounded by external solution at atmospheric pressure.

Water moves into and through the plant because of water potential differences or differences in a component of the water potential. When a potential difference exists across the plasmalemma, water moves at a rate determined by the conductivity of the plasmalemma and the size of the potential difference. Using the potentials of Eqs. 4.1 and 4.2 for the protoplast and apoplast (Fig. 4.3), the water movement can be described by the transport equation

$$J_v = Lp(\Psi_{m(a)} - \Psi_{p(p)} + \sigma(\Psi_{s(a)} - \Psi_{s(p)})), \quad (4.5)$$

where J_v is the steady rate of volume movement (mostly water) across the membrane per unit membrane area $(dV/dt)(1/A)$ and has units of $m^3 \cdot m^{-2} \cdot sec^{-1}$, Lp is the hydraulic conductivity of the membrane ($m \cdot sec^{-1} \cdot MPa^{-1}$), $(\Psi_{m(a)} - \Psi_{p(p)})$ is the pressure difference across the membrane (the matric potential on the outside minus the turgor pressure on the inside of the membrane in MPa, see Fig. 4.3), $(\Psi_{s(a)} - \Psi_{s(p)})$ is the osmotic potential difference across the membrane (MPa, see Fig. 4.3), and σ is the reflection coefficient (dimensionless) indicating the fraction of solute prevented from crossing the membrane. The Lp represents the frictional effects encountered by water as it crosses the membrane, and a larger Lp shows that water crosses the membrane more rapidly for a given potential difference. The pressure probe can evaluate Lp by injecting or removing cell solution and observing the rate that water moves through the plasmalemma (and cell wall) in response.

For most cells, there is solute transport as well as water movement across the plasmalemma. Active metabolism usually is

required for the solute but not the water. The solute also moves passively through the membrane. Both active and passive movements usually are slow compared to water, and the net movement of solute is largely independent of the movement of water. Therefore, the plasmalemma can be considered to be an ideal differentially permeable membrane with a reflection coefficient of essentially 1 for the solutes normally present inside a cell, and the hydraulic conductivity can be considered to apply only to water. Under these conditions, Eq. 4.5 reduces simply to

$$J_v = Lp(\Delta\Psi_w) \quad (4.6)$$

and water is driven across the plasmalemma/cell wall by the water potential difference ($\Delta\Psi_w$) between the two sides.

In special situations, this simplification may not hold. For small lipophilic solutes such as ethanol or isopropanol, σ is less than 1. Other solutes can alter membrane properties and cause σ to be less than 1 in which case internal solute may leak out. Cells that are suddenly subjected to high concentrations of solutes may shrink enough to cause the plasmalemma to separate from the cell wall (plasmolysis) and disrupt the plasmodesmata. Passive movement of solute becomes rapid and can be influenced by the rate of water movement. As a consequence, the exposure of cells to external osmotica can sometimes lead to these artifacts, and great care must be exercised. In these situations, it cannot be assumed that $\sigma = 1$ and the pressure probe, by measuring membrane properties, can detect when σ is less than 1.

SIGNIFICANCE OF REFLECTION COEFFICIENTS

The reflection coefficient is not a permeability coefficient but rather determines how much of the osmotic potential is harnessed in water transport. When σ is less than 1, the osmotic potential is similarly less than fully effective. If σ is less than 1, solute moves across the membrane but its rate is determined by a permeability coefficient for the solute and by any drag exerted by solute moving out of the cell while water moves in.

The osmotic potential can be measured inside and outside of cells but it does not indicate the reflection coefficient because the coefficient is a membrane property. There is no way to determine how

much of the measured potential is contributing to flow without measuring the properties of the membrane. Depending on the reflection coefficient of the membrane, the osmotic effect of the solute can vary dramatically. Moreover, because the reflection coefficient describes a condition of the membrane, its effects are always present and cannot be avoided by making rapid measurements or allowing only small water flows. For this reason, osmotica generally do not simulate the natural dehydration of cells and are rarely used for measurements of cell water status.

WALL ELASTICITY

Sometimes it is useful to know how much water is required to change the cell turgor or water potential by a particular amount. The turgor pressure of the cell arises from the elasticity of the wall. The elasticity can be measured in terms of the bulk modulus of elasticity (ϵ in MPa, Chap. 2) according to

$$d\Psi_{p(p)} = \epsilon \frac{dV}{V}, \quad (4.7)$$

which relates a change in internal pressure to the fractional change in water content (dV/V) of the cell. A larger ϵ indicates that a larger change in turgor will occur when the water content of the cell changes. In other words, a larger ϵ indicates that the wall is less elastic. It can readily be imagined that a rigid container (large ϵ) will experience a large pressure increase when the volume increases inside.

Similarly, the effect of dV/V on the water potential of the cell is described by the expression

$$\frac{dV}{d\Psi_{w(p)}} = \frac{V}{\epsilon - \Psi_{s(p)}} = C, \quad (4.8)$$

which has been called the capacitance C of the cell (see Kramer and Boyer, 1995; Molz and Ferrier, 1982; Steudle, 1989 for derivations of Eq. 4.8). By injecting or removing cell solution rapidly with the pressure probe, it is possible to change the turgor and observe the volume change thus determining ϵ and C .

CELL KINETICS

Because the membrane properties in Eqs. 4.5 and 4.6 affect the rate of water flow, but the volume of water lost or more precisely the capacitance in Eq. 4.8 determines how fast the cell swells or shrinks, the effects of flow on swelling and shrinking provide a way to determine the hydraulic conductivity of the plasmalemma/cell wall complex and can be found by substituting Eq. 4.8 into Eq. 4.6

$$t_{1/2} = \frac{0.693V}{LpA(\epsilon - \Psi_s)} = 0.693rC \quad (4.9)$$

to give an expression describing how fast a cell shrinks or swells. In this equation, $t_{1/2}$ is the time for half the change in water potential or turgor (sec), A is the surface area of the cell (m^2), and r is the frictional resistance to water movement through the plasmalemma/cell wall ($1/LpA$). Kramer and Boyer (1995) give the derivation, which assumes the reflection coefficient equals 1.

Equation 4.9 shows that the cell acts much like an electrical circuit with a resistance and capacitance in series. The resistance r is mostly determined by the plasmalemma/cell wall and controls how fast water enters the cell. The capacitance C (Eq. 4.8) is determined by the size of the cell interior, the elasticity of its wall, and the internal osmotic potential, and these control how fast the potential changes for a unit change in the volume of water. The rate at which the water potential or turgor changes is the product of the resistance and capacitance, and an increase in either resistance or capacitance makes the change slower ($t_{1/2}$ longer).

Because the pressure probe moves water through the plasmalemma and cell wall during the Lp determination, Lp probably includes at least part of the cell wall conductivity as well as the plasmalemma conductivity. The contribution of the cell wall to this conductivity is not expected to be large, but measurements are needed to test this assumption. For many species, Lp ranges between 10^{-6} and 10^{-8} $m\cdot sec^{-1}\cdot MPa^{-1}$ (Kramer and Boyer, 1995). The range of values suggests that the plasmalemma/cell wall can vary in conductivity.

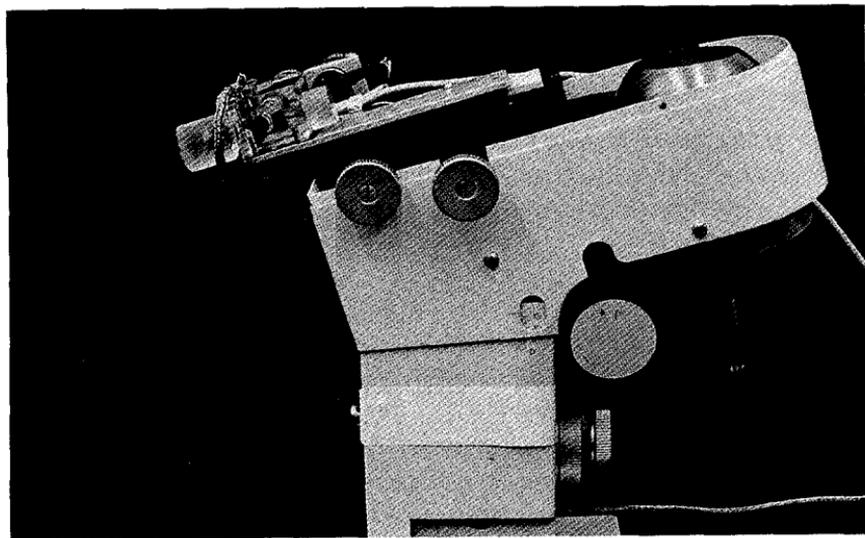


Figure 4.4. Miniature pressure probe mounted on a Leitz micromanipulator.

Using the Probe

After mounting the pressure probe on a micromanipulator (Fig. 4.4), place it on a vibration-free table. A stereomicroscope also should be on the same table and equipped with an eyepiece reticle to allow the solution/oil meniscus to be seen and measurements of the microcapillary dimensions and position to be made. The output of the pressure probe should be attached to a strip chart recorder to provide a continuous record of the pressures. If available, a data logger can be used to store the data for later analysis. In some advanced systems, it has been possible to view the meniscus and acquire and analyze the data with a computer. With your electrical equipment operating, go through the following procedures to ensure that accurate measurements are achieved.

FILLING WITH OIL

The pressure probe should be filled with silicone oil of the least viscous grade (Wacker AS 4 or equivalent). The oil should be clean and may need to be filtered if it contains significant foreign particles.

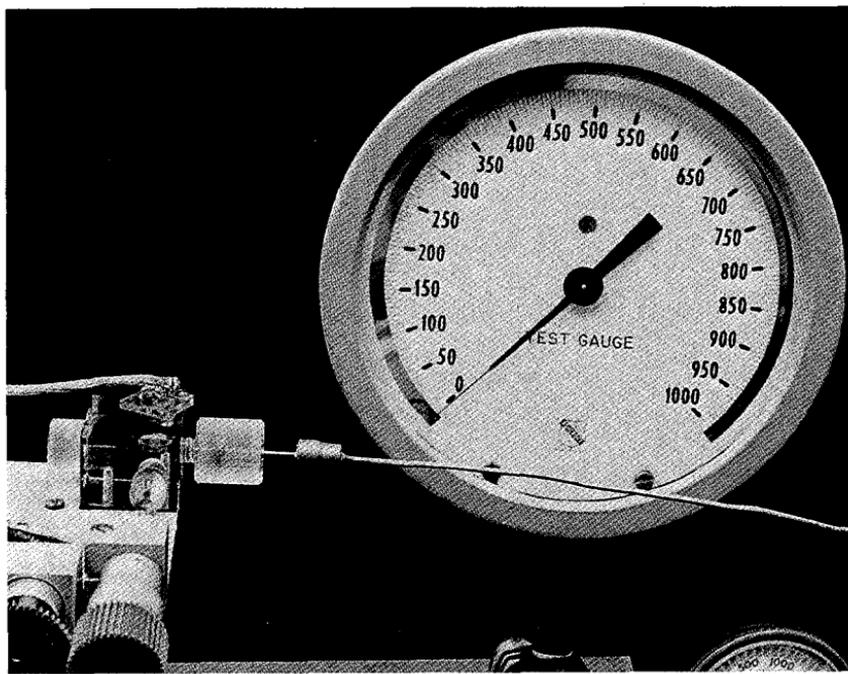


Figure 4.5. Calibration system for the pressure transducer mounted on a miniature pressure probe. A gas line replaces the microcapillary. The probe is filled with silicone oil and gas pressure is applied to the meniscus. The gas pressure is measured with a test gauge.

Usually, a syringe having a fine needle is filled with the oil and the borings are filled in an order that drives out all air. Be careful not to expose the pressure transducer to large pressures or bump it with the needle. Mount the rod and transducer only partially, and fill the borings until oil comes out. Then screw the rod and transducer mountings into place tightly, being careful to have an opening present to release the pressure generated as these fittings are tightened. After filling with oil, inspect all parts of the probe to ensure that no air bubbles are present. If bubbles are observed, repeat the filling procedure.

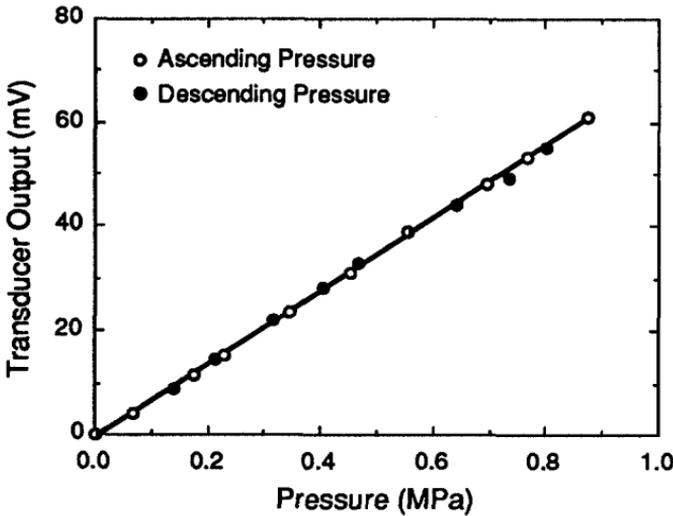


Figure 4.6. Calibration of the output of a pressure transducer with a test gauge shown in Fig. 4.5. From Nonami (1986).

CALIBRATION

The pressure transducer should be calibrated by placing a tube in the socket usually occupied by the microcapillary (Fig. 4.5). The open end of the tube is connected to a compressed gas cylinder. The pressure from the cylinder is increased and applied to the oil-filled probe. The pressure is measured with a high quality pressure gauge (test gauge or master test gauge, see Fig. 4.5) and the electrical output of the transducer is noted. After a series of pressures, a calibration curve can be constructed as shown in Fig. 4.6. The plot should be linear and, because the transducer should be the type to indicate gauge pressure, the output should be zero at atmospheric pressure.

MICROCAPILLARY

The microcapillary is made by heating a capillary (1 mm outside diameter, thin wall) at a point about halfway between the ends and rapidly pulling the ends apart. The melted part is drawn to a fine point that seals, and the two halves of the capillary are formed into near identical microcapillaries.

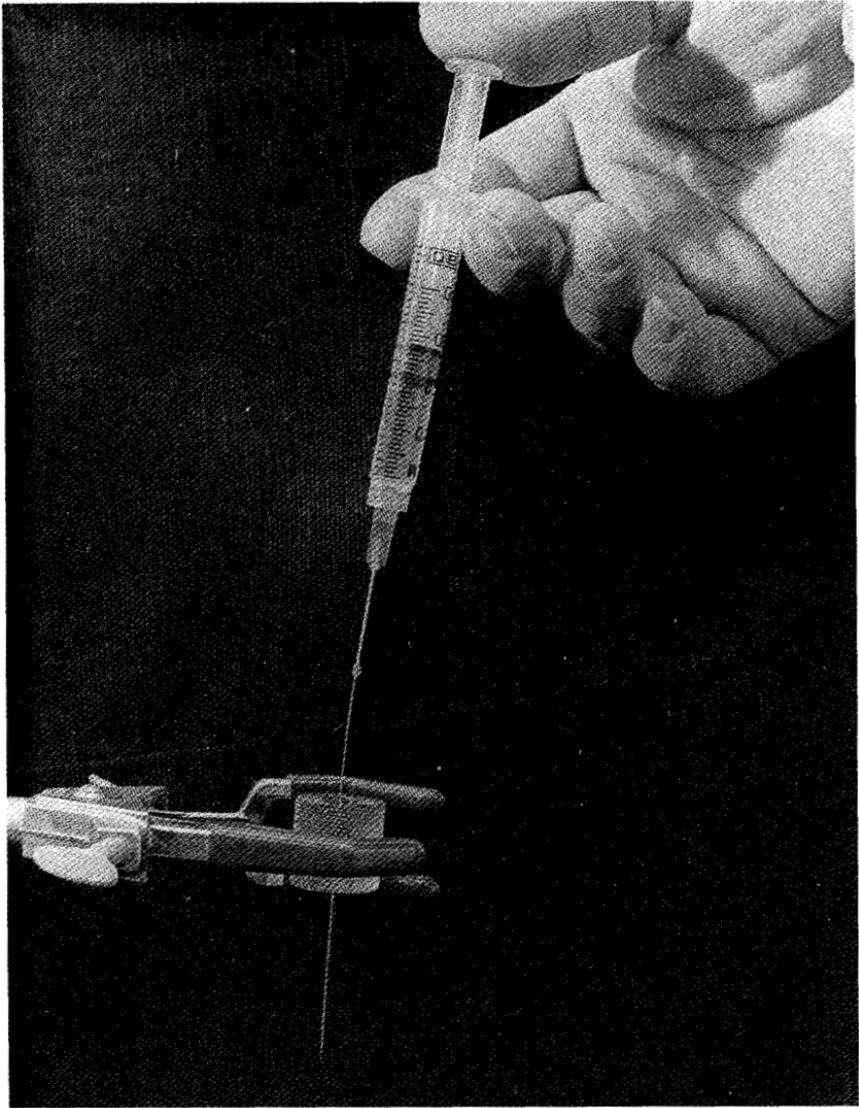


Figure 4.7. Filling a microcapillary with silicone oil. The syringe needle has been lengthened by inserting a fine tube into the needle and sealing with a drop of epoxy.

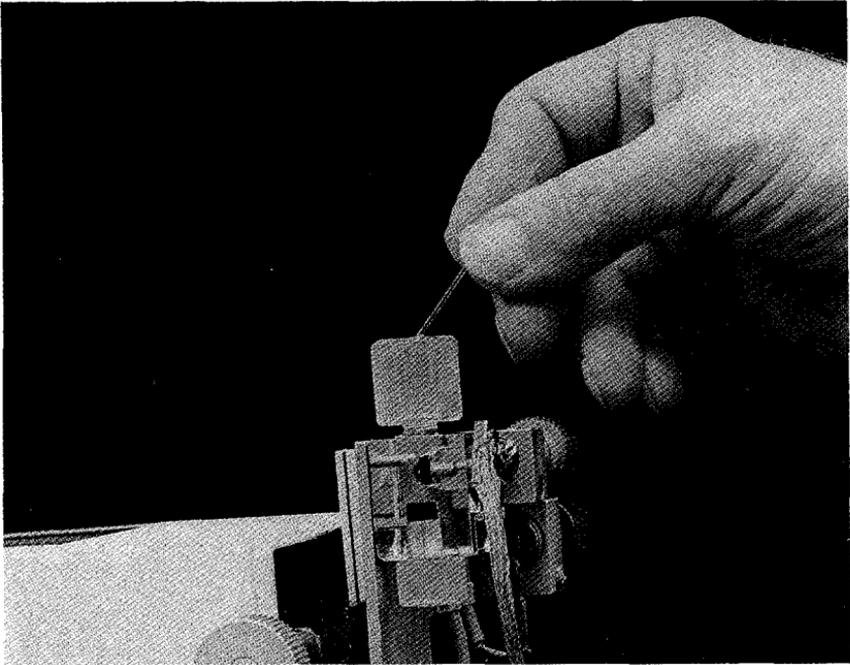


Figure 4.8. Mounting a microcapillary on a miniature pressure probe. The probe has been filled with silicone oil sufficiently to flood the surface of the holder, and the microcapillary has been filled with oil. Holding the open end of the microcapillary under the oil on the flooded surface excludes all air bubbles. The microcapillary is then placed upright in the holder, mounted in the seal, and the holder is tightened while watching the output of the pressure transducer on a strip chart recorder. Be careful not to overpressurize the oil when sealing the microcapillary in place.

The rapid pull of the capillary is most reproducible when a vertical electrode puller such as that made by Kopf (Model 720) is used to heat and do the pulling. Horizontal pullers have the disadvantage that the microcapillary tip sags under the force of gravity during the pulling, which bends the tip. A vertical puller has the advantage that the pull of gravity is symmetrical and no sagging occurs.

FILLING THE MICROCAPILLARY WITH OIL

Mount a microcapillary in the seal of the pressure probe (Fig. 4.7) for filling with oil. A long thin syringe needle can be constructed

by gluing a thin metal tube (such as a thin-walled stainless-steel tube part number Q-HTX-27TW, Small Parts Company, Miami Lakes, Florida) into a syringe needle. Attach the needle to a syringe filled with silicone oil and insert the thin tube into the microcapillary. Move oil into the microcapillary while slowly withdrawing the tube until the microcapillary is completely filled. Place the filled microcapillary on the pressure probe and seal without admitting air bubbles (Fig. 4.8).

LEAKS

Test the sealed pressure probe for leaks by screwing the rod into the oil until the output of the pressure transducer becomes high (up to 1 MPa). After high pressure is established, observe the pressure for several hours. Only a slow decline in pressure should be observed. If the pressure decreases rapidly, double check that the tip of the microcapillary is still sealed (observe that no oil is leaking using a stereomicroscope). Provided the tip seal is good, the leak is most likely to be in the seal for the microcapillary, transducer, or rod. These seals are rubber gaskets that degrade after extended use and need to be replaced. If excessive leaks are observed, replace the seals and repeat the leak test. Seals remain good for 2-3 years.

BEVELING THE MICROCAPILLARY TIP

The tip of the microcapillary needs to be opened to allow the silicone oil to contact the cytoplasm inside the cell. Although the tip can be forced against a surface and broken open, the jagged edge reduces the rate of success in measuring cell pressures. Typically, we obtain success rates of 20-30% with tips opened in this fashion. Success rates of 80-90% are achieved when the tip is opened by beveling.

For beveling, the tip is brought against a rotating disk coated with diamond dust (such as that made by Narishige Model EG-4) while the silicone oil is under pressure. The tip is angled at about 45° to the surface. When an oil streak appears on the rotating disk, the tip has been beveled sufficiently to open and is usually ready to insert into a cell. Tip openings are about 1 μm . For some cells such as the large internodal cells of *Chara*, the tips tend to plug and beveling at a larger angle and for a longer time reduces plugging. Tip openings of 5 μm or more may be required (Zhu and Boyer, 1992), and the large diameter

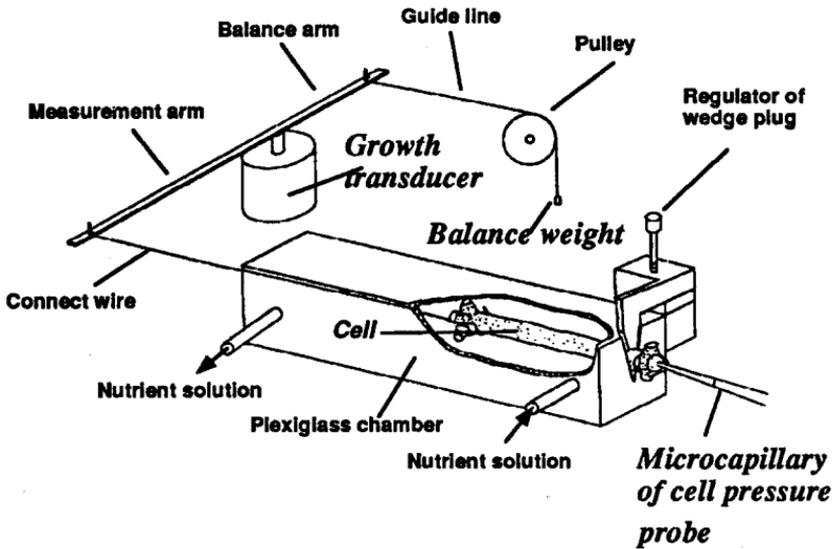


Figure 4.9. Support for a *Chara* cell. The microcapillary tip is inserted in the rigidly mounted end of the cell, and nutrient solution fills the chamber. In this setup, a position transducer is attached to the other end of the cell. From Zhu and Boyer (1992).

apparently prevents cytoplasm from flowing over the opening and sealing it.

MICROMANIPULATOR

The pressure probe must be mounted on a micromanipulator in order to allow small controlled movements of the microcapillary. A micromanipulator such as the one built by Leitz (Fig. 4.4) has a massive base and a large moving platform that are ideal for minimizing vibration and mounting the probe with its accompanying instrumentation.

CELL SIZE

The minimum cell diameter is 25 μm for routinely measuring turgor pressure, elastic modulus, and hydraulic conductivity with the probe. Occasionally, turgor can be measured with smaller cells (as small as 15 μm diameter). Cell properties other than turgor are difficult

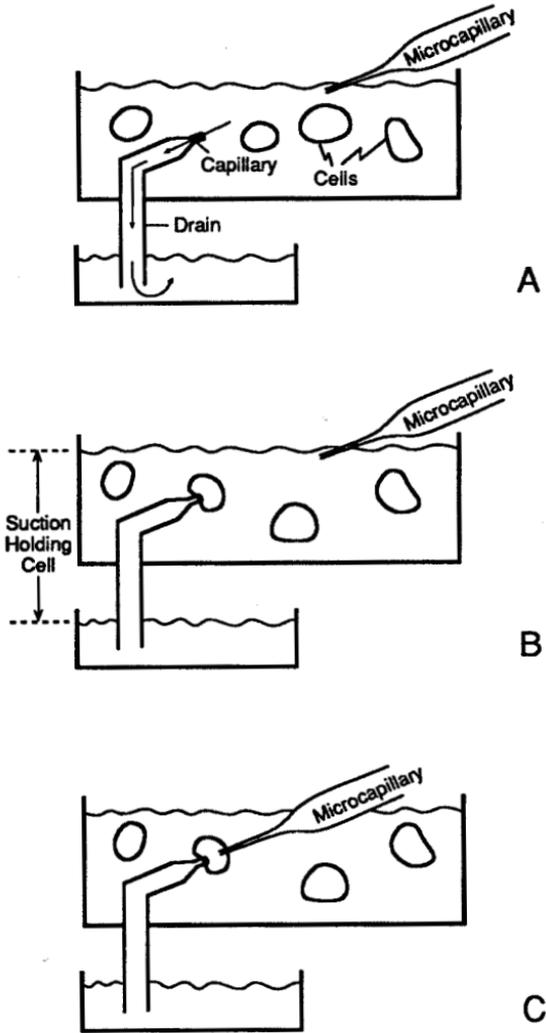


Figure 4.10. Support for a single small cell. Suction is created around the opening of a microcapillary with a fire-polished edge. A cell is swept against the opening by the suction and held there. The pressure probe is advanced into the front of the cell while the microcapillary in the back provides support. The suction can be varied by adjusting the height of the drain tank. The dimensions are not to scale.

to measure because there is more cell disturbance. Apparently, the limiting cell diameter is fixed by the diameter of the microcapillary tip because openings of 1-2 μm in the tips cause relatively large wounds in small cells.

How to Make Measurements

TURGOR PRESSURE

The cell or tissue is typically supported on the side opposite the one for insertion of the microcapillary tip. Mounts for cells or excised tissues may consist of culture vessels with the end of the specimen held immobile for the probe but the rest covered by nutrient solution as in Fig. 4.9. Surrounding the specimen with a nutrient solution is essential to minimize evaporation, and great care must be taken to prevent evaporation from altering the turgor of the measured cells (see Precautions). For single cells, it may not be possible to clamp one end because of the small size of the cell. In this situation, another microcapillary is constructed and the tip is ground until the opening is several micrometers in diameter. The tip is lightly fire polished to provide smooth rounded surfaces at the opening. The tip is inserted into the medium containing the cells and the medium is sucked into the microcapillary by forming a drain with a tube attached to the other end of the microcapillary. Under the microscope, the tip is moved among the cells in the solution until a cell is sucked against the tip, blocking it, and remaining in place because of the suction. The suction should be only strong enough to hold the cell in place without distortion. One design shown in Fig. 4.10 immobilizes the cell from one side and allows the microcapillary of the pressure probe to be pushed from the other side. Another approach is to catch a large number of cells on a screen using slight suction and use the pressure probe on individuals that have been immobilized.

Intact plants may be supported by placing a rigid bar behind the probed region as in Fig. 4.11. A saturated atmosphere can be used to minimize evaporation. Cover the surfaces of the chamber or work room with water and allow the water to vaporize. Saturation of the air can be detected by weighing wet filter paper. When no weight loss is observed, the atmosphere is saturated. However, because part of the

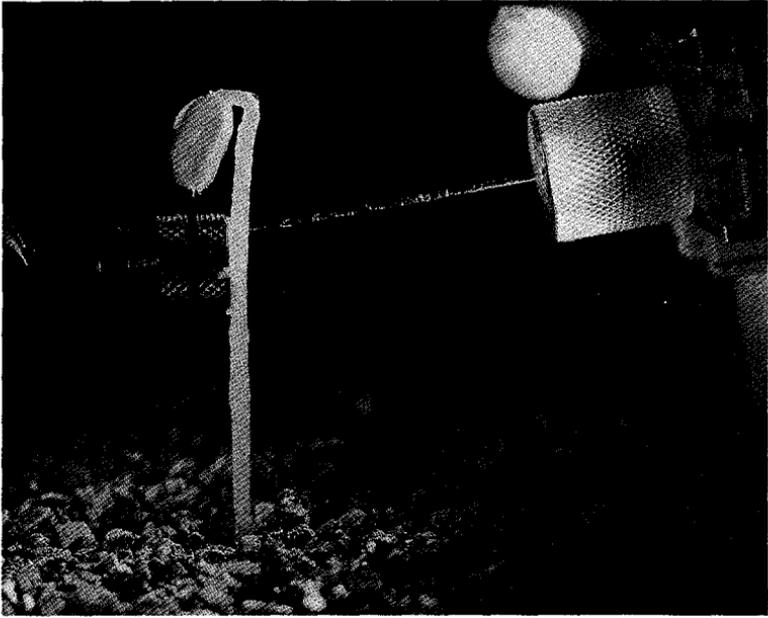


Figure 4.11. Support for a soybean stem. A rigid bar is placed behind the stem to support the tissue into which the microcapillary will be driven. Note the coating of petrolatum (Vaseline) on the surface of the seedling to prevent evaporation. From Nonami and Boyer (1993).

tissue must be illuminated, local heating from the light source can cause evaporation into a saturated environment (Nonami *et al.*, 1987). To avoid this, cover the plant part with a thin layer of petrolatum (Vaseline) and insert the microcapillary through the Vaseline into the tissue as in Fig. 4.11.

The need for illumination arises because the meniscus must be observed between the oil and the cytoplasmic solution. The specimen and microcapillary are illuminated at an angle that causes the meniscus to be seen as a bright surface in the microcapillary. A fiber optic cable provides a simple means of directing light to the microcapillary, and small light-emitting diodes also have been used successfully.

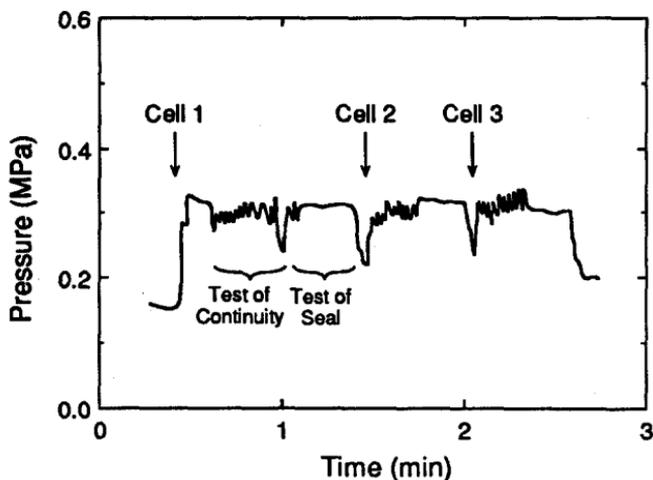


Figure 4.12. Typical recorder tracing of turgor pressure in three cells in a soybean stem. The liquid continuity between the cell and pressure transducer is tested by rapidly varying the pressure in the probe and observing the movement of the meniscus in and out. If the meniscus does not move, no liquid is moving and continuity does not exist. The seal with the cell is tested by observing the pressure for about 10 - 15 sec. If the pressure drops, a leak is present. From Nonami *et al.* (1987).

- 1) With the sample supported and protected from evaporation, the microcapillary tip and sample are brought into view under a stereomicroscope at low magnification. A small amount of silicone oil is usually being released from the tip of the beveled microcapillary. The magnification is increased to about 80X. Although the opening at the tip is too small to be seen at 80x, the tip should appear distinct with no fuzziness. If the tip is fuzzy, there is too much vibration. Determine whether the vibration is caused by table movement or air movement, and take steps to eliminate it (see Precautions).
- 2) Move the microcapillary slowly forward in a straight line and observe the silicone oil close to the tip as the microcapillary enters the cell or tissue. When a cell is penetrated, the cytoplasm will enter the microcapillary abruptly and a bright oil/cytoplasm meniscus should be seen to jump back into the microcapillary. This jump is diagnostic for

penetrating a cell. For a tissue, use the eyepiece reticle to note the depth that the tip has penetrated so that the location of the measured cell can be determined later.

3) Slowly bring the meniscus toward the cell by pushing the rod into the oil of the pressure probe. Stop the meniscus at the original position before entering the cell. If the probe was completely oil filled, this return pressure brings the meniscus to the cell or tissue surface. If the probe contained solution before entering the cell, the meniscus is returned only to the original position before penetrating the cell (Figs. 4.1B and 4.1C). This pressure is the turgor pressure of the cell.

4) With slight movements of the rod, move the meniscus short distances around the original position (Fig. 4.12). Small pressure fluctuations should move the meniscus freely, and the pressures should fluctuate around a mean that is the same as the turgor of the cell, which establishes that hydraulic continuity exists between the cell and the pressure transducer.

5) After testing hydraulic contact, observe the turgor for about 15 sec (Fig. 4.12). The turgor should remain stable, indicating that a good seal exists between the tip and the cell. If the turgor diminishes, there is a leak and the tip should be moved to the next cell repeating Steps 2-5.

6) There can be significant variation in the turgor pressure between cells in a tissue, and representative turgors can be determined only with a statistically relevant sample (an example is in the following chapter, see Fig. 5.2). For most tissues, about 7-10 cells provide a large enough sample to establish a mean and variance.

OSMOTIC POTENTIAL

After measuring the turgor pressure, the osmotic potential can be determined by removing a sample of the cell solution and measuring its osmotic potential in a freezing point osmometer.

1) With the microcapillary tip in the cell, note the position of the meniscus, move the meniscus back into the microcapillary, note the distance, and rapidly withdraw the tip from the cell. The microcapillary now contains a sample of solution from the cell. The time to move the meniscus back and withdraw the tip should be no

more than 1-2 sec in order to avoid diluting the sample with water entering the cell as the turgor decreases (Malone *et al.*, 1989).

2) Swiftly transfer the sample from the microcapillary to a sample well in a nanoliter freezing point osmometer (Clifton Technical Physics, Hartford, NY). By moving small distances, the sample can be transferred to the well within a few seconds. The rapid transfer is necessary to avoid evaporation of the sample from the tip of the microcapillary. The well should be filled with immersion oil of the most viscous grade (Cargille Type B or NVH) to immobilize the sample droplet in the well.

3) View the sample droplet under a compound microscope at 300-400X using an objective having a long focal length. It should be possible to observe volumes as small as 10 picoliters. Make certain that the droplet remains suspended in the oil and does not contact the side of the well. Contact distorts the droplet and alters the freezing point. Although it is possible to calibrate the freezing point for contact with the sides of the well (see Malone *et al.*, 1989; Malone and Tomos, 1992), the simplest approach is to prevent contact.

4) Freeze the droplet and begin the thaw cycle, noting the temperature at which the last ice crystal just disappears. The freezing point is defined as the temperature of disappearance of the last crystal because the presence of significant ice, which is relatively pure water, concentrates the cell solution and prevents it from being the original concentration. The cell solution is closest to its original concentration as the last ice disappears. This procedure should be repeated with the sample and should give reproducible freezing points.

5) Express the freezing point as the number of degrees Centigrade below zero (ΔT). Calculate the osmotic potential from the equation

$$\Psi_{s(p)} = \frac{-2.27\Delta T}{1.86} \quad (4.10)$$

The $\Psi_{s(p)}$ has units of MPa and is at 0°C. For $\Psi_{s(p)}$ at other temperatures, multiply the osmotic potential at 0°C by $T/273$ where T is in Kelvin.

6) If samples have been obtained from the first cell in contact with the microcapillary, there is no other solution in the microcapillary and the

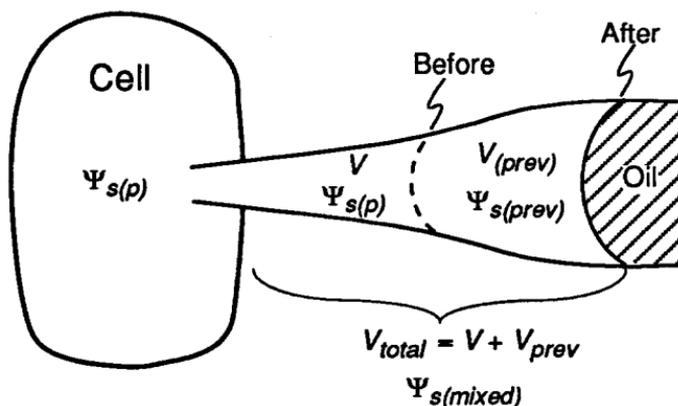


Figure 4.13. Sampling cell solution with a pressure probe when mixing occurs in the microcapillary. If a significant volume of solution $V_{(prev)}$ already exists in the microcapillary before entering a cell, removing a new volume V from the cell can mix with $V_{(prev)}$. By sampling cells around the test cell, the $\Psi_{s(prev)}$ can be measured. Then for the test cell, noting the position of the meniscus before sampling (Before) and after sampling (After) allows the previous volume ($V_{(prev)}$) and sample volume (V) to be calculated. Measuring $\Psi_{s(mixed)}$ from the mixed volume allows $\Psi_{s(p)}$ to be calculated for the test cell according to Eq. 4.11.

$\Psi_{s(p)}$ is calculated directly from Eq. 4.10. If the microcapillary already contains some solution from previously penetrated cells, the sample from the cell can be kept small so that it enters only the tip of the microcapillary where it has little chance to mix with other solution in the microcapillary. In this situation, $\Psi_{s(p)}$ also can be calculated by Eq. 4.10 without correction.

7) If the sampled solution moves into the main body of the microcapillary where other solution is present, there is a chance of mixing with solution already in the microcapillary (Fig. 4.13). Typically, complex tissues require filling the microcapillary with solution from surface cells in order to keep the meniscus in view as the tissue is penetrated more deeply. Determining the $\Psi_{s(p)}$ of these surface cells gives the osmotic potential of the solution already in the microcapillary. A correction for mixing can be made if the volume of this solution and the sample solution also are known (Fig. 4.13). After

measuring the osmotic potential of the cells close to the surface in the vicinity of the deeper cell to be sampled, determine the volume of the solution previously in the microcapillary and the volume from the sampled cell by calculating from the position of the meniscus noted in Step 1 before and after taking the sample into the microcapillary (Fig. 4.13). The volume of the interior of the microcapillary can be determined by approximating the tip as a cone or series of cones and the main axis as a cylinder. The volume of the sample (V) is then determined by subtracting the volume before sampling (V_{prev}) from the volume after sampling (V_{total}). The measured osmotic potential is corrected for mixing with the solution already in the microcapillary to give the original $\Psi_{s(p)}$ of the sampled cell using a relation similar to that in Eq. 3.8 but modified to apply to the microcapillary

$$\Psi_{s(p)} = \Psi_{s(mixed)} + \frac{V_{(prev)}}{V} (\Psi_{s(mixed)} - \Psi_{s(prev)}), \quad (4.11)$$

where the subscripts (*mixed*) and (*prev*) indicate the mixed sample and the previous solution in the microcapillary, respectively. According to this calculation, the $\Psi_{s(mixed)}$ is between the true $\Psi_{s(p)}$ of the sampled cells and the $\Psi_{s(prev)}$ of the previously penetrated cells in proportion to the relative volumes of each in the microcapillary. For example, for $V_{(prev)} = 0.4V_{(total)}$ and $V = 0.6V_{(total)}$, and a measured $\Psi_{s(prev)}$ of -1.2 MPa and $\Psi_{s(mixed)}$ of -1.4 MPa, the calculated $\Psi_{s(p)}$ is -1.53 MPa. As you can see, the corrections are usually small (a few hundredths of an MPa) but they should be made for accurate work.

Note that it is incorrect to avoid this correction by expelling the solution in the microcapillary before entering the sampled cell. If the solution in the microcapillary is expelled, the meniscus becomes invisible and the microcapillary must be refilled with the solution from the sampled cell. The total sampled volume is thus large and can be larger than the volume of the sampled cell. The osmotic potential is markedly diluted by water entering the cell in this situation. Instead, short times (Malone *et al.*, 1989; Malone and Tomos, 1992) and small sample volumes corrected for mixing (Nonami and Boyer, 1993) should be used to accurately determine cell osmotic potentials with a pressure probe.

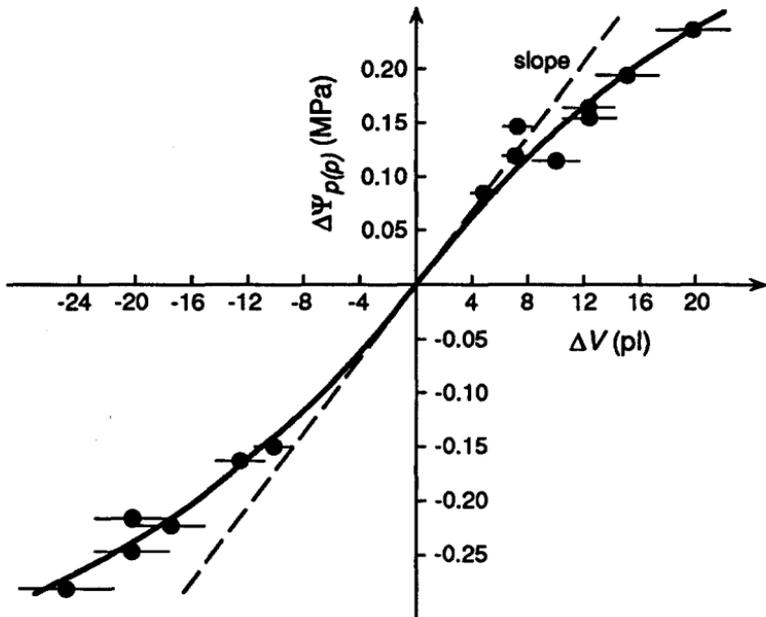


Figure 4.14. Change in turgor pressure with change in volume of cell solution measured as in Fig. 4.2A. In this example, small volumes ΔV are injected into the cell and cause positive $\Delta \Psi_{p(p)}$, and small volumes are removed causing negative $\Delta \Psi_{p(p)}$. The dashed line gives the slope which is $d\Psi_{p(p)}/dV$ for calculating the elastic modulus ϵ . The deviation from the dashed line is caused by finite amounts of water moving through the plasmalemma when the pressure change is large. From Tomos *et al.* (1981).

The microcapillary tip is usually in the central vacuole of the cell, and the measured osmotic potential is for the vacuole to the largest extent. However, the osmotic potential is essentially the same throughout the cytoplasm and vacuole (see Kramer and Boyer, 1995), and the results with the pressure probe sample should apply throughout the cell. Using various micromethods of analysis, the sample of cell solution may be used for other vacuolar determinations such as the elemental content (Malone *et al.*, 1989), metabolite levels (Harris and Outlaw, 1991; Outlaw and Lowry, 1977), or enzyme activities (Hite *et al.*, 1993).

ELASTIC MODULUS

The wall ϵ can be determined by changing the volume of the cell with the pressure probe while measuring the pressure, then measuring the cell volume microscopically.

- 1) Measure the turgor pressure as described earlier.
- 2) Note the position of the meniscus in the reticle of the eyepiece of the stereomicroscope and rapidly pull a small amount of solution from the cell into the microcapillary, note the new position of the meniscus and the new turgor pressure, and rapidly return the meniscus to its original position. This step should take no more than 1-2 sec to minimize water movement across the plasmalemma (see rapid pressure transients in Figs. 4.2A and 4.2C).
- 3) Repeat Step 2 with larger volumes of cell solution, working to decrease and increase the pressure as rapidly as possible. This allows $d\Psi_{p(p)}/dV$ to be calculated for decreasing $\Psi_{p(p)}$ (Fig. 4.14).
- 4) Reverse the procedure in Step 2 by rapidly injecting a volume of solution into the cell from the microcapillary and noting meniscus positions and pressures. Inject increasing volumes of solution as in 3). This allows $d\Psi_{p(p)}/dV$ to be calculated for increasing $\Psi_{p(p)}$ (Fig. 4.14).
- 5) Measure the total volume of the cell. If the test cell is directly visible, the volume can be determined accurately. If the test cell is not visible because of other cells in the tissue, make cross sections and longitudinal fresh sections at the measured depth of the microcapillary. Express the volume as the mean of the measured volumes.
- 6) Calculate the elastic modulus from the $d\Psi_{p(p)}/dV$ measured with injections and removals of cell solution, and the mean cell volume according to Eq. 4.7. The relation between the change in volume and the change in pressure may be S-shaped as in Fig. 4.14 because of water entry and exit through the plasmalemma at large $d\Psi_{p(p)}$. Significant water movement occurs at the extremes because of the longer times necessary to move the meniscus larger distances. Sometimes the curve is not symmetrical (Tomos *et al.*, 1981). The linear part of the curve close to the origin gives the most accurate value of $d\Psi_{p(p)}/dV$.

The largest uncertainty is the volume of the cell. Replicate cells generally have different volumes. Moreover, ϵ becomes larger in larger cells (Steudle *et al.*, 1977). One might expect this relation because larger cells will be required to have walls that withstand larger force (pressure

has units of force per unit area). Therefore, if the volume of the probed cell cannot be directly measured, use a mean value for similarly sized cells. Calculate the variation in ϵ using the method of Propagation of Errors described in standard statistics texts.

CAPACITANCE

The capacitance (C) can be calculated from the measurements already described. Use the data for $\Psi_{s(p)}$, ϵ , and V to calculate $V/(\epsilon - \Psi_{s(p)})$ as in Eq. 4.8. The largest uncertainty is in V and ϵ . Calculate the statistical uncertainty with the Propagation of Errors method described in standard statistics texts.

HYDRAULIC CONDUCTIVITY

The hydraulic conductivity (L_p) is determined by measuring the flow of water through the plasmalemma/cell wall with a pressure probe. Injecting or removing cell solution changes the water potential because the turgor changes. Water leaves or enters the cell in response (see Fig. 4.2B). The flow occurs until the cell returns to equilibrium with its environment, and the time for half of the return can be measured (half-time, $t_{1/2}$ in seconds) as in Fig. 4.2D. Using the $t_{1/2}$ and the other measurements described earlier, the L_p can be determined according to Eq. 4.9.

- 1) Measure the turgor in the cell as described earlier. For a tissue, note the distance the microcapillary tip has penetrated the tissue.
- 2) Raise and lower the turgor rapidly to determine ϵ as above (Figs. 4.2A and 4.2C).
- 3) Raise the turgor but do not lower it (Fig. 4.2B). Allow the turgor to relax as water flows out of the cell. Lower the turgor but do not raise it. Allow the turgor to relax as water flows into the cell. Determine the $t_{1/2}$ for each relaxation by noting the time for half the relaxation to occur as in Fig. 4.2D. The $t_{1/2}$ should be the same for efflux and influx.
- 4) Determine the Ψ_s by rapidly removing a sample of the cell solution and measuring its freezing point in a nanoliter osmometer. The Ψ_s does not vary greatly and contributes only slightly to the calculated value of the hydraulic conductivity. This step sometimes is simplified by determining the Ψ_s of a tissue extract that gives an average for the tissue.

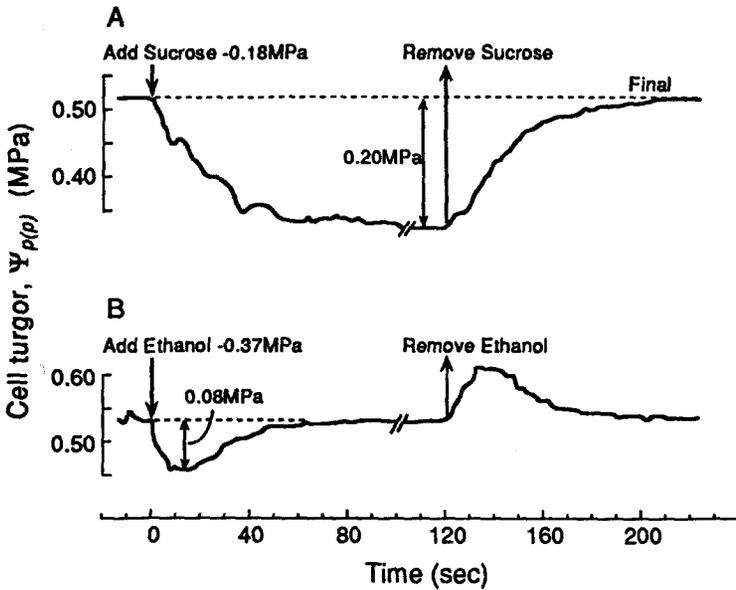


Figure 4.15. Measuring reflection coefficients σ with the miniature pressure probe. A) Sucrose ($\Psi_s = -0.18$ MPa) is placed outside a *Tradescantia* cell and the change in turgor is measured (-0.20 MPa) as the cell reequilibrates, giving a σ of 1.1. B) Ethanol ($\Psi_s = -0.37$ MPa) is placed outside the same *Tradescantia* cell and the change in turgor is measured (-0.08 MPa) giving a σ of 0.21. Note that only water flows out of the cell in sucrose but water flows out and solute flows in with ethanol. From Tyerman and Steudle (1982).

- 5) Measure the volume (V) and surface area (A) of the sampled cell from the dimensions determined microscopically. If the position of the sampled cell is not known precisely, the mean V and A should be determined at the estimated position of the microcapillary tip.
- 6) Calculate the L_p for the plasmalemma/cell wall complex of the cell according to $L_p = 0.693V/(\epsilon - \Psi_s)At_{1/2}$ from Eq. 4.9. An example for a fairly large cell (Hüsken *et al.*, 1978) gives V of 11×10^{-12} m³ and $d\Psi_{p(p)}/dV$ of 1.1×10^{11} MPa·m⁻³ so that ϵ is 1.2 MPa. The Ψ_s measured with extracts of cell solution is -0.25 MPa. The mean A determined from cell dimensions is 3.0×10^{-7} m², and $t_{1/2}$ is about 300 sec. The L_p calculated from Eq. 4.9 is then 5.8×10^{-8} m·sec⁻¹·MPa⁻¹, which is within the range of values expected for cells (Kramer and Boyer, 1995).

REFLECTION COEFFICIENT

The reflection coefficient depends on the solute and membrane and can be most simply measured by determining the change in cell water potential that is caused by solute placed on the other side of the membrane. In the equilibrium state

$$\sigma = \frac{d\Psi_w}{d\Psi_s} \quad (4.12)$$

which indicates that solute supplied externally to change Ψ_s by 1 MPa will change the Ψ_w internally by 1 MPa when $\sigma = 1$.

Figure 4.15A shows that sucrose having Ψ_s of -0.18 MPa caused a decrease of -0.20 MPa in cell turgor in *Tradescantia* epidermal cells (Tyerman and Steudle, 1982). Assuming that the turgor change causes the same change in the water potential, this indicates that $\sigma =$ essentially 1 for sucrose in this cell. Note that Fig. 4.15B for ethanol having Ψ_s of -0.37 MPa shows a water potential change of only -0.08 MPa, which gives $\sigma = 0.21$. Ethanol passes through the membrane and its osmotic effectiveness is thus only a small fraction of that for sucrose. The movement of ethanol through the membrane can be seen by the two-phase pattern of the turgor response: at first the cell shrinks as water leaves the cell more rapidly than ethanol enters but after some time water movement slows and ethanol entry predominates, causing the cell to reswell. This reswelling is diagnostic for solute entry. For sucrose, there is no sign of reswelling. Note also that the ineffectiveness of ethanol is apparent from the beginning even though the concentration difference for ethanol is largest at that stage across the plasmalemma. No matter how fast measurements are made, the osmotic effectiveness is low.

Equation 4.12 has been used to measure reflection coefficients around 1 (e.g., Tyerman and Steudle, 1982) but, for σ less than 1, the two-phase behavior of the cell causes experimental difficulties. As Tyerman and Steudle (1982) point out, permeating solutes can be dragged along by the water moving through the membrane and swept away from the membrane surface. There are unstirred layers of water and solute next to the membrane and these can limit solute and water

transfer. The results depend on how fast the solute penetrates the membrane. Thus, a σ below 1 clearly indicates that the membrane is nonideal but the actual value of σ is usually approximate.

Precautions

VIBRATION

Even moderate vibration can cause pressure probe measurements to fail. Test for vibration by observing the tip of the microcapillary at 80X with the stereomicroscope. If the tip is not clear but rather appears blurred, and if it becomes clearer when it touches a heavy immobile surface, there is too much vibration. The clarity of the free probe should be the same as that of the probe immobilized against the surface. Test whether the source of vibration is air movement by constructing a container to cover the probe, thus preventing the air from stirring.

If the problem is not caused by air movement, it likely is caused by the vibration of the floor on which the probe bench sits. Locate the probe close to a part of the room having a structural support for the building or, better yet, work on a floor that is directly in contact with the ground.

Further stability can be achieved with a vibration-damping table. Electronic and pneumatic designs work well and operate in particular frequency ranges. If the frequency of vibration is unknown, obtain a steel plate about 2 cm thick and place it on some foam rubber or similar vibration-damping material. Mount the probe on the steel plate. Be sure to make the plate large enough to hold the sample and the microscope so that all movement will be as a unit.

EVAPORATION

Evaporation is induced because of the need to illuminate the probe tip and meniscus inside the microcapillary. The illumination causes local heating of the tissue and evaporation even when the surrounding air is saturated with water vapor. The loss in turgor is most apparent in surface cells but can affect cells deep inside the tissue.

The control of evaporation is one of the most important principles of pressure probe use. The degree of saturation should not

be tested with a humidity detector because most do not give reliable readings around 100% humidity. Instead hang wet filter paper in the air and determine the weight loss. If there is any weight loss, the air is not saturated. Even small sources of dry air can decrease humidities sufficiently to allow weight loss. Sometimes these are minimized by saturating the air in the room, then saturating the air in a small chamber around the probed plant part.

Saturating the air will eliminate evaporation only if the evaporating surface has the same temperature as the air. Above air temperature, evaporation can be rapid. To avoid this problem, the tissue can be coated with petrolatum (Vaseline) to create a vapor barrier. Another approach is to use minimal light to illuminate the sample, thus reducing the temperature of the tissue.

TEMPERATURE

The probe and oil expand and contract with changes in temperature. To avoid temperature effects, the instrument should not be exposed to large thermal gradients or rapid fluctuations in temperature. The temperature control of most laboratories is stable enough to allow the probe to be used but you should avoid air ducts, direct sunlight, nearby compressors, and so on.

Temperature also affects the pressures in cells because the osmotic potential varies with temperature, as discussed in Chap. 1 and shown in Appendix 3.2 for sucrose solutions, and turgor responds accordingly. However, the effects are not large because the osmotic potential responds to the Kelvin temperature (see Kramer and Boyer, 1995).

PLUGGED TIP

Most measurements with the pressure probe are made in the central vacuole of the cell. Cells that have a small vacuole or a thick layer of cytoplasm can cause plugging of the tip of the microcapillary with cytoplasm. A plugged microcapillary is detected from the extreme changes in pressure that occur when the metal rod is moved in or out of the silicone oil, and from the lack of response of the meniscus to the pressure change. It is essential to test for an open tip during a measurement because the pressure in a plugged tip can be mistaken for

the turgor pressure of the cell but bears no relationship to the turgor (see Turgor Pressure, step 4, and Fig. 4.12).

Sometimes the tip can be unplugged by raising the pressure and blowing the plug away. With large cells, it is sometimes possible to work with a tip having a larger opening. When this is not possible, the tip should be moved to another cell. If plugging persists, it may be necessary to replace the microcapillary.

REPLACING SEALS

Instrument leaks are usually caused by faulty rubber seals. If a test of instrument leaks fails (see Leaks), install new seals and repeat the test. New seals can be made from a sheet of rubber gasket material of low to intermediate hardness. Obtain a punch of the diameter required and cut several disks. Place the disks in a freezer at -80°C or on dry ice or in liquid N_2 to harden the rubber, then rapidly drill a hole in the center to admit the microcapillary, metal rod, or other part of the pressure probe. The rubber should remain hard as long as it is cold. The hole should be the same diameter as the part passing through the seal (1 mm for microcapillary) or slightly smaller.

After several years of use, cracks may develop in the main body of the probe because of pressures in the borings. The cracks will gradually work their way to the outside as the probe is used, and a leak results. These cracks will be visible and their progress can be followed. As the crack nears the surface, the probe body will need to be replaced with a new one.

STATISTICS

Measurements of turgor among individual cells of a tissue typically vary by ± 0.1 MPa or more (see Fig. 5.2), and single cells may show a similar variation. It is essential to measure a minimum of 7-10 cells for a statistically relevant sample of the turgor. A similar sample size is needed for measuring cell dimensions. The mean ± 1 standard deviation is the usual statistical test, and for reporting the mean of several means of 7-10 cells each, the standard error is used.

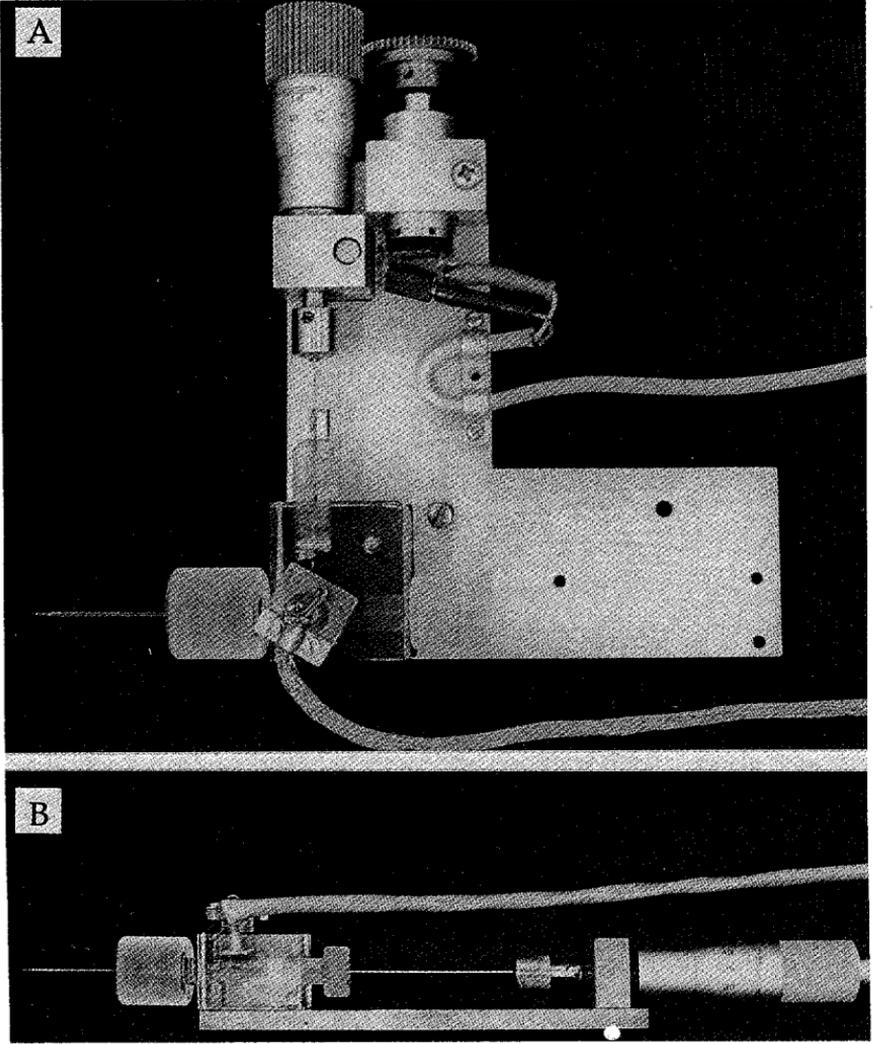


Figure 4.16. A) Miniature pressure probe and B) pressure probe for large cells.

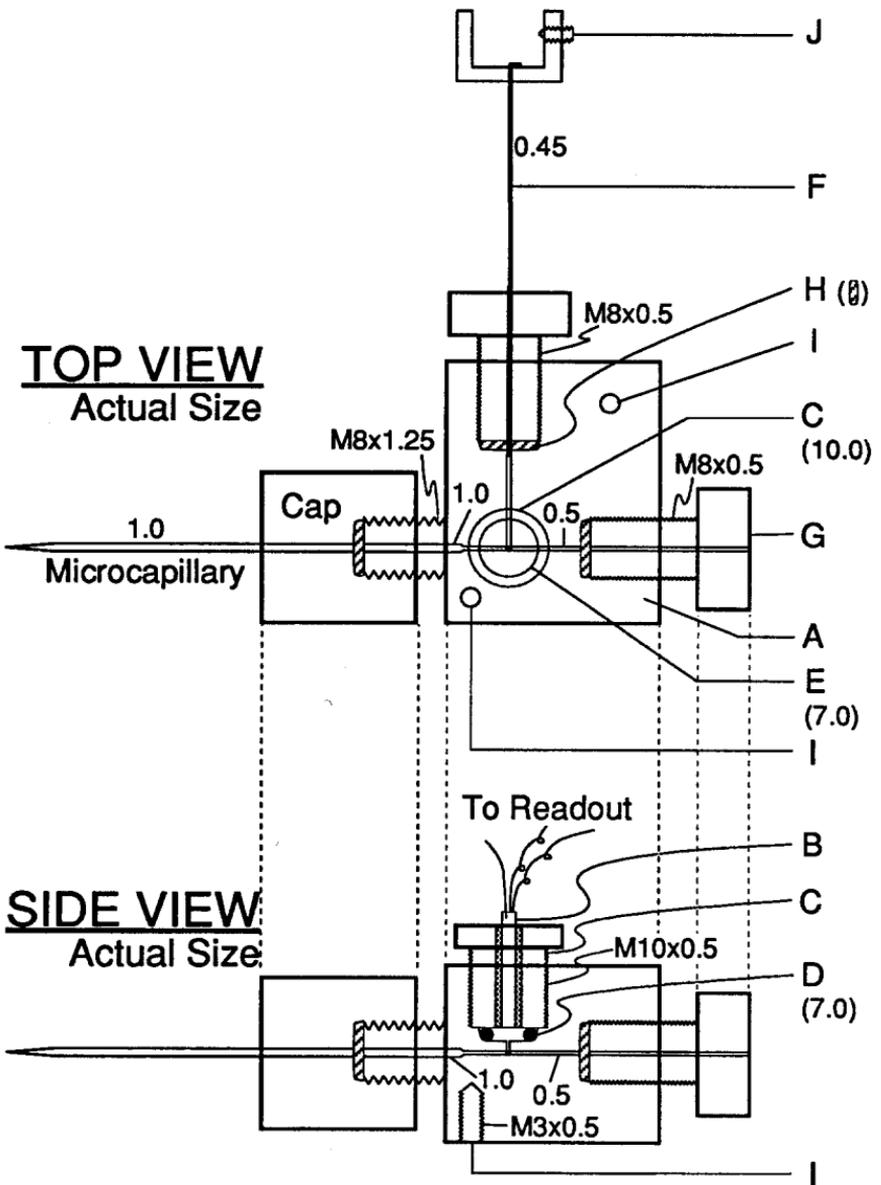
Appendix 4.1-Building a Pressure Probe

The pressure probe consists of a block of Plexiglas that has been bored to allow silicone oil to move between the microcapillary, metal rod, and pressure transducer. The pressure probe can be made for cells of various sizes by changing the diameter of the borings in the block. For the smallest probe, the borings have a diameter of 0.5 mm which is suitable for the smallest cells. For larger cells, borings are large to accommodate the larger volumes of liquid that are moved in and out of the cell. Figure 4.16 shows a detailed view of the miniature probe and a larger one suitable for large internodal cells of *Chara* or *Nitella*. The miniature probe uses a motor to drive the metal rod and thus minimizes vibration from handling the micrometer head. The large probe allows the metal rod to be driven by hand.

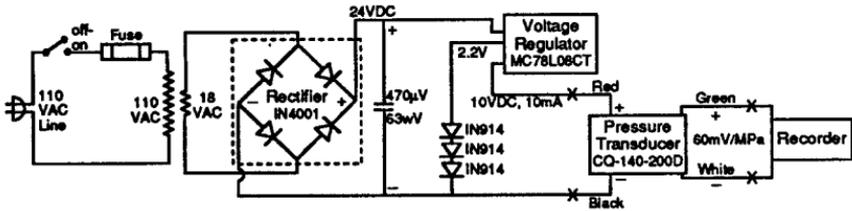
Upon settling on a design for the borings, such as shown in Fig. 4.17, have the block of Plexiglas machined and polished so that the borings can be easily seen from the outside. Mount the block on black paper on a metal plate to allow air bubbles to be easily seen in the silicone oil when filling. Mount the metal rod, micrometer head, and motor on the metal plate as shown (Fig. 4.16 and 4.17). Mount this assembly on a micromanipulator (Fig. 4.4). For various parts for the probe, see Table 4.1.

The power supply provides 10 V DC to the pressure transducer mounted on the Plexiglas block (Fig. 4.18A). The transducer returns a small DC voltage (60-70 mV/MPa) that can be detected with a strip

Figure 4.17. Construction details for a miniature pressure probe shown at a scale of 1:1. Top view extends to the plane of the microcapillary but does not show the transducer. Side view extends to the microcapillary and shows the transducer. The body of the pressure probe is made of a Plexiglas block (A) that is mounted on black paper on a metal plate. The pressure transducer (B) is sealed with epoxy (stippled) in a Plexiglas mounting (C) on the upper side of the block. An O-ring (D) in a recess (E) seals the mount in the block. The metal rod (F) and microcapillary mount on the sides of the block. The seals are punched out of rubber sheet (H) and are drilled to the size of the metal rod or microcapillary after hardening the rubber at low temperature. Two screws (I) hold the block on the metal plate. A set screw (J) holds the metal rod (F) to the micrometer screw. Metric dimensions are shown in millimeters or in millimeter diameter x number of threads per millimeter (M_x__). Not shown are the micrometer screw, motor drive, or power supply and readout.



A Transducer Power Supply



B Motor Power Supply

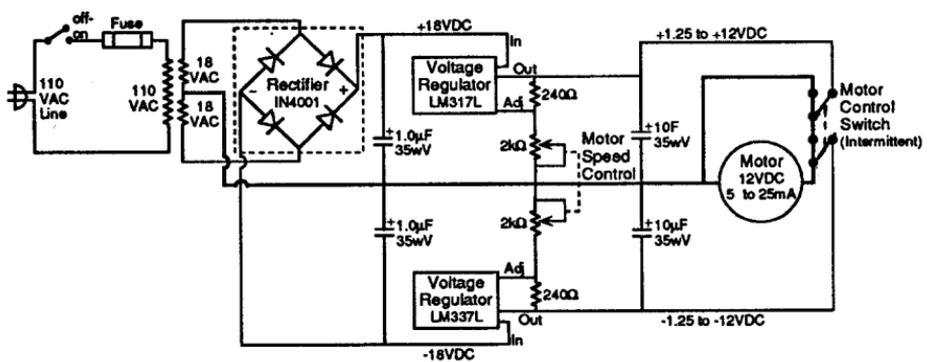


Figure 4.18. A) Power supply for the pressure transducer on the pressure probe. The transducer requires 10 VDC and consumes about 10 mA from the power supply to give an output of about 60-70 mV/MPa (see Fig. 4.6). B) Power supply for the drive motor for moving the metal rod (required for miniature probe, see Fig. 4.16). The speed control is used to vary the voltage supplied to the motor from ± 1.25 VDC to ± 12 VDC and the intermittent motor control switch reverses the voltage to turn the motor forward or backward. The motor consumes as much as 25 mA.

chart recorder (Fig. 4.6). Power consumption is small (about 10 mA) so that an inexpensive power supply can be used. In some systems, the DC output of the transducer is amplified so that a digital meter readout can display the pressure (not shown).

The drive motor for the metal rod in the small transducer uses 0-12 V DC and can be driven forward or backward by reversing the polarity of the voltage. The power consumption of the motor is about 25 mA at 12 V (Fig. 4.18B). The speed of the motor is varied by altering the voltage supplied. The power supply of Fig. 4.18B is variable and the voltage is adjusted with a dual potentiometer (motor speed control).

Table 4.1. Parts Manufacturers

Part	Manufacturer	Part No.	Recent Cost
Differential Pressure Transducer	Kulite Semiconductor Products, Inc. 1039 Hoyt Ave. Ridgefield, NJ 07657 (201) 461-0900	CQ-140-200-D	\$466.00
Motor (12 VDC), Gear, Micrometer (Motor Mike)	Oriel Corporation 250 Long Beach Blvd. Stratford, CT 06497 (203) 377-8282	18040	\$398.00
Micromanipulator	E. Leitz, Inc. Rockleigh, NJ 07647 (201) 767-1100	Leitz micromanipulator	\$2500.00
Vertical Pipette Puller	David Kopf Instruments 7324 Elmo St. P.O. Box 636 Tujunga, CA 91042 (818) 352-3274	Model 720	\$2695.00
Microcapillaries (thin-walled, 1.0 mm OD)	World Precision Instruments 175 Sarasota Ctr. Blvd. Sarasota, FL 34240 (813) 371-1003	TW100F-4	\$50.00
Silicone Oil	Stauffer-Wacker Corp. 3301 Sutton Rd. Adrian, MI 49221 (517) 264-8500	AS-4	Free sample
Small Tubing for Filling Probe with Oil, 6" length, 0.016" OD	Small Parts, Inc. 13980 NW 58th Ct. P.O. Box 4650 Miami Lakes, FL 33014 (305) 557-8222	Q-HTX-27TW	\$1.00

Table 4.1. Parts Manufacturers (continued)

Part	Manufacturer	Part No.	Recent Cost
Microcapillary Beveler	Optical Apparatus Co. 136 Coulter Ave. Ardmore, PA 19003 (800) 648-6622	EG-4 Narashige tip grinder	\$1181.00
Microscopes	Optical Apparatus Co. 136 Coulter Ave. Ardmore, PA 19003 (800) 648-6622	Optiphot: Nikon Compound Microscope with reticle, long focal length 40x objective and 10x eyepiece	\$2500.00
		SMZ-2T: Nikon Stereomicroscope with reticle, 2X objective and 15X eyepieces to give magnification of 30-189X	\$2500.00
Nanoliter Osmometer	Clifton Technical Physics Box 181 Hartford, NY 12838 (518) 632-5260	Clifton Nanoliter Osmometer	\$3000.00
Immersion Oil for Osmometer Wells	Fisher Scientific 50 Valley Stream Pkwy. Malvern, PA 19355 (800) 766-7000	12-370B: Cargille Immersion Oil, Type B, 4 oz.	\$10.00
		12-370-1B: Cargille Immersion Oil, Type NVH, 4 oz.	\$10.50