

Turgor Pressure: Direct Manometric Measurement in Single Cells of *Nitella*

Abstract. A small capillary, fused at one end, serves as a micromanometer when the open end is inserted into a large *Nitella* cell. The cell's ability to compress the gas reveals its turgor pressure directly—save for a small correction due to capillarity. The method gives a lower limit to turgor pressure for the same cell in the normal state. The common method, incipient plasmolysis, gives an upper limit. On *Nitella axillaris* cells the two methods limit the turgor pressure at 5.1 to 5.7 atmospheres. The manometric method is also applicable to growing cells, where osmotic equilibrium is not present.

Manometric measurement of pressure in plant systems has been confined to large objects such as fruits (1) and tree trunks (2). Use of this method for determination of pressure in single cells is possible, however, when the cell is unusually large (internodes of *Nitella axillaris*) and the manometer is unusually small. A micromanometer is made by fusing one end of a capillary (diameter 40 μ) and fashioning the other end into a tip like that of a syringe needle. The technique can be mastered in a few hours on a microforge (3). The sequence of a typical measurement can be seen in Fig. 1, a-c. When the manometer is put under water, capillarity reduces the gas volume from V_1 to V_2 . Atmospheric pressure, P_1 , when multiplied by V_1/V_2 gives P_2 , the pressure generated by capillarity.

The manometer, glued to a cover slip which glides across a slide on a layer of grease, is then pushed into one end of a clamped cell, and the further reduction in gas volume to V_3 is ob-

served. The pressure in the gas is P_3 , which is found by multiplying P_2 by V_2/V_3 . The pressure in the cell is $P_3 - P_2$, which is 1 atm greater than the turgor pressure present. This pressure is slightly less than the turgor pressure of the undisturbed cell, because when the manometer becomes partially filled, the sap concentration is reduced. To obtain the natural pressure, one must multiply the observed gas pressure by the ratio of sap volumes. This appears to be the only correction that need be made, because loss of sap at puncture is negligible. One can follow the effects of puncture by observing a working manometer while a second one is inserted into the opposite end of the same cell. Significant decreases in pressure (0.2 atm) occur only upon removal of the manometer.

The manometric method, uncorrected for sap dilution, gives a lower limit for the normal turgor pressure. On the other hand, the common method for turgor estimation, incipient plasmolysis (approached from the turgid state) gives an upper limit for normal turgor pressure. Both methods can be used on the same cell (Fig. 1, a-f, and Fig. 2). The point where continued increase in the osmotic potential of external medium no longer gives a decrease in pressure is the point of true incipient plasmolysis (ip). From the osmolality (C) of the medium at this point (C_{ip}), the osmolality of cell sap for the fully turgid state (C_t) may be calculated. When C_{ip} is multiplied by V_{ip}/V_t (ratio of cell volumes at incipient plasmolysis and full turgidity), C_t is determined. From data on relative volumes for *Nitella* cells (4), the osmolality of the turgid cell should be 3 to 4 percent less than that at incipient plasmolysis ($C_t = C_{ip}/1.03$). With our data, C_t is 0.213. At 23°C, the osmotic value of such a solution ($\pi_t = C_t RT$, where π_t is the osmotic potential; R , the gas constant; and T , the absolute temperature) is calculated to be

5.15 atm, a value in good agreement with the manometrically measured turgor pressure.

The concentration of the medium which brings on incipient plasmolysis detected manometrically is less than that required to bring on incipient plasmolysis detected visually (plasmolysis approached from the turgid state). For shrinkage of the cytoplasm to be visible, the true plasmolytic concentration must be exceeded. In our material, the latter method gives an estimate in excess of the true value by about 7.5 percent. The plasmolytic method, with or without corrections for volume changes, results in an estimate for normal turgor which is too high. The two methods, when applied without the corrections, therefore bracket the true turgor of a single cell.

The manometer measures turgor pressure without significantly lowering the turgidity of the cell. This is also true of Tazawa's method, in which the compressibility of cells is measured as a function of turgor (5), but our method does not require calibration through application of solutions of increasing osmotic potential. The manometric method can be applied to growing *Nitella* cells because, unlike the plasmolytic and plasmometric (6) methods, it does not require the cell to be in osmotic equilibrium.

In measurements lasting several hours, the gas in the bubble dissolves slowly in the cell sap so that V_2 can no longer be used in calibration. The pressure P_3 can readily be ascertained,

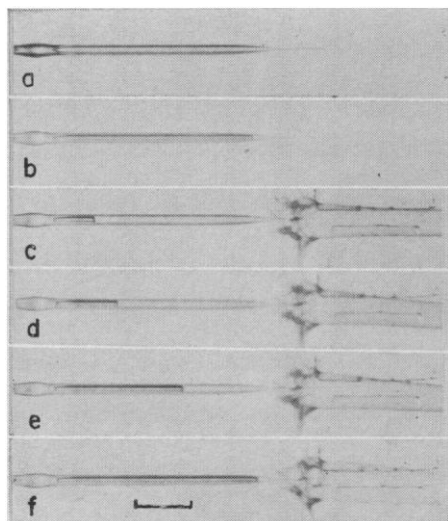


Fig. 1. Manometer at various stages of experiment ($\times 12$). (a) Manometer in air, (b) in water, (c) in cell in "ion-free" water—full turgor, (d) in cell in 0.15 osmolal sucrose, (e) in cell in 0.20 osmolal sucrose, (f) in cell in 0.25 osmolal sucrose—plasmolysis.

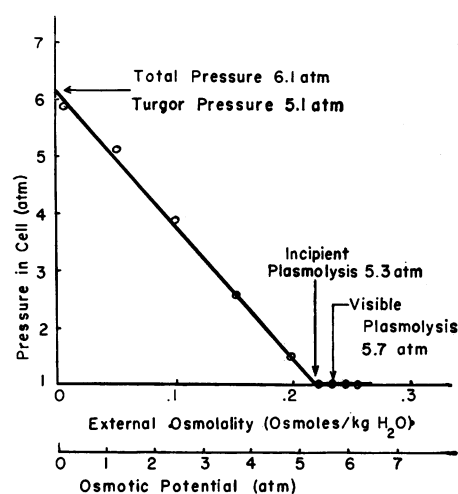


Fig. 2. Cell pressure, measured by manometer, plotted against external concentration of sucrose; pressure corrected for capillarity, data averaged for three cells. Cell pressure is 1 atm greater than turgor pressure. Calculated osmotic potential of external medium at 23°C is indicated.

however, if the cell and manometer are in a vacuum chamber (with window). A known drop in hydrostatic pressure in the chamber, brought on by a pump, will cause the bubble to expand, and the relative change in volume can be used to calculate the pressure in the bubble, regardless of its absolute volume. This calibration does not disturb the protoplasmic streaming of the cell.

The capillary method could be modified to give a method which would operate indefinitely. An oil drop could be put in an unfused capillary with one end in the cell and the other connected by a pressure valve to highly compressed gas. Changes in turgor would be matched by (recorded) changes in applied pressure needed to maintain a constant position for the drop.

Our method could be applied to large cells where the elasticity of the cytoplasm makes the methods requir-

ing osmotic equilibrium all but impossible (for example, large cells of red algae). It also appears to be practical for measurements on cells not in osmotic equilibrium. It could thus provide data pertinent to the physics of the growth process (7).

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Chromosomal Breakage Induced by Extracts of Human Allogeneic Lymphocytes

Abstract. *A high frequency of chromosomal breakage and rearrangement has been found in fibroblasts treated with extracts of allogeneic human lymphocytes. The fact that these changes were not observed when extracts of autologous lymphocytes were tested indicates that mechanisms involving self-recognition are operative. Abnormalities, such as chromosome lagging and bridges, occurring in anaphase were also served.*

The increased frequency of thyroid antibodies in mothers of children with Down's syndrome could be interpreted as an indication that factors related to immunological aberrations in a parent predispose the offspring to chromosomal abnormalities such as Down's syndrome (1). For study of the effect of immunological or self-recognizing reactions on mitoses in vitro, fibroblasts were treated with extracts prepared from circulating human lymphocytes from either the same subjects who donated the fibroblasts (autologous) or from genetically unrelated subjects (allogeneic). In earlier studies, abnormalities in chromosomal distribution as evidenced by variability in chromosome number were noted in experiments with allogeneic extracts (2). We have since turned our attention to the question of whether lymphocyte extract causes more discrete chromosomal aberrations that can be scored in metaphase or anaphase.

Extracts of lymphocytes from seven subjects were tested in a total of 17

combinations of allogeneic extracts and fibroblasts (Table 1) (3). Three of these extracts were also tested on autologous fibroblasts (Table 2). The cultures were harvested at both 24 and 48 hours after the addition of extract. Anaphases were scored for chromosome lagging and bridges, and metaphases were scored for chromosomal breakage and rearrangements (4). Two kinds of breaks were scored, those in which both chromatids were broken (chromosomal breaks) and those in which a single chromatid was broken (chromatid breaks) (Fig. 1). In both instances the lesions were scored only if they were obvious (as opposed to gap lesions). The cells were further subdivided into those containing one and those having more than one broken chromosome. Included in the rearrangements are quadriradials and other complex chromosomal formations (Fig. 1). For each experimental point, a duplicate culture treated with buffer served as a control.

High frequencies of cells with bro-

ken and rearranged chromosomes were observed in all experiments with allogeneic extracts (Table 1). Extracts from the four mothers of children with Down's syndrome, each of whom had circulating thyroid antibodies, seemed to induce greater responses than those from the three subjects who lacked evidence of immunological aberrations. Approximately one-third of the abnormal cells contained more than one broken chromosome. Chromosomal rearrangements such as quadriradials were less common but occurred in up to 10 percent of the dividing cells (up to 21 percent of the abnormal cells) in some treated cultures. In the control cultures in these experiments, about 2 percent of dividing cells had one broken chromosome; only two cells with more than one broken chromosome were observed, and rearrangements were not seen.

In anaphase, 0.5 to 2.8 percent of cells in untreated, control cultures contained abnormalities. In treated cultures these frequencies increased three- to tenfold.

Extracts prepared from lymphocytes of the three subjects (Table 1, subjects 5, 12, and 13) without known immunological aberrations were tested autologously at the same time that they were tested allogeneically. All three produced changes in genetically foreign fibroblasts, but an increased frequency of chromosomal breakage in autologous fibroblasts was not observed (Table 2).

The maximum frequency of chromosomal aberrations was observed in the first mitosis following the addition of extract (24 or 48 hours). Thereafter, the frequency declined rapidly, reaching control levels 2 to 3 days later. The maximum increase in anaphase abnormalities paralleled the increase in chromosomal breakage observed in metaphase. When extract was incubated at 37°C for 3 days and then added to fibroblasts, it had only 50 percent of the activity of an equal amount of the same extract stored for 3 days at -20°C. Thus, the decline in the frequency of aberrant cells is at least partially due to inactivation of the extract at 37°C. The effect of lymphocyte extract on growth and viability was studied with duplicate cell counts made in a hemocytometer at the time of harvest immediately after treatment of cultures with trypsin. Most cultures exposed to lymphocyte extract contained 15 to 50 percent fewer cells than did untreated con-