wounded and kept at 25°C or 32°C for 3, 6, 9, or 16 hours. The plants were then inoculated with *Agrobacterium tumefaciens* strain B6 and placed at 25°C. Twenty-four hours later the plants were placed and left at 32°C to prevent further tumor-induction. Three weeks after inoculation the plants were scored for tumor size by comparison with the photographs shown in Fig. 2. These data are presented in Fig. 3, which shows that plants maintained at 32°C after wounding and before inoculation form larger tumors than comparable plants maintained at 25°C. However, plants conditioned at 32°C for more than 32 hours form significantly smaller tumors than comparable plants conditioned at 25°C. For instance, plants conditioned for 40 hours at 25°C have a mean tumor score of 1.4, whereas those conditioned for 40 hours at 32°C have a mean tumor score of 0.5 after exposure to the bacteria as described above (*5*). It is thus possible to increase or decrease the host response by exposing wounded but uninfected plants to 32°C for various lengths of time.

Although no anatomic differences between plants maintained at 25° or 32°C for the first 24 hours after wounding are detectable by the techniques described, physiological differences are discernable. The significant differences in the conditioning response at 25° and 32°C indicate that the host is affected by even short exposures to 32°C. These findings challenge the tacit assumption that the rate of conditioning is roughly equal at 25° and 32°C.

The conditioning process has been correlated with wound healing; plants infected during the early or late stages of the wound-healing process form smaller tumors than plants infected during the middle of the process, provided the period of exposure to bacteria is limited (*2*). Both wound healing and conditioning have been shown to be accelerated by temperature. Numerous workers have reported that plant cells go through division cycles more rapidly as the temperature increases (*6*). If crown-gall tumorigenesis can occur only during certain parts of the mitotic cycle and requires that this part of the cycle have at least a minimum duration, it is possible that the thermal arrest of tumorigenesis may be due to a decrease of the time available for tumor-induction. This new hypothesis is consonant with the available data and with the reports that progressively smaller tumors are formed in infected plants incubated at temperatures ranging from optimal to the point of thermal cut-off (*7*).

The data presented here do not yield any direct evidence on the actual nature of the tumor-inducing principle. They do, however, challenge two of the premises upon which the concept of a thermolabile tumor-inducing principle with a high molecular weight rests. Although at least part of the thermal arrest of crown-gall tumorigenesis can be explained in terms of host effects, the full elucidation of this phenomenon requires further investigations.

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Osmotic Flow in a Rigid Porous Membrane

Abstract. *The concept of an internal pressure gradient in a rigid porous membrane has been proposed as the basis for osmotic flow. The origin of the pressure in terms of the theory of the chemical potential implies also the existence of states of negative pressure, that is, tension. These states have been observed experimentally by means of a Hepp-type osmometer.*

The classical analysis of swelling in cross-linked charged colloids was based on the idea that the equilibrium state of the interstitial solvent could be regarded as closely related to the Donnan equilibrium. Since in the Donnan equilibrium the crucial role of a pressure difference was well recognized both from theory and experiment, workers in the field of colloids argued convincingly that there was an excess hydrostatic pressure within a colloid phase with respect to its ambient solution. In modern terminology, the excess internal pressure is a consequence of the difference in the mole fraction component of the chemical potential of the solvent between the two phases. The analysis has been applied to equilibrium states of ion-exchange resins. In extending the Teorell-Meyer-Sievers theory of transport processes in

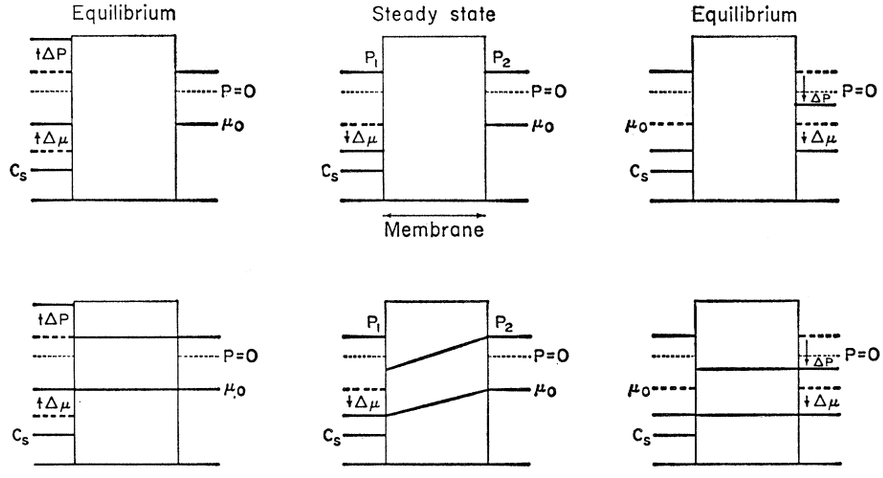

Fig. 1 (top). Thermodynamic variables in the external phases. Fig. 2 (bottom). Profiles of thermodynamic variables throughout the membrane. The zero of the pressure scale is indicated by the fine dotted line and so designated in all diagrams.

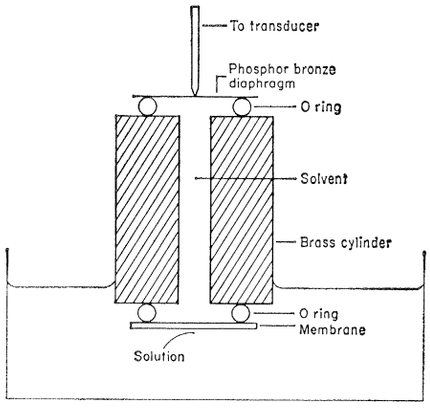

Fig. 3. Hepp-type osmometer. A variant of Hansen's design.

charged membranes, Schlögl (*1*) has taken into account the variation of the internal hydrostatic pressure—in addition to the more familiar electrostatic potential and ionic concentrations—and has predicted "anomalous" transport for both the solvent and solute species. Subsequently, the role of hydrostatic pressure has been extended to explain "ordinary" osmotic flow arising in a rigid porous membrane in the face of an impermeant uncharged solute (*2*). The essential difference which should be noted between the uncharged and the charged barrier is that in the former the pressure falls, whereas in the latter it rises with respect to the outer solution. I now offer further evidence for the validity of the hypothesis of an internal pressure and a pressure gradient and show experimentally that, as predicted by the theory of the chemical potential, the internal pressure can fall even to an absolute negative value, namely, a state of tension (*3*).

Before discussing the profiles of energy and pressure within the membrane, I will review the thermodynamic variables of the solution and the solvent phase associated with the elementary osmotic system. In Fig. 1 the middle diagram depicts the fall in chemical potential of the solvent from the level μ_0 as a consequence of adding impermeant solute at concentration C_s, both phases being at the same pressure, $P_1 = P_2$ (for example, atmospheric pressure). A state of equilibrium can be obtained in two ways, namely, either by constraining the solution phase whereupon pressure rises by ΔP (left diagram), or by constraining the solvent phase whereupon pressure of the solvent phase falls by ΔP (right diagram). In either case, the chemical potential of the solvent becomes equal in both phases. The conditions depicted on the left occur in the classical Pfeffer osmometer and those on the right in the osmometer of the Hepp type (*4*). The thermodynamic quantities can be specified by invoking the necessary and sufficient condition for equilibrium of a given permeant species according to Gibbs. Thus, for the solvent species

$$\mu_1 = \mu_2$$

and, explicitly, for an ideal solution

$$RT \ln N_1 + P_1\overline{V} = RT \ln N_2 + P_2\overline{V} \quad (1)$$

or, for a pure solvent in phase 2

$$RT \ln N_1 + P_1\overline{V} = P_2\overline{V} \quad (2)$$

where N is the mole fraction of the solvent, and $\overline{V}$ its partial molar volume, T, temperature in degrees Kelvin, and R, the gas constant. Thus P_2 must take on a negative value if the mole fraction term falls below a certain value (Fig. 1, right). The physical interpretation of a negative value of P_2 is that for a sufficient $\Delta\mu$ a tension stress will develop in the pure solvent as the system tends toward equilibrium. This state can be realized only if there is continuity of the liquid phase with respect to the walls of the constraining vessel.

The thermodynamic variables of the macroscopic regions of either external

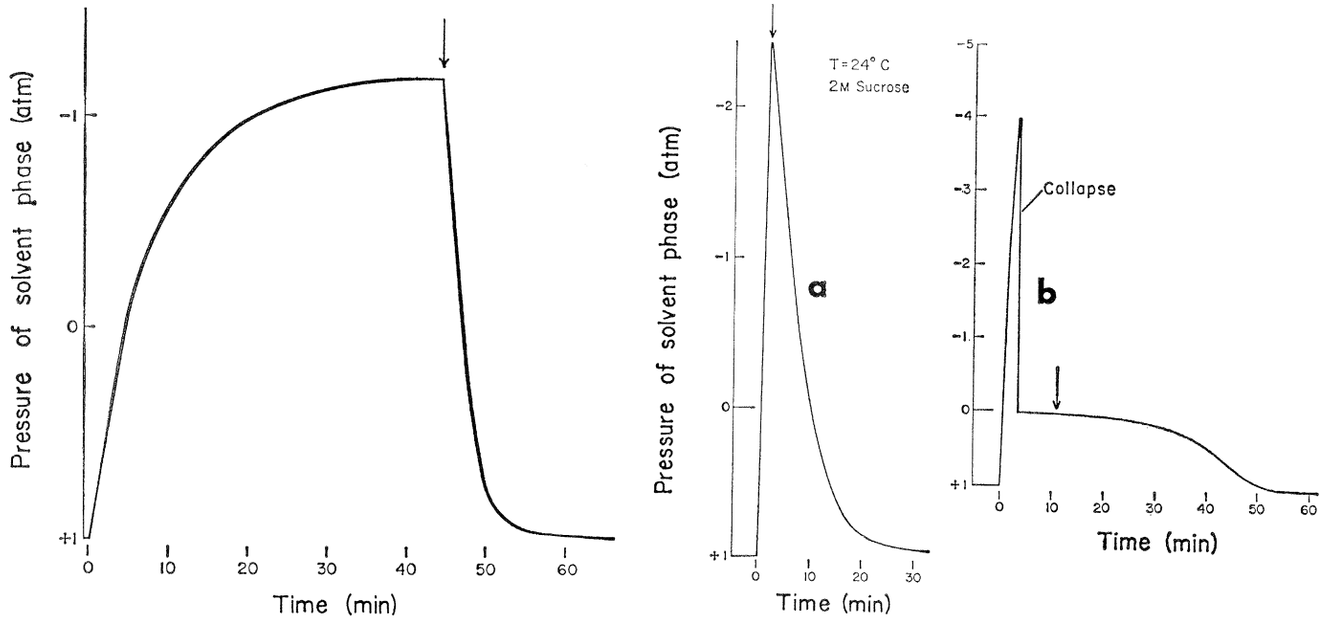

Fig. 4 (left). Pressure response in solvent phase to a solution of polyethylene glycol (120 g/1000 ml; molecular weight, 6000–7500; temperature, 24°C). Arrow indicates moment when solution was replaced by distilled water, and the relaxation began. The asymmetry in the "rise" and "fall" of pressure is an indication of the difficulty in establishing boundary conditions at the membrane with a viscous solution. Fig. 5 (right). Pressure response to sucrose solution. (*a*) The pressure was allowed to rise only to minus 2.4 atm and then to relax by replacing the solution with distilled water at the moment indicated by arrow. (*b*) Experiment repeated but allowed to attain higher negative pressure until cavitation set in. Although not indicated on this scale, pressure fell ("instantaneously") to +22 mm-Hg with osmotic flow continuing at this constant pressure. Arrow indicates moment when solution was replaced with distilled water and relaxation of pressure began.

phase having been considered, the analysis may be extended to the membrane phase both for the equilibrium and the steady state by invoking the assumption that permeant species are locally at equilibrium in the membrane-solution interface. It follows that the chemical potential of the solvent is continuous in this region (Fig. 2, middle), namely,

$$\Delta\mu_1 = \Delta\mu_{\text{membrane}} \quad (3)$$

Since only solvent species are present in the membrane, the change in the chemical potential of the solvent can be satisfied only by a change in the pressure component and, accordingly, if we make the further assumption that the thermodynamic expressions for the macroregion hold for the microregion of the membrane

$$RT \ln N_1 = \Delta P \overline{V} \quad (4)$$

Thus just within the "pore" the pressure must fall from P_1 by the amount

$$\Delta P = \frac{RT}{\overline{V}} \ln N_1 \quad (5)$$

or, for a very dilute solution, $\Delta P \approx RTC_s$. As predicted by this expression, the mole fraction of water in an ideal solution of about 0.04M concentration at 24°C should result in a drop in pressure of 1 atm. The middle diagram in Fig. 2 depicts the case in which the concentration of solute is greater than 0.04M so that the absolute pressure just within the barrier should fall to a negative value, since P_1 is 1 atm.

Throughout the membrane the pressure will vary with distance depending on the hydraulic resistance. The linear rise in pressure to P_2 as shown (Fig. 2) holds for a membrane with uniform hydraulic resistance. So far as the membrane is concerned, solvent will move down the pressure gradient from the pure solvent phase to the solution phase. The main point is that the $\Delta\mu$ of the solvent gives rise to a nonisobaric regime within the membrane even though the external phases are at the same pressure. In porous membranes a pressure gradient gives rise to solvent flow which is predominantly nondiffusional (*5*).

I have attempted to verify the hypothesis of a pressure drop within the barrier by means of a Hepp-type osmometer. This design (Fig. 3) is a variant of the unit developed by Hansen (*6*). Most of the experimental work has been conducted with cellulose acetate membranes of the type used for desalination. O-rings seal the membrane with respect to the solvent chamber. The chamber containing the solvent is provided by a 1-mm cylindrical hole in a brass rod 3 cm long and 1 cm in diameter. By terminating the other end of the chamber in a 0.010-cm phosphor-bronze diaphragm by means of O-rings, the solvent phase is fully constrained and the pressure is reflected in the movement of the diaphragm, which can be recorded by a suitable displacement transducer. It is important to emphasize two crucial points in the experimental procedure: (i) the brass chamber must be cleaned thoroughly to promote wetting of the walls, and (ii) the system must be pumped down thoroughly and assembled under water in order to minimize the onset of cavitation due to presence of "gas nuclei" (*7*).

The tracing in Fig. 4 shows the pressure of the solvent phase as a function of time brought about with an aqueous solution of polyethylene glycol of average molecular weight 6000 at the concentration of 120 g per 1000 ml of water, (approximately 0.02M). This molecule is absolutely impermeant, and accordingly the negative pressure attained is an equilibrium state. That this solution is not ideal is evidenced by the observed ΔP of approximately 2.2 atm as against 0.4 atm for an ideal solution. This markedly nonideal behavior is typical of solutions containing solutes with long-chain molecules (*8*). Since the flow is zero, the gradient of pressure must be zero; and thus pressure in the solvent phase can be extended back into the membrane phase, giving a flat pressure profile throughout as seen in Fig. 2 (right). In this manner one, in effect, probes the pressure of the solvent throughout the membrane. Although a tracing is not included, it should be understood that a more dilute solution gives rise to a positive pressure.

In Fig. 5*a* the pressure recording with time is seen for a solution of 2M sucrose. Osmosis proceeded for about 5 minutes; at the moment indicated by the arrow the solution was replaced by distilled water, whereupon the system relaxed from the pressure of minus 2.4 atm to the initial pressure of 1 atm. The experiment was repeated with the recorder amplification reduced by one-half. The sudden collapse of negative pressure from the peak of minus 4.0 atm (Fig. 5*b*), far short of minus 42 atm as required by Eq. 5, to a positive pressure of approximately 22 mm-Hg (the vapor pressure of water at 24°C) is a typical result when cavitation occurs owing to residual gas nuclei. In my experiments peak pressures as high as minus 10 atm have been observed. As is well known, in the conventional Pfeffer-type osmometer, the peak pressure attained would have been less than the minus 42 atm, since the sucrose species is permeant, that is, sucrose has a "reflection coefficient" of about 0.85 for this membrane. Moreover, in this case, the pressure would not have attained an equilibrium state but instead would have risen to a maximum and relaxed back to the initial pressure. Unfortunately, the "continuous" treatment of the thermodynamic variables (*9*) for the case of permeant solute is a difficult problem which remains to be solved. In general a finite pressure gradient will exist throughout the regime. For increasingly permeant species the gradient approaches zero, resulting in an isobaric regime with ordinary binary diffusion.

As shown by theory and confirmed by experiment, in the elementary osmotic system the pressure gradient causing flow of solvent through the membrane is established by a drop in pressure just within the membrane at the membrane-solution interface. Moreover, the drop in pressure is not restricted to positive values but indeed can fall even to negative values, implying a tension state of the solvent within the barrier.

Tension states have been postulated and measured in plants (*10*) and can be accounted for by similar consideration of the leaf-xylem system by means of the chemical potential.

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