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How Münch's adaptation of Pfeffer's circulating water flow became the pressure-flow theory, and the resulting problems — A historical perspective

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ABSTRACT

Long-distance transport of photoassimilates in the phloem of vascular plants occurs as bulk flow in sieve tubes. These tubes are arrays of cells that lose nuclei, cytoskeleton, and some organelles when they differentiate into mature sieve elements. Symplasmic continuity is achieved by perforations that turn the cell walls between adjoining sieve elements into sieve plates. These structural features are interpreted as adaptations that reduce the resistance sieve tubes offer to cytoplasmic bulk flow. According to the common reading of Ernst Münch's pressure-flow theory, the driving forces for these flows are osmotically generated gradients of hydrostatic pressure along the sieve tubes. However, the significance of pressure gradients in the flow direction has also been questioned. Münch himself stated that no detectable pressure gradients existed between the linked osmotic cells that he used to demonstrate the validity of his ideas, and the earliest explanation of osmotically driven flows by Wilhelm Pfeffer, on which Münch based his theory, explicitly claimed the absence of pressure gradients. To resolve the apparent contradiction, we here reconstruct the history of the idea that osmotically driven transport processes in organisms necessarily require steps or gradients of hydrostatic pressure along the transport route. Our analysis leads us to conclude that some defects of overly simplifying interpretations of Münch's ideas (such as the sieve plate fallacy) could be avoided if our descriptions of his theory in textbooks and the scientific literature would follow the logics of the theory's earliest formulations more closely.

1. Pressure flow

The distribution of photosynthates throughout the bodies of vascular plants occurs as bulk flow in the sieve tubes of the phloem. These tubes consist of sieve elements, cells that are symplasmically coherent due to large pores in the sieve plates, the perforated cell walls between them (Behnke and Sjolund, 1990; Knoblauch and Peters, 2013). Today, sieve tube transport is commonly explained by the pressure-flow theory of German forestry botanist Ernst Münch (1876–1946). It seems widely accepted, as Zimmermann (1964, p. 23) put it, that "there has to be a turgor gradient within the sieve tubes from source to sink areas" for Münch's mechanism to operate. The dominating view is expressed in the popular textbook that we have adopted for our plant physiology classes:

> "The mechanism of phloem translocation in angiosperms is best explained by the pressure-flow model … The pressure-flow model explains phloem translocation as a flow of solution (mass flow or bulk flow) driven by an osmotically generated pressure gradient between source and sink …. The pressure-flow model, first proposed by Ernst Münch in 1930, states that a flow of solution in the sieve elements is driven by an osmotically generated *pressure gradient* between source and sink ($\Delta\Psi_p$). Phloem loading at the source and phloem unloading at the sink establish the pressure gradient" (Taiz and Zeiger, 2010, p. 281; original emphasis).

Münch actually presented his version of the model first in 1926, but this is a minor inaccuracy. Our concern is the portrayal of axial pressure gradients as the immediate causes of the observed translocation. While significant gradients of hydrostatic pressure doubtlessly exist along most working sieve tubes (Turgeon, 2010; Mullendore et al., 2010; de Schepper et al., 2013; Knoblauch et al., 2016; Stanfield et al., 2018), the role of these gradients in the transport mechanism is far less obvious than the above quotations suggest. Münch's work provides a case in point. On one hand, he postulated that in osmotic systems, every concentration gradient implied a corresponding pressure gradient. On the other hand, he maintained that pressure gradients could not possibly

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exist in the linked osmotic cells that he used with great success to demonstrate osmotically driven 'pressure flow' (Münch, 1930, pp. 9, 40).

One may assume that apparent contradictions like this merely represent linguistic ambiguity. In fact, classical texts exhibit a fair degree of terminological vagueness, since various key concepts had not been defined yet when biologists began to investigate osmotic phenomena in organisms. However, the idea that hydrostatic pressure gradients are essential for sieve tube transport has been questioned repeatedly based on experimental as well as theoretical grounds, indicating that the problem lies on the conceptual rather than the linguistic level.

Here we characterize this conceptual problem by evaluating criticism of the notion of pressure flow that was raised over the last 50 years (chapter 2). On this basis, we reconstruct how Münch's predecessors explained osmotically driven intracellular fluxes without invoking pressure gradients (chapter 3), before we analyze Münch's adaptation of the older ideas and their transformation into the pressure-flow theory (chapter 4). Finally, we discuss how a misunderstanding of pressure gradients in sieve tubes fosters misconceptions like the sieve plate fallacy (chapter 5), and offer a remedy (chapter 6).

2. Pressure gradients and sieve tube flow — cause, effect, both, or none?

Half a century ago, Walter Eschrich, Ray Evert, and John Young studied solute transport in tubes made of semipermeable dialysis membranes. In their standard setup, a tube of 7 mm diameter and about 20 cm length was immersed vertically in a water-filled glass cylinder (Fig. 1A; Eschrich et al., 1972). The top end of the tube was either closed or open. The tube was filled with water in its upper part but with concentrated sucrose and dye solution at the bottom. Therefore the semipermeable tube wall was exposed to a large osmotic gradient between the internal and external medium in the lower part of the tube (marked 'x' in Fig. 1A), but to no such radial osmotic gradient along the rest of its length. As a result, water entered the tube at the bottom following its concentration gradient. This increased the hydrostatic pressure in the tube, which drove water out in the upper part of the tube where the radial pressure gradient across the semipermeable wall was not balanced by an opposite osmolarity gradient. In tubes with open upper ends, the increased internal hydrostatic pressure also showed as a rise of the fluid level. The osmotically driven circulation of water — into the tube on the bottom, out of the tube on the top — implied an upward flow within the tube, which manifested itself as an upward movement of the front between the stained sucrose solution and the unstained water. The velocity of this movement depended on the initial concentration of the sucrose solution (Fig. 1B). Eschrich et al. (1972, p. 288) estimated the axial pressure gradient that arose in the semipermeable tubes due to viscous flow to be some 10^5 times smaller than the pressure gradient in the fluid caused by gravity and concluded:

> "Gradients of hydrostatic pressure along the direction of flow due to gravity and resistance to viscous flow are entirely negligible and offer no explanation for [the] present results. The results obtained can be explained solely in terms of hydrostatic and osmotic pressure differences across the semipermeable membrane without consideration of any hydrostatic pressure gradient along the direction of flow" (Eschrich et al., 1972, p. 281).

Consequently, Eschrich et al. (1972) suggested to call the mechanism they had characterized *volume flow*, to distinguish it from Münch's *pressure flow* that appeared to require axial pressure gradients.

Criticism arrived promptly. Paul Weatherley bluntly rejected the idea of renaming the mechanism of phloem transport as "merely confusing". Since the flow rate (J_V) in a tube connecting two osmotic cells depended on the difference of the hydrostatic pressures in the cells

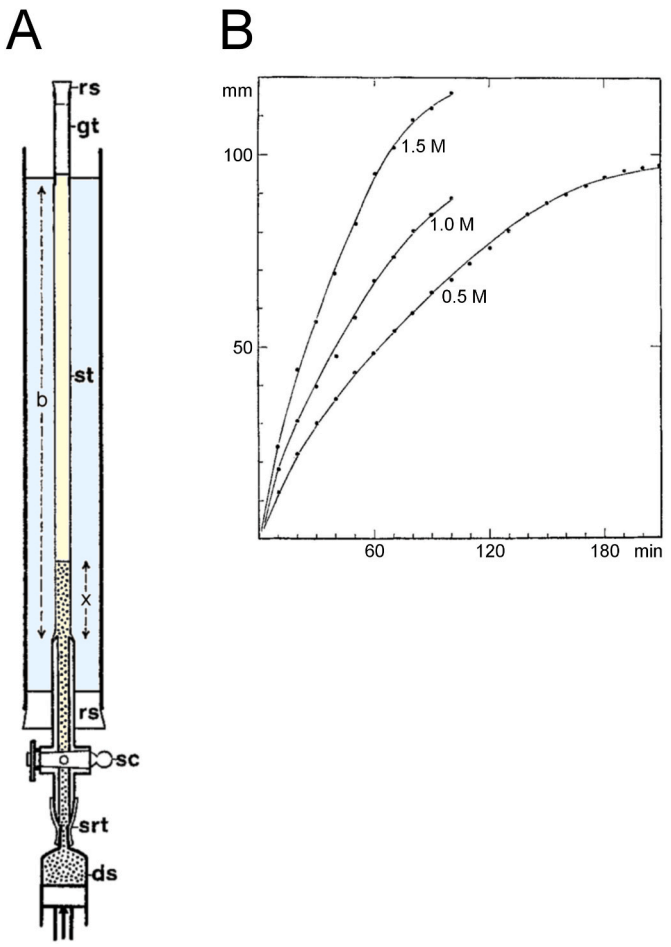

Fig. 1. (A) Device for studying osmotically induced mass flow (Eschrich et al., 1972, Fig. 1). A tube made of semipermeable membrane (*st*) is immersed in a water-filled glass cylinder. The tube is filled mainly with water, but with a concentrated sugar and dye solution (indicated by stippling) at the lower end. The length of the tube that is in contact with the external medium is marked *b*, while the zone that experiences the steep osmotic gradient between external water and internal sugar solution is marked *x*. In this zone, water enters the tube following its concentration gradient, causing an increase of hydrostatic pressure within the tube and consequently an efflux of water where no osmotic gradient exists. This circulation of water drives an upward movement of the front between stained sugar solution and water; typical time courses for tubes with initial sugar concentrations of 0.5 M, 1 M, and 1.5 M are reproduced in (B) (Eschrich et al., 1972, Fig. 2; labels redrawn). Coloration (semipermeable tube, yellow; water bath, blue) added to the original figures for clarity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

($P_1 - P_2$) and on the hydraulic conductivity coefficient (L_t) of the tube (Fig. 2B),

$$J_V = L_t\,(P_1 - P_2) \qquad \text{(Eq. 1)}$$

he argued that "mass flow can only occur … in response to a difference in hydrostatic pressure … however small it may be" (Weatherley, 1973, p. 184).

There are two problems with this argument. First, equations like the above define relationships between physical parameters, but do not establish causality as Weatherley implied. Equation (1) simply states a correlation between ($P_1 - P_2$) and L_t: if one decreases, the other must increase to result in the same J_V. Thus, if the output of the mechanism that drives flow in sieve tubes is regulated to maintain a required flow rate (J_V) independently of a tube's conductivity (L_t), pressure differences ($P_1 - P_2$) of magnitudes that depend on the L_t of each tube will occur. But

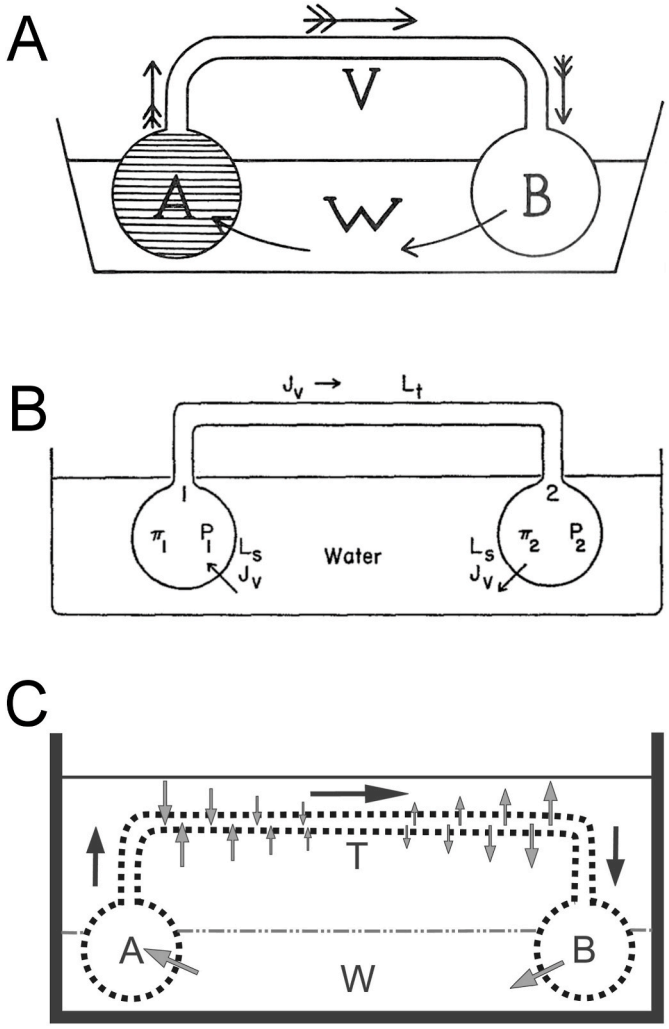

Fig. 2. Simple models representing the core idea of the pressure-flow theory. (A) Münch's *Grundversuch* (basic experiment) of 1930 (Fig. 2 in the original). Two osmotic cells *A* and *B* with semipermeable walls are connected by a tube (*V*) and immersed in water (*W*). As long as the solution in cell *A* is more concentrated than that in cell *B*, water will circulate through the system as indicated by arrows. (B) Version from Weatherley (1973, Fig. 1), in which supposedly relevant physical parameters are indicated. Flow from cell 1 to cell 2 via the connecting tube implies a difference in hydrostatic pressure between the cells, $P_1 > P_2$. (C) In the version by Stanfield et al. (2018, Fig. 7), the conducting tube has semipermeable walls and is an active part of the osmotic system rather than an osmotically passive connector. The entire system is immersed in water, in contrast to (A) and (B). Gray arrows indicate water fluxes across semipermeable membranes.

since these pressure gradients would be of little help in explaining the generation of flow and its regulation, they could be considered mere side-effects of flow. Responding to Weatherley, Eschrich and colleagues stressed that "pressure gradients arising from resistance to viscous flow exist in any real system, but these pressure gradients, irrespective of their magnitude, are in no way an essential feature of solution flow" (Young et al., 1973, p. 355). This obviously affected the interpretation of phloem function:

> "It is generally acknowledged 1. that the plasmalemma of the sieve tube is a differentially permeable membrane, and 2. that sugars are actively secreted into and absorbed from the lumen of the sieve tube. We have demonstrated in this paper that solution flow in the sieve tube follows as the *inevitable* consequence of these two factors. Thus if one accepts these two points, it is not meaningful to ask whether phloem translocation occurs by a volume-flow mechanism or by some other mechanism. Rather, it is only meaningful to ask whether some other mechanism *in addition* to the volume-flow mechanism is operative. This important point has apparently not been recognized in previous discussions of phloem translocation mechanisms" (Young et al., 1973, p. 364; original emphasis).

The second problem concerns the distinct characters of the models used by Weatherley (1973; Fig. 2B) and by Eschrich et al. (1972; Fig. 1A) to represent sieve tubes. Weatherley followed Münch in conceptualizing sieve tubes as osmotically passive connectors between two independently acting osmotic cells, one representing source and the other sink tissues (Fig. 2A and B). Sieve tubes in this concept were functionally analogous to pipes used in plumbing, and their behavior could be evaluated quantitatively using the same mathematical approaches. In contrast, the sieve tube analogs in the models of Eschrich et al. (1972) *were* the osmotic cell in their experiments (Fig. 1). Their reasoning built on — or produced? — a conceptualization of sieve tubes in plants as osmotically active components of a large, coherent osmotic system. Due to the nature of their model, and unlike Weatherley (1973), Eschrich et al. (1972) could not simply borrow a quantitative formalism from what engineers knew about plumbing to describe the functioning of semipermeable tubes; they rather had to develop the required formalism themselves. The importance of the osmotic properties of the sieve tube membranes for the quantification of phloem transport has been recognized in subsequent studies (e.g., Tyree et al., 1974; Thompson and Holbrook, 2003; Lacointe and Minchin, 2008; Cabrita et al., 2013), and was visualized by Stanfield et al. (2018) who re-drew Münch's model as a single, internally differentiated osmotic cell (Fig. 2C).

Weatherley (1973, p. 186) agreed that bulk flow could occur with practically negligible pressure gradients when L_t is large as in the macroscopic tubes used by Eschrich et al. (1972). But this conclusion, he insisted, could not be transferred to microscopic pipes such as sieve tubes with much lower L_t, i.e., with much larger hydraulic resistance. Ironically, this criticism applies also to the macroscopic models Münch (1926, 1927, 1930) had presented to bolster his ideas, models that Weatherley unhesitantly adopted to clarify his understanding of bulk flow in real sieve tubes (Fig. 2B). In this context, work by Jensen and colleagues is of interest. Having expanded the analysis by Eschrich et al. (1972) using similar macroscopic setups (Jensen et al., 2009a), this group studied osmotically induced bulk flow in microfluidics systems (Jensen et al., 2009b). The cross-sectional areas of the tested micro-channels were reduced by factors of up to 3800, compared to Eschrich et al. (1972). Nonetheless, axial gradients of hydrostatic pressure were not required to explain the results (Jensen et al., 2009b).

Animal cells differ biomechanically from plant cells in lacking cell walls that could counteract intracellular hydrostatic pressure (Peters et al., 2000). Thus it may surprise that Young et al. (1973) suggested that their volume-flow mechanism also worked in animal tissues, referring in particular to work by Diamond and Bossert (1967). These authors had attempted to resolve the long-standing enigma of efficient water transport across epithelia in the absence of trans-epithelial gradients of hydrostatic pressure or osmolarity. Such counter-intuitive water fluxes could be generated in artificial, macroscopic three-compartment setups, where they were linked to active osmolyte transport (Curran and MacIntosh, 1962). But what the natural equivalents of the artificial structures might be had remained elusive. Epithelia generally consist of coherent layers of polarized cells with two plasma membrane domains of distinct composition, an apical and a basal one facing extra- and intracorporeal fluids, respectively (Matlin and Caplan, 2013). The cells are connected to their neighbors apically by tight junctions and link basally to a sheet of specialized extracellular matrix, the basement membrane (Fig. 3A). So-called lateral intercellular spaces exist between adjacent cells (Fig. 3A). Diamond and Bossert (1967) modeled these 'lis' as channels with semipermeable walls that were closed at one end by tight junctions but open at the other (Fig. 3B). Solutes were actively

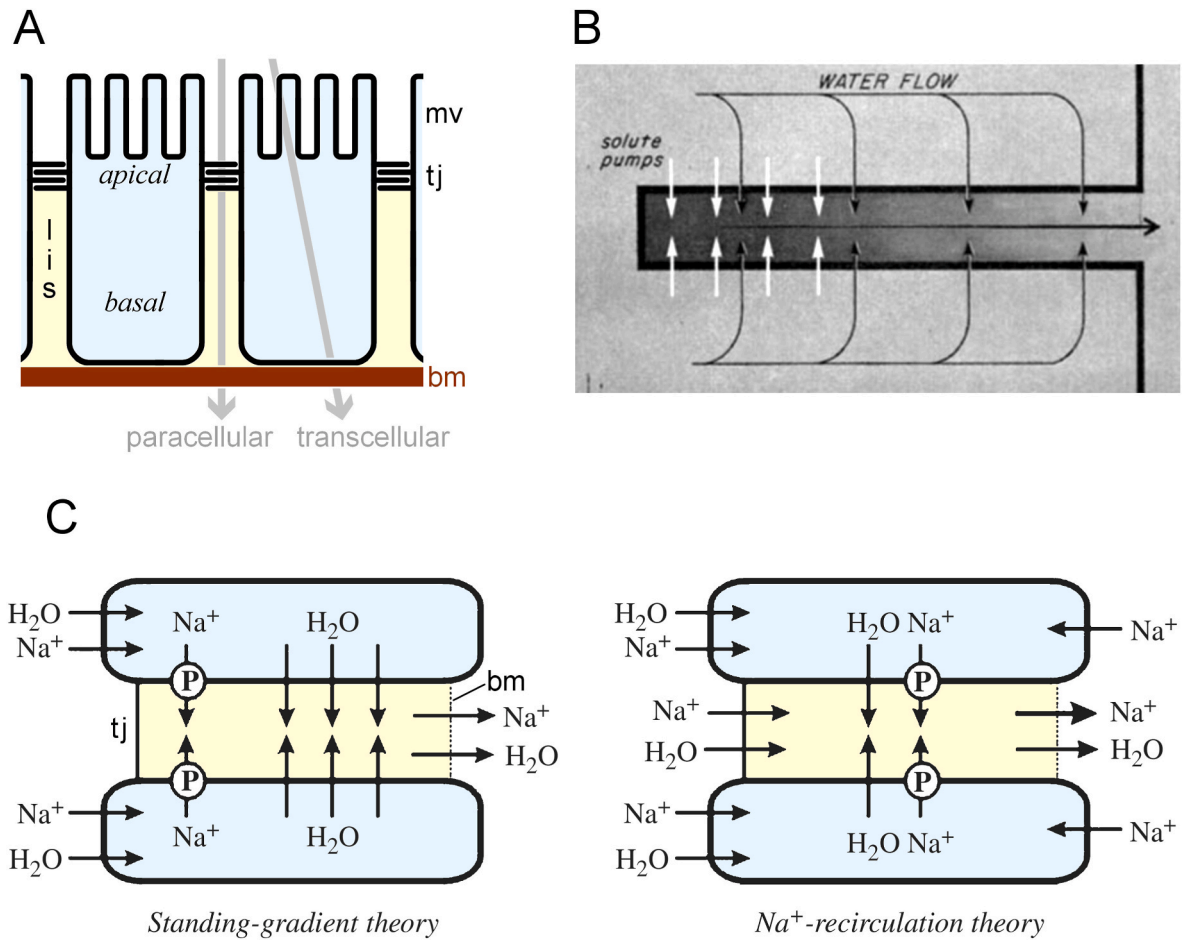

Fig. 3. Water transport across epithelia. (A) Epithelium structure. Cells with apical microvilli (mv) are linked by tight junctions (tj) apically and connect to the basement membrane (bm) basally; the cells enclose lateral intercellular spaces (lis). Water entry into the tissue may be transcellular, paracellular, or a mixture of both. (B) The standing-gradient model (Diamond and Bossert, 1967, Fig. 2). The lateral intercellular space opens basally (right) but is closed apically (left) by tight junctions. Solutes are actively transported into the apical intercellular space, causing osmotic water uptake along the channel and bulk flow (straight black arrow) out of the basal opening. (C) The standing-gradient theory (left) compared to a more recent model (right) with water and solute fluxes across tight junctions and a basal Na^+-recirculation (Larsen et al., 2009, Fig. 10; with modifications. *P*, active membrane transport).

translocated from the cells into the channel near its closed apex, and water would passively follow along the entire length of the tube. Consequently, the interstitial fluid in the channel would be diluted continuously as it moved, driven by its own volume gain, towards the open channel end. In steady state, a standing gradient of osmolarity would be observed in the channel, apparently stable and motionless but in fact the signature of steady, osmotically driven flow (Fig. 3B). Solutes and water translocating across the epithelium in this manner ultimately originated from the extracorporeal fluid from where they were taken up over the apical plasma membrane, and left the system across the porous basement membrane to join the intracorporeal fluid (Fig. 3C, left). No hydrostatic pressure or osmolarity gradients were required between the fluids on the two sides of the epithelium to fuel this *standing-gradient flow*.

Conceivably, standing-gradient flow (Fig. 3B) could be established also in the experimental system of Eschrich et al. (1972; Fig. 1A), if sucrose were added continuously at the lower end of the semipermeable tube, and if the tube contents were allowed to escape without having to cross a semipermeable membrane at the upper end. Yet the model of the zoologists (Diamond and Bossert, 1967) sparked the interest of the botanists (Young et al., 1973) for another reason in the first place: no axial gradients of hydrostatic pressure were required for the model to explain empirical observations. The velocity of the channel contents at a given position depended on the total amount of water driven osmotically into the length of the tube on the apical side of that position. Increasing the hydraulic resistance, for example by partially occluding the basal opening of the lateral intercellular space with the porous basement membrane, would cause increased hydrostatic pressure in that space as a whole, but since this could not affect the governing osmotic parameters, the system would still function as it did before (Diamond and Bossert, 1967, p. 2067). In other words, modulations of the pressure in the flowing fluid were side-effects of changes in the hydraulic resistances along the path of flow, which as such had nothing to do with the physical processes that drove the flow. The correspondence between this model and the ideas of Young, Evert, and Eschrich is obvious.

Over time, the original-standing gradient model became modified by including the basement membrane as a basal closing structure of the lateral intercellular space, by allowing solute and solvent transport across tight junctions, by assuming a homogenous distribution of active solute transporters (mostly Na^+/K^+-ATPases) in the laterobasal plasma membrane, and by adding a Na^+ recirculation loop at the basal side of the cells (Fig. 3C, right). Intriguingly, review articles documenting this progress never even mentioned axial gradients of hydrostatic pressure in the lateral intercellular spaces (Larsen and Møbjerg, 2006; Fischbarg, 2010; Whittamore, 2012; Larsen et al., 2014; Larsen and Sørensen, 2020a,b). Weatherley's (1973) argument that mass flow can only occur in response to differences in hydrostatic pressure could be applied to lateral intercellular spaces just as well as to sieve tubes, of course. Nonetheless, researchers in the field made progress towards a quantitative understanding of transporting epithelia although they ignored

practically irrelevant pressure gradients that might have been required for theoretical consistency.

Twenty years after the debate around the volume-flow model (Eschrich et al., 1972), Phillips and Dungan (1993) demonstrated that fluid transport in tubes with semipermeable walls could be characterized by two dimensionless numbers. These parameters were, first, the ratio between axial resistance to viscous flow and the resistance of the semipermeable membrane to water permeation, and second, the ratio of the osmotic pressure of the transported solution to the axial pressure gradient along the transporting tube. A decade later, Thompson and Holbrook reached similar conclusions concerning the usefulness of the two dimensionless ratios (Thompson and Holbrook, 2003: see also Thompson and Holbrook, 2004; Thompson, 2005, 2006). These authors showed that since live sieve elements almost always were in water potential equilibrium, the first ratio (called $\widehat{R}$) became practically irrelevant compared to the second ratio ($\widehat{F}$) in describing sieve tube transport (Thompson and Holbrook, 2003). So what exactly is $\widehat{F}$, in simple words? Phillips and Dungan (1993, p. 468), who called it H, described it as "a measure of the relative importance of osmotic forces and frictional losses as a result of flow of a viscous fluid". Thompson and Holbrook (2003) stressed that sieve tubes worked most efficiently when $\widehat{F}$ was large. Notably, $\widehat{F}$ increases with increasing osmolarity of the sieve tube sap and with decreasing magnitudes of turgor gradients along the transporting tubes. The latter point appears intuitive as maintaining pressure gradients steeper than required to achieve the desired transport rates would be a waste of energy and demand unnecessarily robust tube structures. The conclusion also accords with the interpretation of the simplified internal structure of sieve elements as an adaptation to the requirement for low hydraulic resistance to cytoplasmic bulk flow (Ehlers et al., 2000; Heo et al., 2017). High $\widehat{F}$ indicates that turgor pressure is similar along the entire sieve tube. *Osmoregulatory flow* proceeds nonetheless in this tube as a consequence of turgor regulation that is executed autonomously by multiple elements linked into a symplasmic unit:

> "… the transport phloem is conceptually better conceived of as a turgor regulating 'unit' than as a conduit for transport. This distinction highlights turgor regulation as the primary means of controlling translocation, rather than turgor *gradient* regulation, and greatly simplifies the mechanistic demands placed on membrane solute transport. Turgor drops were once sought as confirmation of the pressure flow hypothesis (Fisher, 1978), but a very large turgor drop would actually indicate that the sieve tube is transporting solute inefficiently. Thus, it is probably misleading to emphasize turgor drops too much in our conceptual description of the pressure flow hypothesis" (Thompson and Holbrook, 2003, p. 1573; original emphasis).

Weighing the arguments discussed so far, we can only agree. But how did the notion that axial gradients of hydrostatic pressure were essential for phloem transport originate in the first place?

3. Münch flow before Münch — Pfeffer's thought experiment

In 1855, Carl Nägeli reported that when a large, cylindrical internode cell of *Nitella* was exposed to water at one end but to a strong sugar solution at the other, the usual, cycling cytoplasmic streaming was replaced by "a stream from the water- to the sugar-bathed end" (Nägeli, 1855b, p. 27). This stream carried with it all "movable parts" and deposited them at the sugar-exposed end of the cell. It would seem far-fetched, though, to celebrate Nägeli as the true discoverer of what our textbooks call pressure flow, as contemporary interpretations of osmotic processes differed significantly from more recent views. Botanists generally thought that the boundary layer responsible for osmotic phenomena in plant tissues was the cell wall, which was called *Membran* by scholars who, like Nägeli, published in German. Nägeli (1855a, 1855b) additionally recognized the "diosmotic properties" of the protoplasm as a whole, interpreting it as a second boundary that in series with the cell wall mediated osmotic interactions between the cell sap (i.e., the vacuole contents) and external media.

Nägeli's interpretation became modernized, as it were, in 1877, when Wilhelm Pfeffer published *Osmotische Untersuchungen — Studien zur Zellmechanik* (Osmotic investigations — studies on cell mechanics). Interested in the relationship between the composition of solutions and the hydrostatic pressure they could generate osmotically, he built what became known as *Pfeffersche Zellen* (Pfeffer cells): osmotic cells consisting of water-permeable clay pots of 6–9 mL volume, with a semipermeable layer of copper ferrocyanide precipitated onto their inner surfaces (Fig. 4B). The clay pots, originally manufactured to serve as electrical cells (batteries), were necessary to contain the hydrostatic pressure that developed when cells containing a defined solution were submerged in hyposmotic media (Fig. 4A). Results he obtained, combined with theoretical considerations and the structural analogies between plant cells and his artificial osmotic cells, led Pfeffer to postulate that the most important osmotic barrier in living cells was a thin surface layer on the protoplast. He called this layer the *Plasmamembran*, apparently the first application of the term that closely resembled the modern usage of 'plasma membrane' (accounts of the history of the modern biological membrane concept often underestimate Pfeffer's contribution; for a more realistic assessment, see Liu, 2019). But for Pfeffer, the term was a generic one, as he postulated that additional *Plasmamembranen* separated the protoplasm from any vacuoles that a cell may form (see also Pfeffer, 1891).

Pfeffer's *Osmotische Untersuchungen* were crucial for the development of the modern understanding of osmotically driven bulk flow in biological systems. While discussing the "origin and distribution of growth-mediating materials", he evaluated possible mechanisms that might drive intracellular "streams" of these substances:

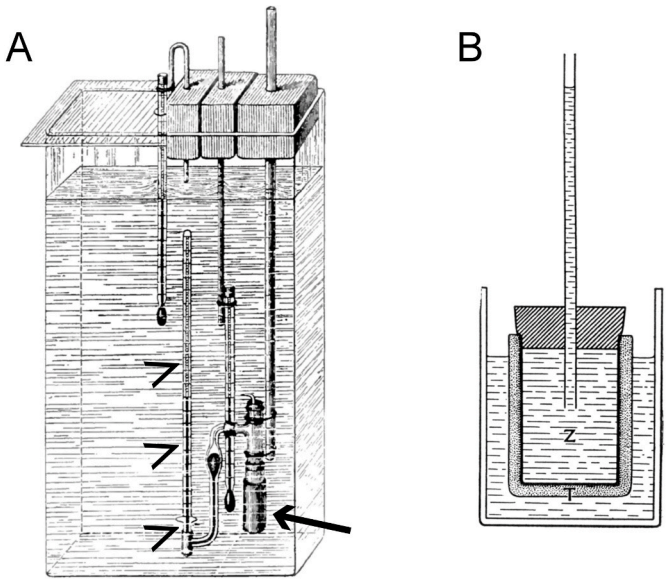

Fig. 4. Osmotic experiments by Wilhelm Pfeffer. (A) Experimental setup for studying osmosis (Pfeffer, 1877, Fig. 5). A *Pfeffersche Zelle* (Pfeffer cell), an osmotic cell made by precipitating semipermeable copper ferrocyanide membranes onto the inner surfaces of clay pots, was filled with a defined solution, closed, and then submerged in another solution; we added an arrow to indicate the cell in Pfeffer's figure. Hydrostatic pressure within the cell was monitored with a manometer (arrowheads added). Temperature at two depths in the bathing medium was monitored by thermometers. (B) Schematic section of a *Pfeffersche Zelle* from a later textbook; the clay pot is labeled *T*, the dark line on the pot's inner surface represents the semipermeable membrane, and *Z* is the solution in the cell (Troll, 1948, Fig. 136).

> "… such flows can be generated by osmotic means, if the osmotically active bodies are not distributed homogeneously in the solution … To clarify the issue, let us imagine a hollow glass cylinder, with both of its open ends closed by membranes of the same type, and placed vertically under water. A concentrated solution of a non-permeant substance is present in the lower part of the glass cylinder, while a more diluted solution extends to the upper membrane. The more concentrated solution by itself would generate a higher osmotic pressure than the more diluted one. The final magnitude of the pressure in the cell, however, will lie between these values, and will be reached, of course, when the amount of water filtrated by pressure through both membranes combined equals that taken up through osmotic effects. In our scenario, the osmotically generated influx per unit time of water across the lower membrane exceeds that occurring across the upper membrane. On the other hand, equal amounts of water per unit membrane area filtrate through the two membranes *under the pressure that is more or less the same in the entire cell*. Thus, water must flow from the lower to the upper wall of the cell as long as solutions of different osmotic effect touch the two membranes. In short, we are observing a circulating water flow" (Pfeffer, 1877, p. 222; our emphasis. Compare our graphical representation of this thought experiment in Fig. 5A–D).

We have to add three comments for clarification. First, Pfeffer used the verb *filtrieren* (to filtrate) exclusively for water fluxes across membranes that are driven by hydrostatic pressure. So when he speaks of a situation in which as much water is filtrated as is osmotically attracted, he means the equilibrium condition in which a gradient of hydrostatic pressure across a semipermeable membrane and an opposite osmolarity gradient cancel each other. Second, the German *Zelle* has an almost identical connotational field as the English *cell* (both are derived from the Latin *cella*); the German terms for prison cell, electrical cell, storm cell, etc., are literal translations of their English equivalents. Unsurprisingly, Pfeffer consistently called the clay pots he used in his osmotic studies *Zellen*, as they were osmotic cells. To avoid misunderstandings, we emphasize that the word *Zelle* (cell) in Pfeffer's quotation above refers to the glass cylinder, not to a biological cell.

Third, and most importantly, Pfeffer's statement that pressure "is more or less the same in the entire cell" carries special importance; in fact, the *absence* of significant gradients of hydrostatic pressure was essential for his explanation of the mechanism of osmotically driven bulk flow. Precisely because hydrostatic pressure is the same everywhere in the glass cylinder, it can be too small to balance the osmotic gradient at the lower membrane while simultaneously being too large to be balanced by the osmotic gradient at the upper one — which results in bulk movement through the tube and "circulating water flow". Of course, there is an implicit assumption behind Pfeffer's claim: the resistance of the glass cylinder to bulk flow has to be negligible. If this resistance increases, for example when the cylinder becomes very narrow so that friction between fluid and cylinder wall can no longer be ignored, the flow away from the lower membrane over which water enters will be inhibited. Osmotic water influx will continue nonetheless, resulting in increasing hydrostatic pressure (until the equilibrium is reached), and as long as the increased pressure is large enough to overcome the hydraulic resistance of the cylinder, bulk flow will continue. In this scenario, gradients of hydrostatic pressure are side-effects of increased hydraulic resistance, but they are not the causes of bulk flow; osmotic flow across semipermeable membranes is. If it were otherwise, the extreme case presented in Pfeffers's thought experiment, in which no pressure gradients build up since the hydraulic resistance of the system approaches zero while bulk flow driven by osmosis proceeds regardless, would remain inexplicable. The logic of Pfeffer's thought experiment mirrors the conclusion by Eschrich et al. (1972, p. 288): "the concept of a hydrostatic pressure gradient providing the driving force for solution flow is misleading because gradients of hydrostatic pressure arise simply as a consequence of viscous flow".

Pfeffer never applied his insights into osmotic mechanisms to long-distance phloem transport (Knoblauch and Peters, 2017a). This does not mean, though, that he failed to grasp the potential significance of osmotically driven bulk flow for transport over distances much greater than the size of a typical plant cell – after all, Pfeffer cells were macroscopic devices. He considered a role for 'circulating water flow' in the generation of root pressure and water exudation (Pfeffer, 1877, pp. 223–234), and expanded on the subject in his textbook *Pflanzenphysiologie* (plant physiology; Pfeffer, 1897, pp. 234–267). The idea was developed further by Blackman (1921), who visualized Pfeffer's thought experiment in a way that appears strikingly similar to the *Grundversuch* Ernst Münch would present six years later (Fig. 5E; compare Fig. 7B). The modification of Pfeffer's model by Romell (1918) had additional source and sink compartments at the ends of the tube. Glucose was continuously produced from starch in the source, and degraded in the sink (Fig. 6A). Immersed in water, "such a system obviously would generate a water flow from *A* to *B* and maintain it as long as degradable polymer is present" in the source compartment (Romell, 1918, p. 353). This is, of course, the mechanism Münch postulated a decade later to drive phloem transport from sugar-producing sources to sugar-consuming sinks (Fig. 6B) — but Romell (1918), whom Münch never cited, evidently saw no reason to include gradients of hydrostatic pressure in his explanation of the process.

4. Münch's adaptation of Pfeffer's thought experiment

At the time Romell (1918) and Blackman (1921) employed Pfeffer's theory of circulating water flow to explain root pressure, bleeding, and exudation, an unchanged second edition of *Osmotische Untersuchungen* appeared (Pfeffer, 1921); the book was still considered up-to-date! When Münch presented his hypothesis of phloem transport a few years later, he stressed that "the physical basis of our theory … is *the same* as that of Pfeffer's exudation theory" (Münch, 1926, p. 69, our emphasis; analogous statements are found in Münch, 1927, p. 344; Münch, 1930, pp. 224–225). The reasons why plant physiologists like Pfeffer had not applied their models of osmotically driven flow to the phloem while the forest scientist Münch did, are complex and have been analyzed elsewhere (Knoblauch and Peters, 2017a). Here we will focus on how Münch's adaptation of Pfeffer's model acquired axial hydrostatic pressure gradients as an apparently essential component.

Münch presented his ideas in a short note (Münch, 1926), a full paper (Münch, 1927), a textbook of forest botany (Büsgen and Münch, 1927), and in an extended monograph (Münch, 1930). The paper of 1927 (pp. 340–342) provides the first complete description of the argument that eventually led to the idea of *Druckgefälle* (pressure slopes) acting in sieve tubes. Münch started by considering two osmotic cells containing solutions of different concentrations, immersed in the same water bath. The cells carried vertical tubes that served as manometers to monitor hydrostatic pressure in the cells (Fig. 7A). When the cells were lowered into the water bath at the beginning of the experiment, the fluid in the tubes would assume different levels in accordance with the hydrostatic pressure required to balance the osmotic water influx into each cell. Münch reasoned that …

> "… if one connects the cells with a pipe [reference to the drawing reproduced here as Fig. 7B], and if *A* contains a solution of higher concentration than *B*, *A* will attract water and press solution through the connecting pipe into *B*. The solution in *B* thus experiences mechanical overpressure, which drives out water that will flow to *A* where it will be taken up again" (Münch, 1927, p. 341).

This setup, Münch's *Grundversuch* (basic experiment), is Pfeffer's thought experiment (Fig. 5A–D) turned into reality. Münch evidently did not think of the hydrostatic pressure difference between the two cells in osmotic equilibrium (shown in Fig. 7A) as the driving force for bulk flow from *A* to *B*. When the cells were connected, *A* rather drove *B* out of

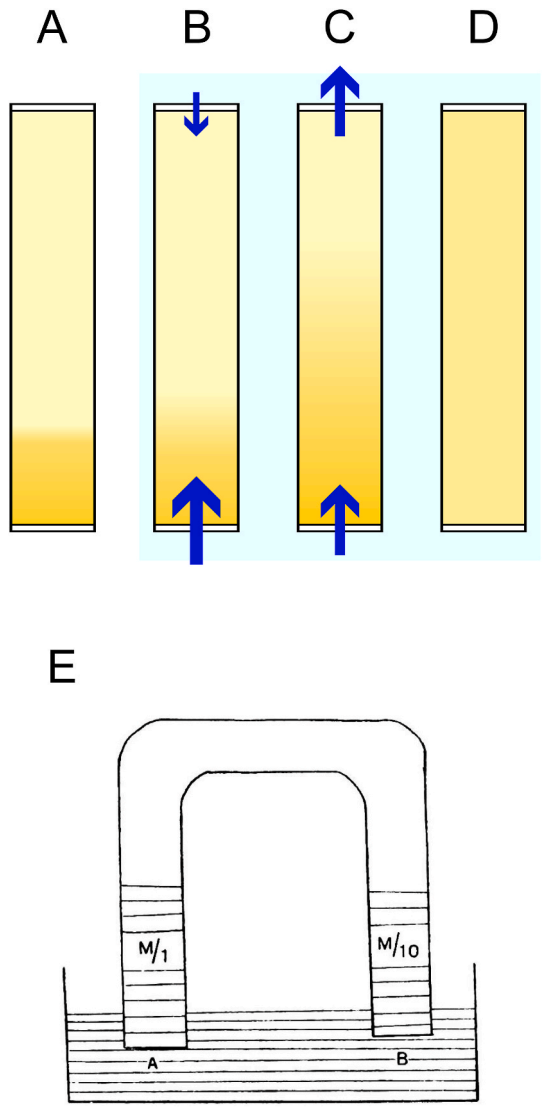

Fig. 5. "Circulating water flow". (A–B) Our representation of Wilhelm Pfeffer's (1877) thought experiment concerning the mechanism of osmotically driven bulk flow through osmotic cells (redrawn with modifications from Knoblauch and Peters, 2017b, Fig. 1b). (A) Glass cylinder closed at both ends with semipermeable membranes (double lines), filled with a highly concentrated solution at the bottom and a less concentrated one at the top. (B) When the tube is put under water, the osmotic gradients drive water influx across both membranes but more vigorously so at the bottom where the gradient is steeper. (C) Due to net water influx, the hydrostatic pressure in the tube will soon exceed the equilibrium pressure balancing the osmotic gradient across the upper membrane, while it will not yet be sufficient to balance the osmotic gradient at the lower membrane. Consequently, water will enter the tube at the bottom but exit the tube at the top. The resulting unidirectional bulk flow in the tube erodes the concentration gradient. (D) Net flow of water across the membranes ceases when the osmotic gradients across both membranes have become identical. (E) Drawing representing Pfeffer's thought experiment by Blackman (1921, Fig. 3). A U-shaped glass tube is filled with a concentrated sugar solution (*M/1*) on the left and a ten-fold diluted solution (*M/10*) on the right, before the two openings of the tube are closed by semipermeable membranes (*A* and *B*) and placed in water. The shape of the glass tube renders Blackman's model strikingly similar to Münch's *Grundversuch* (compare Figs. 2A and 7B).

osmotic equilibrium by imposing "overpressure" on it, causing water efflux from *B* and thus decreasing the hydrostatic pressure in the entire system. In other words, hydrostatic pressure was above that required for osmotic equilibrium in *B* but below the equilibrium pressure in *A* (otherwise *A* would not take up water). This would work, of course, in

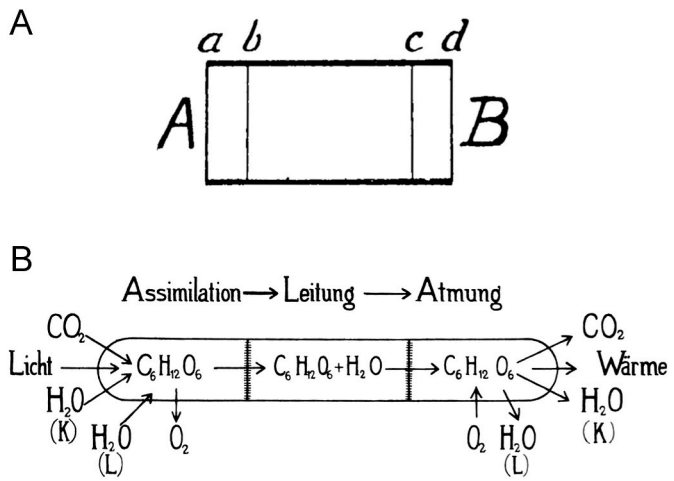

Fig. 6. Sugar metabolism driving bulk flow. (A) Romell's (1918, Fig. 8) model of the osmotic mechanism of plant bleeding. Horizontal lines represent impermeable walls of a water-filled tube, while vertical lines are semipermeable membranes. Membranes *a* and *d* are permeable only for water, while membranes *b* and *c* are permeable also for small molecules. Starch present in compartment *ab* is slowly broken down by enzymes into osmotically active glucose. Glucose but neither starch nor enzymes can cross membranes *b* and *c*. Enzymes in compartment *cd* turn glucose into osmotically inactive forms, by degradation or polymerization. Immersed in water, the system takes up water at *a*, bulk flow occurs from left to right, and water leaves at *d*. (B) Münch's (1930, Fig. 5) model of the metabolic basis of the pressure flow mechanism. Photosynthesis generates sugar in the compartment on the left, sugar is transported from source to sink via the compartment in the center, and respiration breaks sugar down in the compartment on the right (German terms: *Licht*, light; *Assimilation*, assimilation; *Leitung*: conduction; *Atmung*, respiration; *Wärme*, heat).

the absence of any noticeable hydrostatic pressure gradient along the transport route from *A* to *B* — it is the same process as in Pfeffer's thought experiment, after all. In fact, Münch (1927) did not mention hydrostatic pressure gradients between *A* and *B*, and his drawing of an experimental setup modified for "simpler handling" (our Fig. 7C) confirms that pressure gradients did not concern him at the time. This setup has a connection to which a manometer pipe could be attached to monitor pressure (Münch, 1927, p. 341). A second connection could easily have been added, in analogy to the two manometers in Fig. 7A. But there was only one, not two as would have been needed to detect a pressure gradient. Münch's *Grundversuch* soon appeared in lab manuals for physiology classes, and sometimes a second connection for easy filling of the two osmotic cells was included (Fig. 7D). But attempts to measure pressure differentials were never reported. The simple reason is that no detectable axial pressure gradients could be expected to build up in the *Grundversuch*. Münch certainly understood this, as we will see.

Readers aware of Pfeffer's *Osmotische Untersuchungen* (1877; 1921) obviously realized that the mechanism of Münch's *Grundversuch* was not novel, and some seemed unimpressed by its (lacking) originality. In his botany textbook, Wilhelm Troll (1948, pp. 485, 489–490), for instance, portrayed the *Grundversuch* as "two coupled Pfeffer cells" (Fig. 7E) and explained the pressure-flow model without ever mentioning Münch.

The description of the mechanism Münch had presented in 1927 remained essentially unchanged in his opus magnus of 1930. But there Münch added:

> "If differences of concentration exist, flow of solution in the direction of decreasing concentration will occur. With this insight we have arrived at the core of the problem of material translocation in plants: *in osmotic systems, a concentration gradient implies not only a diffusional gradient, but also a mechanical pressure gradient*" (Münch, 1930, p. 9; original emphasis).

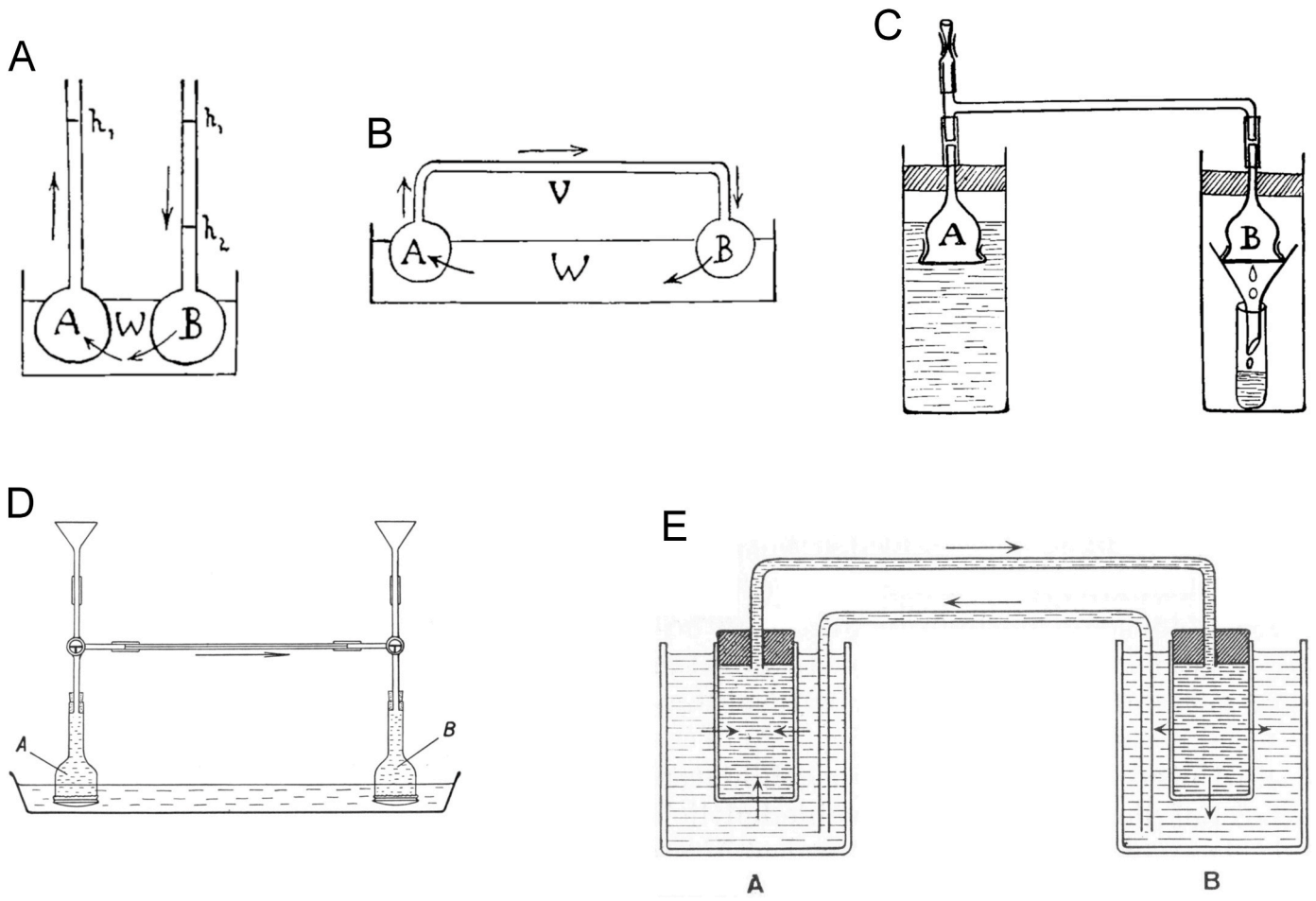

Fig. 7. Models used by Ernst Münch (A–C) and others (D, E) to explain osmotically driven bulk flow. In all models, the solution in osmotic cell *A* is more highly concentrated than that in cell *B*, and arrows indicate water or solution flow. (A) Two osmotic cells with manometer tubes in a water bath (*W*). Fluid levels in the vertical pipes indicate osmotically generated hydrostatic pressure (higher in *A*; Münch, 1927, Fig. 1). (B) By linking the pipes, a system is formed in which bulk flow from *A* to *B* and an overall circulation of water occurs (Münch's *Grundversuch* in the version of 1927; Fig. 2 in the original). (C) Actual experimental setup used by Münch (1927, Fig. 3) made of two glass bells closed with semipermeable membranes. Putting bell *A* under water resulted in exudation from *B*. The hydrostatic pressure in the system could be monitored by a single manometer attached above bell *A*. (D) The *Grundversuch* as described in a lab manual for plant physiology classes, with two connections to which manometers could have been linked but were not (Brauner, 1932, Fig. 29). (E) Two connected Pfeffer cells (compare Fig. 4B) from the textbook by Troll (1948, Fig. 385), used to introduce the "pressure-flow theory" without mentioning Münch.

This juxtaposition of a mechanical (i.e., hydrostatic) pressure gradient to a diffusional gradient is easily misunderstood if taken out of its original context. Compare what Münch had to say about hydrostatic pressure gradients in his *Grundversuch* in 1930 (Figs. 2A and 7B):

> "If in our *Grundversuch* one attempted to measure hydrostatic pressure with manometers in the differently concentrated fluids in the two cells *A* and *B* or at various positions in the connecting pipe, one would *of course* find no differences, as no noticeable flow resistances exist …. However, differences in mechanical pressure will appear in our osmotic apparatus if strong flow resistances are created in the connecting tube by obstructions. In this case, a series of manometers attached to the connecting tube will exhibit higher pressure in *A* than in *B*. The magnitude of this pressure differential would directly indicate the magnitude of the resistances at the prevailing flow velocity" (Münch, 1930, p. 40; emphasis added. Münch's drawings of this modified *Grundversuch* are reproduced in our Fig. 8).

These statements are in full agreement with the conclusions of Eschrich et al. (1972) and Young et al. (1973). It follows that Münch's claim of diffusional gradients *always* implying mechanical pressure gradients was not a conclusion from empirical observation but a theoretical construct. Formally, the claim is valid; per equation (1), any flux from cell *A* to *B* implies different pressures in *A* and *B*. But if bulk flow occurred in settings in which "of course" no gradients in hydrostatic pressure could be detected, just as it occurred in settings in which hydrostatic pressure gradients were in fact observed, then pressure gradients as such had little explanatory value concerning the mechanism(s) of the observed fluxes.

If so, why did Münch stress the existence of pressure gradients even in cases in which it was impossible to detect any? As indicated above, he was concerned about possible misunderstandings of the role diffusion played in long-distance transport, especially since this transport was ultimately driven by osmosis (diffusion across a membrane). Discussing the physics of his *Grundversuch*, he explained: "The purpose of these deliberations is to show that the flow in the connecting pipe possesses a physical character that is distinct from diffusion and osmosis, although in principle the former is due to the same causes as the latter are" (Münch, 1930, p. 12). We conclude that while Pfeffer and Münch described the same process and evidently agreed on its mechanism, they attempted to focus their audiences on different aspects and chose their rhetoric accordingly. Pfeffer neglected theoretically required, minute pressure gradients and treated pressure as a constant to stress that both membranes in his thought experiment were far from their respective osmotic equilibria. Münch, on the other hand, emphasized the

theoretically required pressure gradients even under conditions in which they could not be detected, to convince his readers that the flow observed differed fundamentally from diffusion. Münch's insistence on ubiquitous hydrostatic pressure gradients for theoretical, or rather pedagogical reasons may have decreased confusion in the audience he addressed in 1930. In later audiences, however, it seems to have been *generating* confusion, as the following example shows.

5. Why it matters — the sieve plate fallacy

As Münch evidently had understood in 1930 (Fig. 8), sieve plates are responsible for a large part of the total hydraulic resistance of sieve tubes (Mullendore et al., 2010; Jensen et al., 2012; Stanfield et al., 2018). If transport efficiency were the only factor controlling the evolution of sieve tube structure, one would expect selection to drive the rapid disappearance of sieve plates, analogous to the disappearance of the nucleus, cytoskeleton, vacuole(s), and Golgi apparatus in sieve elements (Heo et al., 2017). However, organismal structures hardly ever are subject to a single type of selection pressure only, and the very existence of sieve plates in sieve tubes of all vascular plants as well as in large brown algae suggests important functions that balance the disadvantage of an increased hydraulic resistance. These functions appear related to the shutdown of individual sieve tubes in response to various stimuli. Callose depositions on sieve plates may block sieve tubes reversibly during attacks by phloem-feeding pests (Hao et al., 2008), following injury (Mullendore et al., 2010), or during seasonal dormancy (Aloni et al., 1991). Forisomes, protein bodies in sieve tubes of legumes, act as cellular stopcocks wherever a rapid but reversible shutdown of a sieve tube is required (Knoblauch et al., 2001). As forisomes seem to move with the stream, most are located close to sieve plates (Peters et al., 2006). Without sieve plates, forisomes and other materials that seal sieve plates in wounding reactions (Fischer, 1885; Knoblauch and van Bel, 1998) would just drift to sink organs and accumulate there.

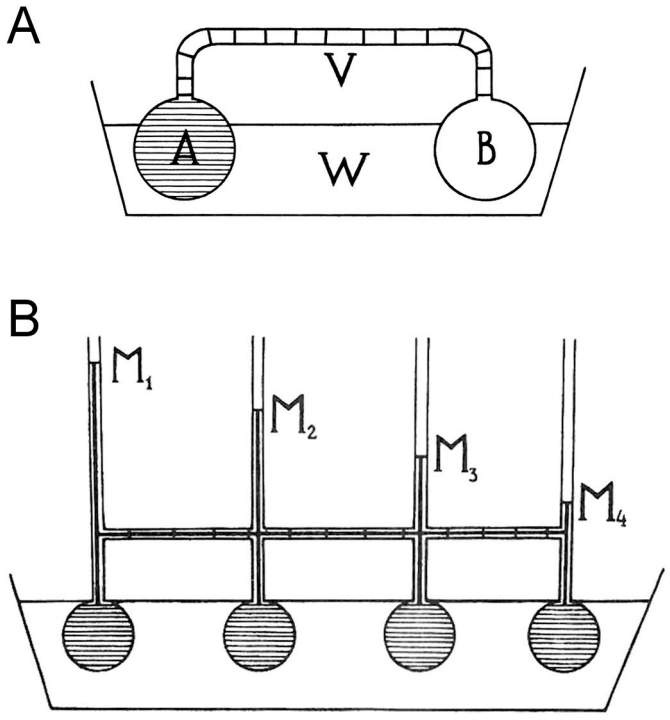

Fig. 8. Gradients of hydrostatic pressure are caused by increased hydraulic resistance in Münch's *Grundversuch*. (A) Modified setup (compare Figs. 2A and 7B) with sieve plate-like obstructions in the connecting tube (*V*; Münch, 1930, Fig. 4). (B) Four connected osmotic cells with manometer pipes (M_1 to M_4). The cells are filled with different solutions, decreasing in concentration from left to right. When the horizontal connecting tube includes obstructions as shown here, flow is impeded and the manometers indicate different pressure values in the four cells. Without these obstructions, measured hydrostatic pressure is uniform in the system (Münch, 1930, Fig. 6 and pp. 40–41).

Now consider what our textbook says about sieve plates and their fluid-dynamic effects:

> "If no cross-walls were present in the translocation pathway … the different pressures at the source and sink would rapidly equilibrate. Sieve plates present a sequence of resistances to the moving phloem sap; the resistance is thought to result in the generation and maintenance of a considerable pressure gradient in the sieve elements between source and sink" (Taiz and Zeiger, 2010, p. 281).

Obviously, this could be understood as a statement of a simple fact: without sieve plates, the hydraulic resistance of sieve tubes would be smaller, and the plant could distribute photoassimilates at much lower energetic expense. But if read in context with the assertion that "the pressure-flow hypothesis demands the presence of a positive pressure gradient, with turgor pressure higher in sieve elements of sources than in those of sinks" (Taiz and Zeiger, 2010, p. 282), a different interpretation seems to be implied. In tests we conducted in sophomore level plant biology courses, a majority of students concluded from the quoted textbook information that without sieve plates, the phloem could not transport photoassimilates: the absence of sieve plates implied an absence of pressure gradients, and without pressure gradients no phloem sap translocation could occur. This is nonsense, of course — no engineer would ever suggest to improve the efficiency of a pipe by adding constrictions that multiply the pipe's hydraulic resistance. The fallacy made its way into the scholarly literature nonetheless:

> "… solutes move through the phloem as the result of an osmotically generated pressure gradient between a source and a sink …. The role played by the sieve plates in this scenario is critical. The pressure difference between the source and the sink would rapidly vanish if the transport pathway operated in a completely open system. The sieve plates provide resistance along the transport pathway that maintains the pressure gradient in the sieve tubes." (Niklas and Spatz, 2012, pp. 99–100).

6. A remedy

Ambiguous terminology often reflects conceptual ambivalence. The term *pressure flow* invites students to view pressure as the causative agent of phloem translocation. *Volume flow* (Eschrich et al., 1972), *osmoregulatory flow* (Thompson and Holbrook, 2003), *standing gradient flow* (Diamond and Bossert, 1967), and even *Münch flow* could help avoiding this misunderstanding. However, the logical structure of Münch's mechanism as introduced in textbooks and elsewhere probably is more influential than terminology. We suggest to follow the logics of Pfeffer's thought experiment more closely while expanding it to osmotic cells with significant internal hydraulic resistance, as shown in Fig. 9.

Two osmotic cells, I and II, contain solutions of different concentrations, $C(\mathrm{I}) > C(\mathrm{II})$. The actual hydrostatic pressure in these cells, P_{act}, equals ambient pressure when the cells are kept in air (Fig. 9A). Immersed in a dilute external solution of $C_{ext} < C(\mathrm{II})$, both cells will take up water as long as their actual pressure, P_{act}, is less than the pressure at osmotic equilibrium, P_{eq} (Fig. 9B). Generally, $P_{act} \neq P_{eq}$ implies a non-equilibrium that provides driving forces for solvent and/or solute fluxes. Specifically, $P_{act} < P_{eq}$ causes osmotic water influx into a cell whereas $P_{act} > P_{eq}$ drives osmotic water efflux. Since hydrostatic pressure at equilibrium depends on solute concentration, we expect $P_{eq}(\mathrm{I}) > P_{eq}(\mathrm{II})$ (Fig. 9B).

What will happen if the cells are merged into a single structure of negligible internal hydraulic resistance, while the concentration drop between zones I and II is maintained? In this enlarged cell, any hydrostatic pressure gradients flatten immediately as compensatory fluid

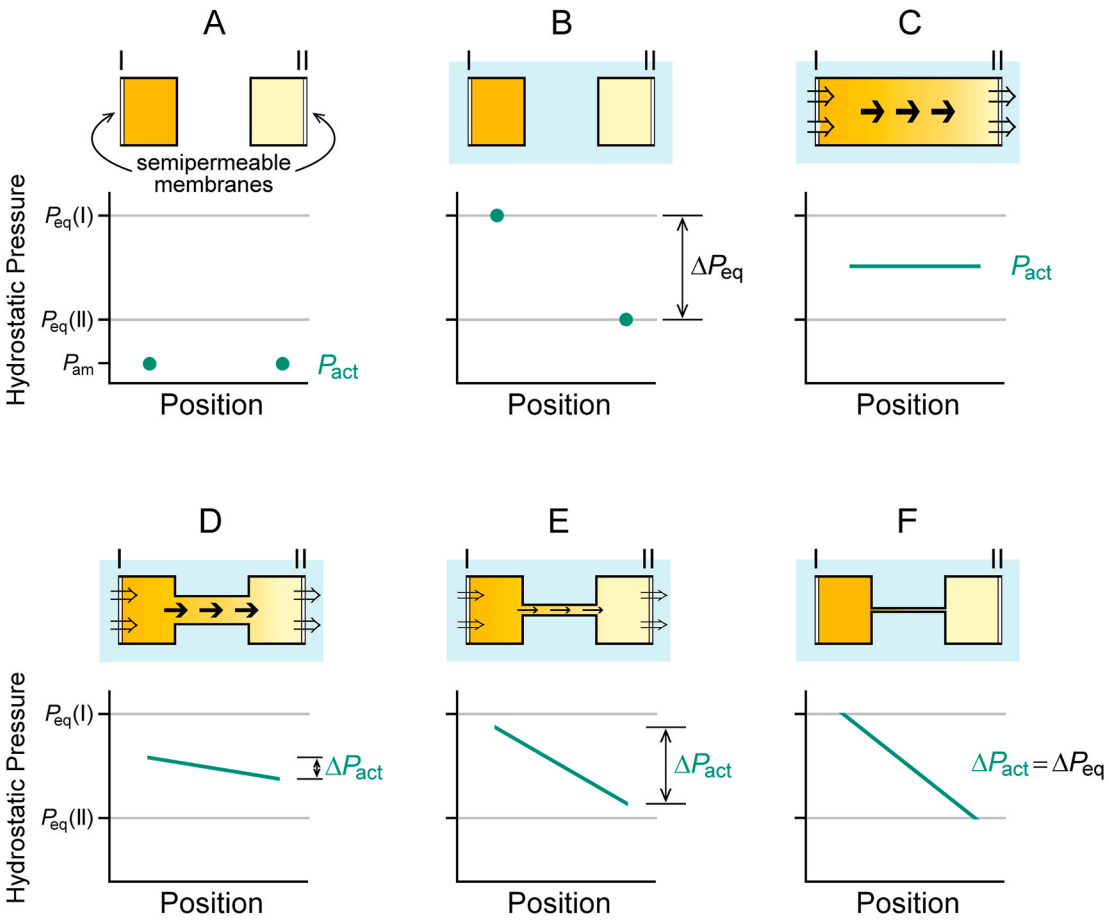

Fig. 9. Münch flow explained by an expanded version of Pfeffer's thought experiment. Osmotic cells are shown on top of each subfigure; double lines represent semipermeable membranes. Darker shading in the cells indicates higher solute concentrations. Open arrows represent osmotic water flux across membranes, solid arrows indicate bulk flow within cells. Diagrams below the osmotic cells show hydrostatic pressure: P_{am}, ambient pressure; P_{eq}, pressure at osmotic equilibrium; P_{act}, actual pressure in a cell. See main text (chapter 6) for detailed discussion.

motion is unrestricted by hydraulic resistance (compare equation (1)). Consequently, the actual pressure, P_{act}, in the entire cell will assume a value between P_{eq}(I) and P_{eq}(II) (Fig. 9C), which can be expressed as:

$$P_{eq}(\mathrm{I}) > P_{act}(\mathrm{I}) \cong P_{act}(\mathrm{II}) > P_{eq}(\mathrm{II}) \quad \text{(Eq. 2)}$$

This means that water will enter the cell over membrane I but leave it over membrane II, which drives bulk flow through the cell from zone I to zone II (Fig. 9C). When the flow path between zones I and II is constricted so that its hydraulic resistance cannot be considered negligible anymore, flow in the cell will be impeded and a P_{act} gradient develops (Fig. 9D):

$$P_{eq}(\mathrm{I}) > P_{act}(\mathrm{I}) > P_{act}(\mathrm{II}) > P_{eq}(\mathrm{II}) \quad \text{(Eq. 3)}$$

The steepness of the P_{act} gradient is determined by the hydraulic resistance of the cell (Fig. 9D and E; compare equation (1)). Osmotic equilibrium will be established at both membrane I and II when the P_{act} gradient required to overcome hydraulic resistance reaches the magnitude of the difference between the equilibrium pressures:

$$P_{eq}(\mathrm{I}) = P_{act}(\mathrm{I}) > P_{act}(\mathrm{II}) = P_{eq}(\mathrm{II}) \quad \text{(Eq. 4)}$$

In this situation, net water fluxes across the membranes and bulk flow through the cell cease (Fig. 9F).

Models of complex processes for teaching purposes necessarily are simplified, and our explanation of phloem flow (Fig. 9) is no exception. We assume impermeable walls of the transporting tube, time-invariant solute concentrations on both sides of membranes I and II, and constant fluid viscosities. It actually may be an advantage that we do not presuppose familiarity with osmotic pressure and water potential, two notoriously difficult concepts for students. Introducing *Münch flow* in this way should prevent the interpretation of pressure gradients as the immediate causes of flows in sieve tubes, and consequent misconceptions like the sieve plate fallacy. This hypothesis will be tested in our plant biology classes.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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