Limiting Diameters of Pores and the Surface Structure of Plant Cell Walls

Tepfer and Taylor (*1*) claimed that our earlier estimates (*2*) of the limiting pore size of plant cell walls were substantially in error. I will demonstrate that the experimental results that Tepfer and Taylor used to make their claim were not feasible and will resolve the controversy that they believe exists.

We used a solute exclusion technique to determine the size of the pores in the walls of plant cells that allow molecules to freely penetrate the wall; we estimated that these pores were about 40 Å and suggested that molecules larger than this would have difficulty passing *through* the walls of living plant cells (*2*). Tepfer and Taylor (*1*) constructed a gel permeation column from cell walls of homogenized bean hypocotyls and found that proteins larger than those we would predict to pass through a cell wall did permeate a sizable portion of the wall matrix. Although they stated that such a gel permeation column could not determine the diameters of pores which allow molecules to pass completely through a cell wall, they did suggest that wall shrinkage in hypertonic solutions reduces the pore size artifactually, and that in turgid cells ovalbumin, a molecule with a diameter much larger than 40 Å, would penetrate the cell wall of a living cell. They reported that epidermal cells of oat roots shrank but did not plasmolyze in solutions of 0.2*M* sucrose, whereas 0.3*M* sucrose caused plasmolysis. They reasoned that for cells placed in 0.2*M* sucrose plus 5 percent ovalbumin, the sucrose would supply most of the osmotic potential required for plasmolysis, and the ovalbumin would cause little osmotic shrinkage. Tepfer and Taylor (*1*) stated that 0.2*M* sucrose plus 5 percent ovalbumin caused plasmolysis and concluded that "under these conditions it appears that the ovalbumin was able to penetrate the cell wall and hence cause plasmolysis."

I determined the osmotic potentials of such solutions by freezing-point-depression osmometry; the 0.2*M* sucrose is about −5.4 bar, and 0.3*M* sucrose is about −8.2 bar (Fig. 1). Observation of plasmolysis by microscopy with a 0.1*M* increase in external solute concentration (about 3.0 bars) is reasonable. Since the osmotic potentials among the individual root cells are heterogeneous, observation of incipient plasmolysis of a population of cells would be difficult with increases in solute concentration much less than 0.1*M*.

Observation of plasmolysis with 0.2*M* sucrose plus 5 percent ovalbumin is another matter. Plasmolysis with only 5 percent ovalbumin is very unlikely; 5 percent ovalbumin alone contributes an osmotic potential of no more than 0.3 bar, and the presence of 0.2*M* sucrose does not result in an interaction that increases the osmotic potential of the solution more than additively (Fig. 1). Thus, the ovalbumin can contribute no more than 10 percent of the osmotic potential needed to observe plasmolysis unequivocally.

Primarily as a result of their gel permeation chromatography, Tepfer and Taylor set out to explain the differences between their data and ours. In doing so, they interpreted our data in a manner that creates controversy when, in fact, there is none. We apparently left the impression that the plant cell wall is a homogeneous, solid matrix with capillaries no larger than 40 Å. This impression should not be construed from our data, particularly in light of the work of many investigators who have shown by purely physical techniques that, although a majority of pits of a cell wall are smaller than 40 Å, pits up to 300 Å in diameter are found (*3*). These investigations were cited in our report but were not discussed in detail since we were interested only in the limiting size of the pores that allowed molecules to pass through a wall. Even though one might argue that Tepfer and Taylor's frozen plant walls ground to a fine powder may not behave the same as the walls of a living cell, it is still not surprising that they found large proteins able to penetrate a sizable portion of their homogenized wall fragments. If the surface structure of a cell wall is crudely analogous to a plane composed of double-sided funnels, we could only determine the diameter of the necks of the funnels. The gel permeation chromatography of Tepfer and Taylor (*1*), on the other hand, could give some clue to the nature of the larger included space. Thus, the limiting diameters of pores approximate the total included volume of a gel permeation column and not the void volume. We would predict that proteins only up to the size of cytochrome c would approach the total included volume; this was verified in the data of Tepfer and Taylor. It is important to note that in neither report can one derive the actual amount of wall space available for diffusion or any directionality of permeation.

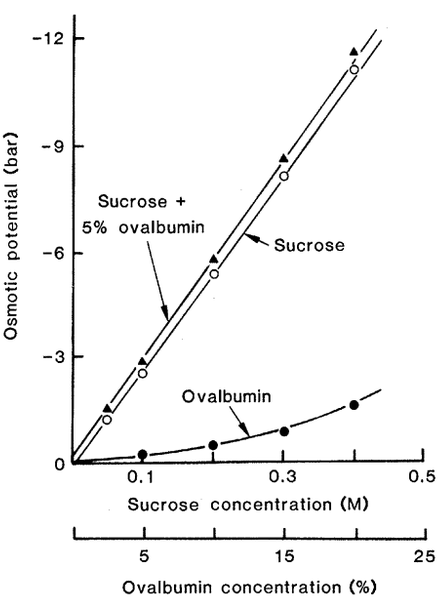

Fig. 1. Comparison of the osmotic potentials of sucrose and ovalbumin solutions. Solutions were made in deionized water, and the osmolality determined by freezing-point-depression osmometry (Advanced Instruments, Newton Highlands, Massachusetts). Osmotic potentials were then calculated for 23°C from osmolality values. (Electrophoresis grade, salt-free ovalbumin was from Sigma Chemical Company, St. Louis, Missouri).

Tepfer and Taylor also state that when cells are placed in hypertonic solutions, water will diffuse out faster than a larger solute can diffuse in, and thus, cytorrhysis (cell collapse) should precede plasmolysis. Although true in theory, this phenomenon played little role in our estimation of the limiting pore size of the wall. As we described in our original report, cells in polyethylene glycol (PEG) 1000 or 1540 exhibited momentary cytorrhysis followed by plasmolysis (*2*). The time required for this plasmolysis was no more than 10 seconds. Diffusivity values for a large size range of dextrans are established (*4*) and were cited in our report. Such data clearly show that the diffusivity values for this range of polymers vary no more than twofold. Thus, if the PEG 1000 caused plasmolysis within 10 seconds, then PEG 4000 should cause plasmolysis within 20 seconds. Cells that excluded PEG 4000 or PEG 6000, excluded it for over an hour.

They also reason that turgid cells have larger pores than flaccid cells, and cell wall shrinkage in hypertonic solutions decreases the limiting diameter of the pores artifactually, much as a circle drawn on a balloon shrinks when the air is released. Since we know little about the surface structure of the cell wall of any living cell, however, there is no reason a priori that the pores should become smaller. Whether or not the cell

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walls of turgid cells have pore diameters different from those of flaccid, plasmolyzed, or collapsed cells, and whether or not such a difference, if any, has any biological significance, are still valid questions. But pertinent to these questions are the results of Janes (*5*), who showed that turgid pepper plants could not absorb PEG 4000 from root solutions even though exposure to PEG 4000 lasted for up to 7 days. Although plant cells are capable of absorbing PEG's smaller than 4000 daltons (*5*), Handa *et al.* (*6*) have recently shown that suspension cultures of cells adapted to grow in concentrated solutions of PEG exclude ^{3}H-labeled PEG 4000 for 12 days even though the turgor pressures of these adapted cells were six- to eightfold higher than those of cells growing in the absence of PEG. Thus, Tepfer and Taylor's hypothesis that the walls of turgid cells have limiting diameters substantially larger than those of flaccid cells has not been supported by any direct experiments.

I contend that our data represent an accurate estimation of the limiting diameters of the pores of the cell walls of those cells that we measured, and that cell-to-cell communication with molecules larger than about 40 Å would be severely restricted.

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Barrier Islands Revisited

It is rewarding to see that our research on the mid-Atlantic barrier islands (*1*) has led to further investigation by Leatherman and his co-workers (*2*). Unfortunately, they have attributed to us several statements that are incomplete, inaccurate, and thus, misleading.

First, nowhere in our report did we postulate, as Leatherman *et al.* state, that "two capes will develop within 100 years along the barrier island chain on the eastern shore of Virginia in response to a theoretically trapped standing edge wave." Our theory is that "If past trends in shoreline change continue, two capelike features may develop within the next century." We used the term "capelike" to distinguish the type of shoreline protrusions that could develop along the Virginia coast [see figure 3 in (*1*)], from the full-scale or true capes, such as Cape Hatteras and Cape Lookout. More importantly, we state in straightforward terms that cape development could be initiated in association "with (i) irregularities in the orientation of the coast due to regional-scale geology or (ii) variations in the intensity of processes occurring along the coast."

Second, in our discussion of standing edge waves, we only pointed out that a modal number 3 edge wave results in an almost perfect fit with the predicted locations of the capelike features. In a previous publication (*3*), we discussed the implications of matching edge wave modal numbers with periodicities in shoreline landforms. But in our *Science* report, where we introduced the concept of edge wave modal number 3, we went on to state that "this does not mean that only edge wave modal number 3 is involved, or that edge waves are responsible for the large sedimentary capes of the Atlantic coast."

Third, it is difficult for us to believe that anyone could read Inman *et al.* and Guza and Inman (*4*) and reach the conclusion of Leatherman *et al.* that "Inman and his co-workers have not shown that edge waves are a primary factor in shaping shorelines." Guza and Inman state (on p. 2998), "It is the contention of the present work that edge waves, both directly and via their interaction with other water motions, are responsible for many cases of cuspate topography." Guza and Inman go on to state (on p. 3006) that "It is conceivable that topographic feedback to edge wave excitation is so strongly negative that there is no important topographic response. We [Guza and Inman] believe this highly unlikely. Edge waves most probably provide an initial longshore periodic perturbation in the topography. . . ." As for the need to have an "effective headlands to trap a standing wave," as Leatherman *et al.* suggest, Guza and Inman state (on p. 3011) that "when the incident wave field is long-crested and of uniform amplitudes, there is no need for such end effects (headlands, groins, or curving shorelines) because any radiative energy losses out of the 'ends' of the system are only a small fraction of the total nonlinear energy input."

One of the most significant developments in coastal science over the last two decades has been the concerted effort by many investigators to explain the regular and periodic variations in landforms that occur along sedimentary coasts. These landforms range in size from beach cusps to capes. We have reported on along-the-coast periodicities ranging in wavelength from hundreds of meters to tens of kilometers (*3*, *5*). Explanation for periodicities in shore zone landforms (cusps, bars, overwash patterns, and so forth) and associated processes have in recent years focused on the role of standing waves and intersecting wave trains. We specified in our report (*1*) that "our studies of shoreline dynamics for the 122 km of coast between Cape Hatteras and Cape Lookout indicate that edge waves may play an important role in regional scale variations in shoreline dynamics." At this time we see nothing to lead us to believe otherwise. The tabulation of classical geomorphological factors cannot, in our opinion, account for either the crescentic shoreline landforms found along most sandy coasts, nor the tripartition of the Virginia barrier islands.

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