placed in a liquid medium in a shake flask. With gentle to moderate agitation, cells or small aggregates of cells will slough off. Typical conditions would be 27°C and a pH of 5.5 in the dark. These suspended cells then replicate. After two or three weeks, the suspended cells are transferred to fresh medium, and large aggregates or residual callus are discarded. Suspensions can grow to high cell densities (similar to applesauce). All suspension cultures contain *aggregates;* however, it is possible to select for fine suspensions by discarding large aggregates at each subsequent transfer.

The aggregates are due not so much to clumping as to the failure of progeny to separate. Cell-to-cell communication is very important in plants. Normally, all cells in a whole plant are connected to one another by small pores called the *plasmodesmata*. The plasmodesmata allow the interchange of lower-molecular-weight compounds (< 800 da) from the cytoplasm of one cell to another. The plasmodesmata are formed principally during cell division. Cells in aggregates can communicate with one another through plasmodesmata and also through diffusable species. For example, plants generate ethylene, which acts as a hormone. In a large aggregate, there will be concentration gradients of such metabolic products as well as nutrients (e.g., oxygen and hormones). These gradients lead to a wide variety of microenvironments and, as a consequence, cells in different positions in the aggregates may have greatly different biochemical and morphological structure. Although plant cell cultures are pure cultures in the sense that only one species is present (ideally one genotype), it behaves very much like a mixed culture. A mix of cell types may be necessary for the formation of some products.

Cells in suspension can be made to undergo differentiation and organization if the correct environmental conditions can be found. Nutrient and hormone levels must be adjusted. Embryos, shoots, and roots can be made from aggregates in suspension. However, let us turn our attention from aggregates back to individual cells.

Plant cells can be very large, with cell diameters typically in the range of 10 to 100 μm. They grow slowly, with doubling times typically ranging from 20 to 100 h. Growth is usually nonphotosynthetic, with sucrose or glucose supplied exogenously as the carbon and energy source. Typical respiration rates are roughly 0.5 mmol O_2/h-g dry weight or about 5% to 15% of that in *E. coli*. Plant cells can often be cultivated at very high densities, up to 70% of the total reactor volume as cells. Although the respiration rate is low, high cell density requires very good oxygen-transfer capabilities in a reactor.

Plant cells contain a higher percentage of water than bacterial cells (90% to 95% versus 80%). This higher water content is due, in part, to the presence of the central vacuole, which can occupy as much as 95% of the intracellular volume in extreme cases. Plant cells tend to secrete relatively few compounds, but sequester them inside the vacuole. Many of these compounds are secondary metabolites of commercial interest; many would also be cytotoxic if not removed from the cytoplasm. Their intracellular storage will have important implications in our later discussions on bioreactor strategies.

One other biological aspect of critical importance is the role of *elicitors*. In whole plants, there are a number of defensive mechanisms that plants use to contain attacks from pathogenic fungi, bacteria, and viruses. Defense mechanisms can be activated if breakdown products of fungal or plant cell walls (oligosaccharides) are present. Other compounds, some even nonorganic, can also elicit a response. Many secondary metabolites are involved in the plant's defensive mechanisms. The exposure of suspension cultures to elicitors has led in many cases to rapid increases in the accumulation of secondary