Patapoff and Overcashier tested freezing methods and annealing on primary drying rates, sample temperature during primary drying, dried product resistance, appearance of the freeze-dried cakes, and protein aggregation (10). The latter will be discussed in section "Mechanisms of Morphological Change During Annealing" below. They tested a recombinant human antibody formulation. Cooling via immersion into a dry ice/ethanol bath resulted in the greatest product resistance and therefore slower sublimation rates and higher product temperatures. Shelf-ramp freezing and ice crystal seeded shelf-ramp freezing each resulted in successively less resistance. Samples that were frozen using the standard shelf-ramp method and annealed had the lowest resistance. The dry ice/ethanol and standard methods also formed a more resistive layer at the top of the sample. They also found that a placebo version of their formulation exhibited completely different freezing and drying characteristics than the active-containing formulation: the placebo dried significantly faster.

Vacuum-flask freeze-dryers are used in many laboratories for bulk lyophilization. They consist of a vacuum and condenser system to which glass flasks are attached externally. The liquid within the flasks is frozen by evaporative cooling. Kramer et al. used this principle to freeze solutions in vials within a freeze-dryer (11). With vials loaded on a 10°C shelf, they reduced the pressure to 760 mT to induce the formation of a 1 to 3 mm thick layer of ice on the top of the solution within five minutes. The shelf temperature was then ramped down to −40°C to complete solidification and lyophilization was continued normally. The drying rates and morphologies of these samples were compared with those frozen by conventional shelf-ramp. In addition, annealing was tested on samples frozen by both means. The evaporative nucleation method resulted in large "chimney-like" ice crystals, and the drying rates of some formulations were up to 20% faster. Annealing also resulted in increased drying rates. Shelf-ramp frozen sample morphology was spherulitic, and annealing of these samples increased the size of those spheroidal ice crystals.

Spray-freeze-drying is a process in which fine droplets are sprayed through an ultrasonic nozzle directly into liquid nitrogen. The frozen droplets are then placed into vials and freeze-dried in a standard lyophilizer. They are of particular interest for pulmonary and epidermal delivery. Sonner et al. recently reported on the use of this technology using trypsinogen as a model protein (48). They produced spherical particles with diameters from 20 to 90 μm that contain high internal porosity. Sonner et al. compared the properties of 20% trehalose samples that were spray-freeze-dried and annealing during primary drying to those that were not annealed. Annealing caused the particles to shrivel. The authors tested the postdrying hygroscopicity of the particles by measuring moisture uptake rates. They found that the longer the particles were annealing during drying, the lower the subsequent moisture uptake rates. They theorized that annealing reduced the specific surface area of the particles, slowing water adsorption. Protein recovery results will be discussed in section "Mechanisms of Morphological Change During Annealing" below.

Webb et al. studied several formulations of human interferon-γ, comparing liquid nitrogen immersion vial freezing with spray-freezing (47). Vial immersion resulted in a directional lamellar morphology, and the freeze-dried cakes were severely cracked. Spray-freezing yielded spherical particles similar to Sonner et al. (48). Specific surface areas for the spray-freeze-dried samples were 6.8 to 15 m^2/g, three to seven times those of their counterparts that were