Preface

Sixty-five years have passed since the publication of Basil Luyet's *Life and Death at Low Temperature*, the first organized attempt to summarize past observations of freezing injury in living organisms and to infer from them the mechanisms of injury. Not surprisingly, attention at that time was focused primarily on mechanical damage from ice, although, as evidence of the immaturity of the science, Luyet considered five other possible mechanisms: the withdrawal of energy, attainment of a minimal temperature, too-rapid thawing, dehydration, and "various physiological, physical, or chemical alterations."

One might have expected that the subsequent years would have led to a clear and functional understanding of freezing injury and, as a result, have enabled efficient cryopreservation of almost anything. But it wasn't that easy. Looking back at some of the hypotheses that emerged — salt concentration (Lovelock), disulfide binding (Levitt), the two factor hypothesis (Mazur), minimum volume (Meryman), membrane depletion (Williams, Steponkus) — one can see that, in retrospect, each in a very general sense was correct, but none provided sufficient insight to lift applied cryopreservation very far from the empirical. In fact, even after those sixty-plus years, our insight is still pretty much limited to the recognition that intracellular ice is generally lethal, that ice nuclei are rare or absent within cells, that extracellular ice concentrates the extracellular solution and the cells lose water osmotically and shrink. However, the precise nature of the lethal event for each cell type continues to be debatable, whether from the seeding of extracellular ice through membrane pores, from membrane stresses leading to the loss of membrane material, from temperature- and concentration-related denaturation or precipitation, or from some as-yet ill-defined phenomenon. The role of cold denaturation of proteins has been largely ignored.

Fortunately — or, some might argue, unfortunately — the advent of penetrating cryoprotectants, primarily glycerol and DMSO, has enabled empirical cryopreservation to leapfrog basic research. Topics that were central to cryobiology research in the 1950s and 1960s have been overshadowed by demonstrations of empirical success. Cryopreservation is increasingly being undertaken by specialists in the subject tissue rather than by cryobiologists, and the practicalities of grant and contract support tend to perpetuate this trend.

However, although the effectiveness of the penetrating cryoprotectants has made many basic questions moot, the challenges are still far from resolved. The cryoprotectants themselves are now the obstacles to easy success. Glycerol penetrates cells slowly and some cells not at all, creating major osmotic problems. But glycerol has the great advantage of being a nontoxic macromolecular stabilizer. DMSO, despite its reputation, does not cross membranes instantly; in fact, at temperatures below 10°C, it enters cells relatively slowly — at least this is true for red cells. And it is a macromolecular destabilizer with demonstrated toxicity.

From the purely practical aspect, it is better cryoprotectants that are needed. Polymers have their own shortcomings and, with some exceptions, enable long-term, low-temperature storage only in the presence of associated penetrating agents. Complete vitrification is a promising approach, but there are still formidable obstacles to be surmounted. For an ideal penetrating agent, there are only two criteria. Such an agent must cross cell membranes readily and rapidly and must be nontoxic at elevated concentrations. Obvious candidates are low-molecular weight organic compounds or cations such as ammonium compounds that are partially dissociated and enter the cell in the uncharged form. However, there are a limited number of low-molecular weight compounds and even the two simple criteria cited above may be too demanding.

This timely and impressive volume shows just how far cryobiology has come in providing frozen preservation for a variety of cells and tissues and also how much dedicated effort it has taken to get this far. It is also apparent that each individual cell type requires an individually crafted cryoprotective regimen that may still achieve only limited recovery, and that the ultimate goals of applied cryobiology have yet to be achieved. It is likely that the limitations of cryopreservation apparent today will not be resolved just by more tinkering, but will require a return to the basic questions that drove cryobiology during its earlier years. In the pharmaceutical industry, examples abound of successful new therapeutics made possible only by an accurate understanding of the process to be addressed. For cryopreservation, the easy answers are not good enough. The time is ripe for more basic science.

Harold T. Meryman