Freeze-drying Systems

In every freeze-drying installation the following facilities must be provided:

1. The initial freezing of the material.
2. The vacuum drying chamber in which the frozen material can be maintained under vacuum conditions without melting, so that the ice sublimes directly to vapor.
3. A pumping system including both a vacuum pump for pumping down and removing noncondensable gases, and either a desiccant or a refrigerated condenser to trap the evolved water vapor from the drying material. The water vapor constitutes the major pumping load in a vacuum tight plant, and is in such great volume that it cannot be handled by the vacuum pump alone, even using a vapor diffusion pump—unless of prohibitively large size. In the case of vapor pumps, incidentally, the rate of drying from the frozen state is not significantly increased by pressures below the mechanical pump range. (For the majority of *biological materials little is gained by employing temperatures* below − 40 C which is well within the range of refrigeration equipment, which is fortunate because the complication of refrigerating systems rapidly increases with lower operating temperatures.)
4. Provision for economical drying in two stages if very low residual moisture content is desired.
5. Ancillary equipment to indicate temperature and pressure —for example, vacuum gages, thermocouples, temperature and pressure recorders.

Initial Freezing

There are three methods of initial freezing: Prefreezing, Shelf Freezing, and Centrifugal Evaporative Freezing.

Prefreezing is suitable for material of sufficient bulk not to melt while being transferred to a drying unit. It includes *shell freezing* in which the bottle containing the liquid is rotated slowly about an almost horizontal axis in a refrigerated liquid, insuring peripheral freezing of the material with a central cavity that reduces thickness and increases the surface exposed to the walls of the container, and subsequently to the vacuum, assisting in heat absorption for rapid drying. *Vertical spin freezing* is a modification of shell freezing which accelerates the freezing process.

Shelf freezing is used for small bottles or vials containing only a few cubic centimeters of liquid or trays containing bulk quantities in a shallow layer. Bottles are placed on refrigerated shelves for freezing within the chamber later used for drying. This method does not give the large surface area and minimal depth of the frozen product so valuably supplied by the two previous means, but is industrially convenient and requires minimal handling.

Centrifugal evaporative freezing is a technique used for ampule-contained quantities of liquid (or material in a single bulk container) and employs a low speed centrifuge for complete suppression of foaming, giving a porous dried product which can readily be reconstituted. In addition, production of thin shells or wedges of frozen material with large surface areas is obtained. About 20 percent of the water evaporates before freezing occurs, thereby reducing the drying period. This degree of liquid concentration is demonstrably not harmful to the product and centrifugal evaporative freezing has been shown to yield a higher reliability than other methods.

Drying Stage

The vital technique of freeze-drying depends upon the fact that ice will sublime with passage directly from solid to vapor if the frozen material is maintained under vacuum. The length of time taken to dry any material is variable and is affected principally by the freezing point of the material; the thickness of the material when frozen; and the restriction to vapor flow which may be offered by bottle necks and filters. Drying is usually accomplished in a vacuum chamber to which heat is applied externally, by direct electric heat or circulation of heated fluid in the walls of the chamber.

Storage of Freeze-dried Materials

Ampule-contained material is conveniently stored in vacuum-sealed ampules, although sometimes it is more desirable to seal under an inert gas atmosphere which is admitted to the vacuum system just before closure. For larger containers, sealing by stoppers rather than glass fusion is generally used along with either inert gas or vacuum, making room temperature storage possible.

Freeze-drying apparatus is manufactured by several companies in the United States and England, and the details of construction are described in their brochures. Simple freeze-dryers are now listed in most catalogs of standard laboratory equipment.

TISSUE BANKS

In recent years a surge of interest in procurement, storage, and scheduled usage of certain human tissues has resulted in the development of suitable means to make such procedures a reality. Several different tissues are included, the most commonly used being segments of bone and various portions of the blood arterial and venous systems. The use of the corneas of the human eye for transplant to other individuals has established the term *Eye Bank*, but the period of corneal viability and hence usefulness is so short that simple ice refrigeration of the container in which the eyes are placed and transported covers this particular subject for purposes of this discussion.

With reference to bone, artery and other tissue banks, some elaboration is necessary since certain facets of the preservation and storage of these materials are concerned with refrigeration and refrigeration processes such as the foregoing freeze-dry technique. Since the preservation and use of these tissues is comparatively recent, there are differences of opinion about methods of storing and processing tissues, all of which have their enthusiasts and detractors, as well as another group which is primarily interested in synthetic or manufactured prostheses. These synthetics have their widest use in the surgery of the circulatory system where nylon and orlon prostheses are used to replace segments of aorta removed from aneurysm (permanent abnormal arterial dilatation) or other disease. The non-reactive metal (Vitallium) plates and screws and nails used in bone surgery are usually supplementary and only occasionally does metal constitute an entire prosthesis.

The use of bone grafts from various sources has a long medical history which is reviewed with a most extensive bibliography by Chase and Herndon.[2]

Principles of Preservation of Tissue for Later Transplantation

1. If possible, cells of tissue should be kept viable.
2. If viability is not possible or necessary, tissues must be preserved from degeneration.
3. Tissues must be maintained in the same aseptic (free of microorganisms) state as when placed in the storage container.

The principles are best fulfilled at − 20 C or below. The chief value of any bone transplant lies in provision of support and fixation with both a cellular framework and mineral salts.

Several methods of bone preservation are presently in use, falling into four categories.

Chemical. The bone to be preserved is placed in aqueous Merthiolate 1:1000 which is a simple, cheap, and aseptic medium. The container is then sealed and kept in a refrigerator at 4 to 6 C until used. The solution is changed every two weeks. The bone tolerates this procedure well and sensitivity to Merthiolate does not seem to preclude subsequent implantation in a patient.

Freeze Drying. This process kills the tissue cells but does not denature the proteins. Various methods and apparatus are on record but follow in general the principles described above in the discussion of freeze-drying. The bone is usually slow frozen at −15 C for four days, placed in block ice for fourteen days, rapidly dried under high vacuum at −40 C and stored at room temperature in vacuum bottles.

Boiling. Must be followed by storage in sealed sterile flask.

Frozen Storage. This is accomplished by storage in a deep-freeze at −20 C or by more extensive cold at −78 C. The bone is procured aseptically, stripped of soft tissues and sealed in a sterile tube. The tube containing the graft is then immersed in a methyl alcohol/solid CO_2 mixture for ten minutes at −78 C. Rapid freezing takes place. The bone is then placed in an insulated box surrounded by solid CO_2 in a deep freezer providing an immediate surrounding temperature of −78 C. When needed it is withdrawn from the bank, placed in a thermos container with solid CO_2, and taken to the operating room where it is thawed in sterile normal saline at room temperature just before implanting.

Sterilization of bone transplants by Cobalt-60 irradiation is described by DeVries,[3] using gamma radiation from a 10,000 curie radioactive cobalt source to render the bone bacteriologically sterile without subsequent harmful effects to the recipient. The radiation does not destroy the ability of the graft to stimulate new bone formation. Since sterilization is one of the major problems in this procedure, this radiation technique is merely supplementary to the usual preservation storage.

Artery banks are set up under similar circumstances, using similar methods and serving an equally necessary purpose. The three principal methods used are *chemical* (wet-cold storage), in which the arterial segments are kept in sterile containers in 70 percent alcohol or beta-proprio-lactate at 4 to 6 C; *deep freezing;* and *freeze-drying*. The arterial grafts are usually obtained from a subject under 40 years of age who has died free of transmissible disease, usually an accidental death. The grafts are taken within 12 hr after death under aseptic conditions and are placed in sterile tubes. Quick freezing follows in a methyl alcohol and solid CO_2 bath for three minutes, and the tube is then placed in a deep-freeze at −20 C or in an inner box (as noted above under bone-banking) at −78 C. A technique for freeze-drying, which is similar to previously described methods, is reported by Lehr and by Fisher *et al*, the latter including a cathode ray sterilization method. It is to be noted that this problem of whether the graft is sterile or not becomes a major part of any of these methods, both for arteries and for bones, since the ability of certain microorganisms—both bacterial and viral—to withstand extremes of cooling is recognized and has been mentioned earlier in this discussion.

Comments on these methods and their general applicability are in order. The chemical or wet-cold storage method is the simplest, and probably the best if sterilization is attained and maintained. It also avoids the loss of the specimen through thawing, if its use is unexpectedly precluded. Simple deep-freezing creates a situation of worry to some surgeons in that the water of crystallization which comes out when the specimen is thawed is thought to cause notable weakening of the arterial wall. In the freeze-drying procedure, sterilization of various forms is resorted to: *X-radiation* which is very expensive; *ethylene oxide*, which must be used in a hood because of its volitility and potential explosiveness; *beta-propriolactate*, which is used as pre-freeze soaking; and *cathode ray* sterilization. The question as to the advisability of effecting any of such procedures plus the additional time-consuming and expensive freeze-drying will usually be answered by the surgeon using the grafts.

Banks of other tissues used in surgical repair such as cartilage, veins, dura, etc. following similar techniques and confront the user with similar problems of sterility and storage. It is evident that several methods for achieving satisfactory results exist, and the one selected will depend in large measure on the thinking of the individual involved, and the space, money and personnel available.

VIRUS PRESERVATION AND STORAGE

In the laboratory studies of viruses, both research and diagnostic, special low temperature refrigeration in the −40 C range is used. While the absolute composition or characteristics of the numerous known viruses still leaves much to be determined, certain aspects of behavior of these microorganisms are known, as well as some of the physical properties that promote their growth, activity and survival in unnatural circumstances. The actual laboratory studies of this group of organisms generally proceeds at room temperature or higher temperatures up to body temperature of 37 C, and are of no particular concern in the consideration of the subject at hand. However, in the preservation of viruses which are found in various human and other tissues, quick freezing and continued subsequent maintenance of the material in the −20 C to −40 C range provide the laboratory with a means of keeping tissue and viruses contained therein for further study. This proves of most value in clinical medicine in investigating possible presence of virus-caused disease and in the research laboratory supplies a ready source of living virus to be used in the production of anti-serum or for animal inoculation to study, in living tissue, modes of operation of the organism in question. By suitable refrigeration these procedures can be done deliberately and in circumstances determined by the investigator.

The type of refrigeration necessary to produce and maintain such temperatures is naturally special. Aside from the necessary increased insulation, the freezer also has two compressors, two coils and a low temperature refrigerant, which not only provide the lower temperature, but also some margin of security in the event of mechanical failure of a single compressor. Needless to say, this refrigerator is not entered with the frequency of the more standard unit, and is used essentially for continuous freezing and low temperature storage. Certain viruses are better preserved in even lower temperatures, and the addition of a solid CO_2 container kept within the mechanical cooler will provide temperatures in the −70 C range.

An alarm system on this type of refrigeration is essential since the materials stored inside are particularly valuable in the research sense, and certainly the human element in some of the material stored for diagnostic study enhances the importance of this facet of operation. Unless the refrigerator is situated in a corridor or other readily accessible area, an alarm system of local lights or bells is not sufficient. An electric system which operates an alarm light and bell in the main engineering or maintenance office of the building, supplemented by another bell in the refrigerator room, becomes quite essential. Maintenance of such a low temperature is not secured with ease, and escape from such a temperature occurs with fair rapidity if mechanical or power failure occurs.

The tissues and their contained viruses when preserved under such conditions are stored in sealed glass vials or bottles and present no hazard to the handler unless the container is broken. Therefore, while a proper respect for the infectious area and the possibilities of contamination is recommended,