

of law that records of refrigeration performance be maintained.

In some instances, it is feasible to use an alarm system which replaces or supplements the recording thermometer and calls immediate attention to temperature variations beyond prescribed limits so that correction may be undertaken immediately. Various simple means are also described for detecting temperature variations, and, in some cases, such ideas have merit.

LABORATORY INSTRUMENTS USING REFRIGERATION

Several laboratory instruments have refrigeration attachments for the performance of unique procedures or to meet certain physical conditions encountered in a process. Since the instruments are generally of standard manufacture, details of power input, compressor size, etc., are listed in most catalogs of laboratory or hospital equipment.

Refrigerated Centrifuge

This instrument is used principally for centrifugation of materials of very volatile nature where evaporation under usual conditions would nullify quantitative studies, where solidification or precipitation of the material being separated depends on the maintenance of a specifically cold temperature, or where heating during prolonged centrifugation would adversely affect the material under investigation. Refrigerated centrifuges therefore embody the usual principles of construction of any such instrument, but have a hermetically sealed compressor unit for low temperature operations, and a cylindrical evaporator of stainless steel forming the spinning chamber interior. It is necessary to pull down the temperature before beginning the normal centrifuging action of the instrument. It is customary to cool the shields and other pieces of equipment that hold the containers and material under investigation, either by leaving them in their positions on the centrifuge head, or by placing them in another refrigerated area, perhaps where the prepared specimens are stored.

These centrifuges are commercially manufactured and equipped with accessories which provide the user with a wide choice of rotation speeds. There is also an adjustable thermostat which maintains an operating temperature $\pm 1^\circ\text{C}$ deg and registers to within 1°C deg of the temperature of the material being centrifuged. Such refrigerated machines are costly (over \$2000), but usually require little maintenance or attention aside from a routine periodic inspection. They are not usually found in the average hospital or clinical laboratory, but are used principally in biochemical, physicochemical and bacteriological laboratories in medical schools and research centers. Purchase of such an instrument for occasional use may be avoided if there is available a large walk-in refrigerator of the desired temperature into which an ordinary unequipped centrifuge may be operated. Moisture condensation may eventually cause difficulty if this suggestion is followed to extremes, but a laboratory needing that constant usage of a refrigerated centrifuge would undoubtedly be among those possessing such a machine.

Freezing Microtome

This is an instrument used principally by pathologists to prepare rapid sections of tissues removed during surgical procedures, so that microscopic examination may be made and a diagnosis offered to the surgeon before he has completed his work. From the medical standpoint, the ability to offer such a consultation and produce this information in the time limits allowed, permits an alternative or a more exten-

sive operation to ensue on the firm basis of known fact, or cessation and closure on the same basis.

The principle involved is not complex, and success of the procedure depends on sound knowledge of a capable pathologist using satisfactory equipment. The tissue is procured by the surgeon and given over to the pathologist who examines it manually and visually and selects suspicious or unusual areas for immediate microscopic examination. This selected tissue is cut into appropriately thin slices, and after formalin fixation accelerated by heat, is placed on the microtome, an instrument used for cutting sections as thin as 4 microns (1 micron = $1/1000$ mm). The plate on which the tissue is placed is attached by semi-flexible metal tubing to a carbon dioxide tank, or is incorporated in a mechanical refrigeration coil, providing a freezing temperature of the desired degree. The tissue is then cut with a knife blade mechanically designed to move through a suitable arc or cycle to cut a section of designated thickness. The section is then mounted on a glass slide, stained and examined under the microscope. This process takes from five to ten minutes and in capable hands offers definite and dependable information. The terms *frozen section* or *rapid section* have evolved from this method of preparation and are commonly used in hospital parlance.

The freezing microtome is also used in pathology and anatomy and other laboratories to prepare tissues for microscopic section in situations where the longer methods of preparation are not feasible, or where damage to certain constituents of the tissue under consideration would occur from routine processing. Examples of the former are laboratories where considerable extra equipment would be needed, entailing expense beyond anticipated usage, and in the latter case, where stains for fat, amyloid, and other tissue constituents, which are soluble or otherwise disturbed by usual tissue processing, are desired.

The instrument itself is usually constructed to utilize the freezing properties of vaporizing liquid carbon dioxide. Emission of the carbon dioxide from the tank by suitable tubing through holes in lateral margins of the metal plate on which the tissue is placed results in prompt freezing and satisfactory firmness and adhesiveness to permit the thin slices to be made. Some experience is necessary to gauge the desired hardness of the tissue, since too enthusiastic cooling results in tissue flaking and crumbling, while insufficient cooling precludes accurate sectioning and adequate tissue adhesion to the plate. The microtome itself may be adjusted to cut various thicknesses, usually in the 4 to 10 micron range.

A mechanical cooling attachment, capable of maintaining and recording any preset temperature between -30°C and -40°C , is now available. It eliminates many disadvantages of the carbon dioxide method, such as under- and over-freezing, awkward tank handling, disturbing gas pressure noises, and the delays caused by possible low tank pressure at critical times necessitating an unexpected switch over.

ELECTRON MICROSCOPE

A recent innovation using extreme cold to reduce specimen contamination in electron microscopy has been reported. The principle in electron microscopy is to photograph the image resulting from the scattering of an electron beam by the specimen under study. Contamination of the image field occurs when organic compounds such as hydrocarbons, adsorbed on the specimen surface, are dissociated by the electron impact, or polymerized, forming a contamination layer. Reduction of vapor pressure by vacuum and extreme cooling of the specimen chamber markedly reduce this contamination.

A copper block, inserted in the chamber and cooled by liquid air below -100°C , captures the contaminating par-

Medical Applications

ticles and removes them from the image field. The resultant photographs and the ensuing study are thus markedly improved by this simple practical procedure. A dry ice and acetone mixture producing a -70°C temperature is also an effective cryogen. Temperature is measured and controlled by a suitably placed thermocouple.

LIQUID SCINTILLATION COUNTER

Liquid scintillation counting is a method now in use in research laboratories for counting pulses from low level beta emitting isotopes. It offers advantages of efficiency, sensitivity and ease of sample preparation in this rapidly expanding field of investigation which relies on radioactive emissions from appropriately used isotopes to produce vital information. This discussion is not concerned with a detailed description of the process, but rather with a unique use of a cooling apparatus which adds considerable accuracy to the method.

In measuring low level beta pulses it is necessary to use photomultipliers to convert the light energy to electrical energy and to provide amplification. Unfortunately, the photomultiplier produces a large and variable number of thermal pulses, the amplitude of which is essentially the same as the amplitude of the pulses due to beta decay events, thus preventing their elimination by the ordinary pulse height selection method. To eliminate these thermal pulses, the use of cooling and a coincidence arrangement of two photomultipliers is necessary.

Cooling in a freezer greatly reduces the rate of thermal pulses, and, by having two photomultipliers view the sample vial containing the solution of radioactive sample and liquid scintillator, legitimate light pulses are seen simultaneously by both. An electronic coincidence circuit is provided to pass such pulses and to reject thermal noise pulses which occur at random in the two photomultipliers and rarely coincide.

The freezer used in conjunction with the counting and recording device to house the samples during processing is a modified chest-type 12 ft capacity commercial freezer with a -20 to -25°C operating range.

Warburg Apparatus

This is essentially a manometric apparatus to measure the amount of oxygen or other gases absorbed or evolved by tissues, microorganisms and organic or inorganic substances, as a function of time under conditions of atmospheric pressure and constant temperature. It is also used to measure transpiration of gases through membranes. The details of construction of these machines are found in manufacturers' literature.

These instruments have both heating and refrigerating units to provide a bath with a temperature range of 0 to 50°C . This bath is constructed of Monel metal in inner and outer shells separated by one-half inch insulation. The cooling system consists of a refrigerating unit, cold water tank, and circulating pump; the last controlled by a thermostat. This pump moves water from the cold water tank to the bath as needed to maintain the desired bath temperature. A close temperature control thus established avoids the accumulation of dirt and scale common with a submerged coil in the bath.

FREEZE-DRY APPARATUS

By freezing organisms and tissue quickly, it is often possible to prevent alteration in their chemical and biological characteristics. Separation at melting or freezing point with resulting concentration of components is avoided. This is in contrast with slow freezing in which two or more phases are obtained, the phase to solidify first consisting of solvent, less rich in solute than the remaining liquid phase. In the case of cellular materials, disruption of cell walls and the like is

usually avoided. Protein molecules, which frequently are large and relatively labile, are locked in place as a result of quick freezing, and denaturation does not occur. Viability of microorganisms (viruses) is maintained and enzymatic changes are retarded. Quick freezing thus is applied for the basic purpose of avoiding change.

Speed of freezing of biological materials depends upon several factors in addition to the temperature of the refrigerant. Thus, surface extent, insulating barriers, phases encountered, are all important. With liquids, agitation or stirring is beneficial.

Principles of Dehydrating Frozen Materials

1. The temperature is below that at which many labile substances undergo chemical change. This applies to labile components in blood, in viruses and other forms of microorganisms, and to other biologicals and pharmaceuticals.

2. Because of the low temperature, the loss of volatile constituents is minimal.

3. Since the product is frozen, there is no bubbling or foaming. Thus changes due to surface action may be avoided. An example is surface denaturation of proteins which occurs in drying protein-containing solutions even at low liquid temperatures under vacuum.

4. In most cases, the solute remains evenly dispersed and distributed without undergoing concentration as the frozen solute sublimates. The remaining dry residue emerges as a highly porous solid framework, which occupies essentially the same total space as the original solution. This residue therefore is a friable interlocking and spongy structure, not a fine powder, and solubility is rapid and complete.

5. The molecules of solute are virtually locked in position in quick freezing and the tendency for coagulation of even lyophobic sols is minimal. The lipid constituents of dry blood plasma do not reconstitute perfectly after drying and produce slight turbidity, but there is far from complete coalescence and the particles are safe for intravenous injection without capillary embolism (lodgment of abnormal particle causing obstruction).

6. During drying the surface of the evaporating frozen ice layer gradually recedes to leave more and more of the highly porous residue of solute exposed. Thus, *case hardening* never occurs, resulting in low moisture content and high degree of stability.

7. Bacteriological growth and enzymatic changes do not take place under conditions of freeze-drying (but bacteriological or other contamination is not necessarily removed).

8. Because of the high vacuum used, the amount of oxygen present is quite small, so even the most readily oxidizable constituents are protected.

The applications of the principles of freeze-drying in medical and other scientific fields are numerous. The following table illustrates many of the present day uses.

Blood	Viruses	Vaccines
Human and animal plasma	Small pox	Small pox
Anti-sera	Babies	BCG
Agglutinating sera	Scrub typhus	Yellow fever
Complement	Eft Valley fever	Newcastle disease
Albumin	Blue tongue	Fowl pest
Fibrin	Coxsackie	Rinder pest
Tissues	Microorganisms	
Arteries	Pathogenic organisms	
Bones	Microorganism suspensions	
Skin	Molds	
Molluscan tissues	Yeasts	
Tumors	Fungi	
Seeds	Cheese starter culture	
	Bacterial cultures	
	Brucella abortus	
Biological Chemicals		
Liver extract	Hyaluronidase	
Rabbit brain	Human thrombin	
Cell extracts	Hormones	
Protein-containing liquids	Hog stomach extract	
Bee venom	Chromatographic separations	
	Enzyme separations	