Air-Steam Mixtures. While air-steam mixtures have a lower temperature and lower thermal capacity than pure steam, the presence of air may be utilized to control the pressure in the chamber when flexible-walled containers of products are being sterilized. For example, plastic bags of large-volume parenterals (LVPs) or collapsible tubes of aqueous jellies would swell and burst in an autoclave utilizing steam only, particularly during the cooling phase. When air is mixed with the steam and the air pressure is independently controlled, the pressure applied to the outside of the containers can be adjusted to equal the internal pressure so that the containers do not burst. Because of the tendency of steam and air to stratify, the mixture must be mixed continuously; this is usually accomplished by means of a blower.

Moist heat sterilization is also applicable to equipment and supplies such as rubber closures, glassware, and other equipment with rubber attachments; filters of various types; and uniforms. To be effective, however, air pockets must be eliminated. This normally requires that the items be wet when placed in the autoclave. They also will be wet at the end of the sterilizing cycle. When moisture can escape without damage to the package, part of the moisture can be removed by employing an evacuation step at the end of the cycle.

Dry heat sterilization is used for containers and equipment whenever possible because an adequate cycle results in sterile and dry equipment. High-speed processing lines recently developed have included a hot-air tunnel for the continuous sterilization of glass containers, which are heated by infrared lamps or by electrically heated, filtered, circulating air. Glass and metal equipment usually withstand dry heat sterilization without difficulty, although uneven thermal expansion may cause breakage or distortion. Rubber and cellulosic materials undergo degradation, however. Certain ingredients, such as chemicals and oleaginous vehicles, to be used

in sterile pharmaceutical preparations are sometimes sterilized with dry heat at lower (usually 140°C or less) temperatures.

Nonthermal Methods

Ultraviolet Light. Ultraviolet light is commonly employed to aid in the reduction of contamination in the air and on surfaces within the processing environment. The germicidal light produced by mercury vapor lamps is emitted almost exclusively at a wave length of 2537 Angstrom units (253.7 millimicrons). It is subject to the laws for visible light, i.e., it travels in a straight line, its intensity is reduced in proportion to the square of the relative distance it travels, and it penetrates materials poorly or selectively. Ultraviolet light penetrates clean air and pure water well, but an increase in the salt content and/or the suspended matter in water or air causes a rapid decrease in the degree of penetration. For most other applications, penetration is negligible, and any germicidal action is confined to the exposed surface.

Lethal Action. When ultraviolet light passes through matter, energy is liberated to the orbital electrons within constituent atoms. This absorbed energy causes a highly energized state of the atoms and alters their reactivity. When such excitation and alteration of activity of essential atoms occurs within the molecules of microorganisms or of their essential metabolites, the organism dies or is unable to reproduce. The principal effect may be on cellular nucleic acids, which have been shown to exhibit strong absorption bands within the ultraviolet wavelength range.

The lethality of ultraviolet radiations has been well established; however, it also has been shown that organisms exposed to ultraviolet radiations can sometimes recover, a fact not surprising if the previously described theroy of lethality is correct. Recovery has been increased by the addition of certain essential metabolites to the culture, adjustment of the pH of the medium, or exposure to visible light shortly after exposure to the ultraviolet radiations. Therefore, adequate exposure to the radiations must occur before reliance can be placed upon obtaining a sterilizing effect.

The germicidal effectiveness of ultraviolet light is a function of the intensity of radiation and time of exposure. It also varies with the susceptibility of the organism. The data in Table 21-4 show some of this range of susceptibility.[20] From these data, it can be seen that if the intensity of radiation on a surface was 20 microwatts per cm^2, the minimum intensity usually recommended, it would require approximately 1100 seconds exposure to kill *B. subtilis* spores, but only approximately 275 seconds to kill S. hemolyticus. The intensity of ultraviolet radiation can be measured by means of a special light meter having a phototube sensitive to the 2537 Å wavelength.

Maintenance and Use. To maintain maximum effectiveness, ultraviolet lamps must be kept free from dust, grease, and scratches because of the large reduction in emission intensity that will occur. Also, they must be replaced when emission levels decrease substantially (about 30 to 50%) owing to energy-induced changes in the glass that inhibits the emission.

Personnel present in areas where ultraviolet lights are on should be protected from the direct and reflected rays. These rays cause reddening of the skin and intensely painful irritation of the eyes. The American Medical Association has recommended that the maximum safe human exposure for 1 hour be limited to 2.4 mw/cm.^{2}

Ultraviolet lamps are used primarily for their germicidal effect on surfaces or for their penetrating effect through clean air and water. Therefore, they are frequently installed in rooms, air ducts, and large equipment in which the radiation can pass through and irradiate the air, and also reach exposed surfaces. Water sup-

Ionizing Radiations. Ionizing radiations are high-energy radiations emitted from radioactive isotopes such as cobalt-60 (*gamma rays*) or produced by mechanical acceleration of electrons to very high velocities and energies (*cathode rays, beta rays*). Gamma rays have the advantage of being absolutely reliable, for there can be no mechanical breakdown; however, they have the disadvantages that their source (radioactive material) is relatively expensive and the emission cannot be shut off as it can from the mechanical source of accelerated electrons. Accelerated electrons also have the advantage of providing a higher and more uniform dose rate output.

output.

Electron Accelerators. Electron accelerators are of two general types, the linear and the Van de Graaff accelerators.

Lethal Action and Dosage. Ionizing radiations destroy microorganisms by stopping reproduction as a result of lethal mutations. These mutations are brought about by a transfer of radiation beam energies to receptive molecules in their path, the direct-hit theory. Mutations also may be brought about by indirect action in

which water molecules are transformed into highly energized entities such as hydrogen and hydroxyl ions. These, in turn, bring about energy changes in nucleic acids and other molecules, thus eliminating their availability for the metabolism of the bacterial cell. Ionizing radiations differ from ultraviolet rays in their effects on matter primarily in that the former are of a higher energy level, actually producing ionization of constituent atoms. Bacterial spores and

Applications for Sterilization. Accelerated electrons or gamma rays may be used to sterilize select products by a continuous process. Most other product sterilization procedures must be performed in batches. Continuous-process sterilization requires exacting control so that there are no momentary lapses in sterilizing effectiveness.

A number of vitamins, antibiotics, and hormones in the dry state have been successfully sterilized by radiation. Liquid pharmaceuticals are more difficult to sterilize because of the potential effect of the radiations on the vehicle system as well as the drug.

Filtration. Filtration may be used for the removal of particles, including microorganisms, from solutions and gases without the application of heat. Ideally, filters should not alter the solution or gas in any way, neither removing desired constituents nor imparting undesired components. This requirement essentially limits the types of filters currently employed to the polymeric types listed in Table 21-5A, B. Furthermore, almost all of those currently in use with parenteral solutions and gases are of the membrane type, that is, tissue-thin material removing particles primarily by sieving. When a filter does remove constituents from a solution such removal is usually due to the phenomenon of adsorption, which being a surface phenomenon, occurs during only the first portion of the filtration, that is, until the surface of the filter is saturated with the adsorbed molecule or ion. The most common attack on the filter itself is due to the solvent properties of the vehicle of certain parenteral products. Since the most common solvent for parenteral solutions is water, and the use of other types of solvents is limited, this usually is not a problem. N

membrane filters are usually composed of plastic polymers, including cellulose acetate and nitrate, nylon, polyvinyl chloride, polycarbonate, polysulfone and Teflon. Occasionally, sintered metals such as stainless steel and silver are used when highly durable characteristics are required.

Since most of the membrane filters are disposable, the problem of cleaning after use is limited to the reusable filter housing and support screen. These are usually made of stainless steel or tough plastic polymers that are cleaned rather easily. Careful attention must be given, however, to disassembly of the housing and scrubbing to remove any residues that might introduce contamination in subsequent use.

The pores, or holes, through any filter medium consist of a range of sizes. For example, if a filter is designated as 0.2 micron porosity, the porosity most commonly used to effect sterilization, the maximum mean pore diameter is 0.2 micron, with many pores much smaller than this and a few larger. The latter may have diameters as large as 0.5 micron, but they are so few in number that the probability of a microbial spore (commonly rated as being 0.5 micron in diameter) finding those few pores is highly remote. However, it must be recognized that there is a probability of this happening, even though remote. Therefore, it is no longer acceptable to consider such filters an absolute means of sterilizing a solution. To increase the probability of achieving a sterile filtrate, some researchers are proposing that the solution be passed through a series of two 0.2-micron porosity filters. Others have suggested that a 0.1-micron porosity filter be used, but this would greatly reduce the flow rate.

Since membrane filters function primarily by sieving, particles of any kind in a solution are retained on the surface. If the content is relatively high, particles may accumulate on the surface and plug the filter so that the flow of solution decreases and perhaps stops. To avoid this problem, when solutions have a high content of solids, particularly when the solids are deformable macromolecules, the solution can best be processed by passing it through one or more prefilters, the first usually being a relatively porous depth filter. With depth filters, particles may gradually migrate through the filter if filtration time is prolonged, if there is a high pressure differential, or if there is frequent fluctuation of the pressure.

Liquid Flow Through a Filter. The flow rate of a liquid through a filter is affected by the size of the pores through the filter, the pore volume (the proportion of open space to solid matrix), the surface area of the filter, the pressure differential across the filter, and the viscosity of the liquid. Of these factors, the two most practical ways to increase flow rate is to increase the surface area of the filter or the pressure differential across the filter. There is a practical limit to increasing the diameter of a disc filter; thus, if larger surface areas are required, a pleated filter in a cartridge form is often used. In this way, a large increase in surface area may be achieved within a relatively small overall dimension of the filter unit. Within the limits of the

physical strength of the filter and its housing, the pressure differential can be increased to several hundred pounds per square inch. In pharmaceutical practice, however, the pressure differential used is rarely more than 25 to 30 pounds per square inch. Usually, positive pressure is applied on the liquid upstream of the filter, but a vacuum may be drawn downstream of the filter. In the case of a vacuum, the maximum differential achievable is one atmosphere, or approximately 15 pounds per square inch. Furthermore, the negative pressure in the filtrate chamber makes it difficult to prevent the ingress of contamination from the environment. Therefore, for filtrations designed to render solutions sterile, it is preferable to apply pressure upstream of the filter using a gas filtered to be free from microorganisms. Any leakage that may occur in such a system causes loss to the outside without contamination of the sterile filtrate.

Solutions having a high viscosity normally have a slow flow rate. In most instances, the rate can be increased by warming the solution, thereby reducing its viscosity provided the warming does not have an adverse affect on the solution.

As previously mentioned, the flow rate through a filter also depends on the relative pore volume of the filter. All filters must have a solid matrix that forms the framework for the pores. The lower the amount of solid matrix is in proportion to the pore spaces, the higher are the pore volume and the flow rate.

Types of Filters. Since the filter membranes are designed to be used once and then discarded, they are disposable; further, filter housings composed of plastic polymers, which are also intended to be disposable, are becoming increasingly available. Thus, all after-use cleaning is eliminated. In addition, the membrane filter is sealed into the housing by the manufacturer, so that the risk of leakage is minimal.

Membrane filters are usually in the form of discs or pleated cylinders (cartridges). They range from 13-mm discs (approximately 0.8 cm^2) to 20-in. or longer cartridges (approximately 0.84 M^2). The housings are usually of stainless steel or of various plastic polymers.

A few years ago, it was rather common practice to use filters that were reusable, such as diatomaceous earth, sintered glass, and unglazed porcelain. Because of the problems of adequate cleaning between uses and of testing, current applications of these filters are limited.

Testing of Filters. Although membrane filters are tested and labeled by the manufacturer, the pore size and integrity of the filter should be checked before use. The least complicated method for doing this is the bubble point test. This test is performed by applying air pressure, or other gas pressure, to the upstream side of a hydrophilic filter in which the pores are filled with water. The pressure is gradually increased until bubbles pass through the filter and are detected in a liquid downstream. This bubble point pressure is inversely proportional to the diameter of the pores, and thus is a measure of the largest pores. The filter manufacturer identifies the appropriate test pressure for each pore size, for example, 55 pounds per square inch gauge for a hydrophilic membrane filter of 0.2 μm porosity as given in Table 21-5. If there is even a pinhole or similar defect in the filter, bubbling occurs at a much lower pressure than expected. For hydrophobic membranes, the filter is usually wet with ethanol or methanol prior to application of the air pressure.

A more direct test with respect to the ability of a filter to retain microorganisms is the microbial challenge test. A standardized culture containing a large number of small microorganisms, such as Pseudomonas diminuta, is filtered. The objective is to provide a high probability of finding oversized pores in the filter by the challenge of a large number of small microorganisms. Therefore, after filtration, the presence of bacteria in the filtrate constitutes a failure of the filter to sterilize the liquid. This type of test is normally a part of the quality control program of the filter manufacturer for each lot of sterilizing porosity membrane from which filters are made; however, it is rarely used in the pharmaceutical plant for individual filters.

Aseptic Processing. Sterilization of a solution by filtration provides an extremely clean solution, removing dirt particles as well as microorganisms in the micron size range. After sterilization, however, the filtrate must be transferred from the receiver and subdivided into the individual final containers. The objective of this process, known as *aseptic processing*, is to

exclude every microorganism from all steps of the process subsequent to filtration. Accomplishing this requires a rigidly controlled aseptic environment and technique. The difficulty of maintaining such an aseptic condition is the greatest problem associated with sterilization by filtration; however, for solutions that are adversely affected by heat, this may be the only way in which sterilization can be accomplished.

Aseptic processing is technically not a sterilization process, but is mentioned here because of its close involvement with sterilization by filtration. It is used for products that cannot be *terminally sterilized*, that is, sterilized after they have been sealed in the final container. (See also Chapter 22.)