Sterilization is the process designed to produce a sterile state. The traditional concept of the sterile state is the absolute condition of total destruction or elimination of all living microorganisms. This concept has given way to the reality that sterile is a term that must be given relative connotation and that the probability of having achieved the absolute can only be predicted on the basis of kinetic projection of microbial death rates. Therefore, sterility in the absolute sense cannot be shown to have been achieved, but rather, can be approached with an increasing probability of success as a sterilization process is improved. With terminal methods of steriliza-

improved. With terminal methods of sterilization of a parenteral product, particularly steam under pressure, a probability of no more than one nonsterile unit in a million (10^{-6}) is readily achievable. Even greater levels of assurance can be achieved with current technology. In this

chapter, *sterile* indicates a probable condition of complete freedom from viable microorganisms with the limitations just expressed; these limitations are developed more fully later in the chapter. The term *aseptic* indicates a controlled process or condition in which the level of microbial contamination is reduced to the degree that microorganisms can be excluded from a product during processing. It describes an "apparently" sterile state.

Persons responsible for carrying out sterilization procedures must be acutely aware of the degree of effectiveness as well as the limitations of each sterilization process. They must also understand that these processes may have a deleterious effect on the material to be sterilized. In the processing of pharmaceuticals, it is often necessary to reach a compromise between the most effective sterilization procedure and one that will not have a significant adverse effect upon the material to be sterilized. For example, it may be necessary to add an antibacterial agent

to a thermally sensitive product to enhance the effectiveness of a low-temperature sterilization process; thereby decomposition is prevented while the combined effect of the antibacterial and the heat provide reasonable assurance that the product will be sterilized.

Microorganisms exhibit varying resistance to sterilization procedures. The degree of resistance varies with the specific organism. In addition, spores, the form that preserves certain organisms during adverse conditions, are more resistant than vegetative forms of the organism. The data given in Table 21-1 illustrate the varying resistance of different spores to moist and dry heat. Therefore, the conditions required for a sterilization process must be planned to be lethal to the most resistant spores of microorganisms normally encountered, with additional treatment designed to provide a margin of safety against a sterilization failure.

Validation of Sterilization Processes

All sterilization processes (thermal, chemical, radiation, and filtration) are designed to destroy or eliminate microbiologic contaminants present in a product. The official test for sterility of the product is a destructive test on a selected sample; thus, the task of proving that all units of a product are sterile must involve the employment of probability statistics. The statistics of probability depend on such parameters as the length or degree of exposure to the sterilant, the type and number of microorganisms present, the desired level of microbial destruction or elimination, and the resistance of the microorganism(s) presented to the sterilization process.

In recent years, the pharmaceutical industry has intensified its efforts to quantitate the rate and extent of microbial destruction or elimination. The Food and Drug Administration has stated in its current good manufacturing practice regulations that sterilization procedures must be validated pertaining to (1) the design of the equipment and the process used to produce batch sterilization and (2) the confirmation with reproducible data of a given probability level of residual microbial contamination upon completion of the sterilization process. Validation of sterilization processes can be facilitated by using quantitative, theoretically sound principles such as microbial death kinetic expressions.

Microbial Death Kinetic Terms

An important term in expressing microbial death kinetics for heat, chemical, and radiation sterilization is the *D value*. The D value is the time (for heat or chemical exposure) or the dose (for radiation exposure) required for the microbial population to decline by one decimal point (a 90%, or one logarithmic unit, reduction). The D value may be estimated graphically, as shown in Figure 21-1, or mathematically, as shown by equation (1):

$$D = \frac{U}{\log N_0 - \log N_u} \qquad (1)$$

where U is the exposure time or exposure dose, under specific conditions, N_0 is the initial microbial population (product bioburden) and N_u is the microbial population after receiving U time

or dose units of sterilant exposure. For example, after 5 min of product exposure to a temperature of 121°C, the microbial population was reduced from 2×10^5 to 6×10^3. Then, the D value at 121°C is:

$$D_{121} = \frac{5 \text{ min}}{\log(2 \times 10^5) - \log(6 \times 10^3)} = 3.28 \text{ min}$$

Thus, at 121°C, the microbial population is decreased by 90% every 3.28 min.

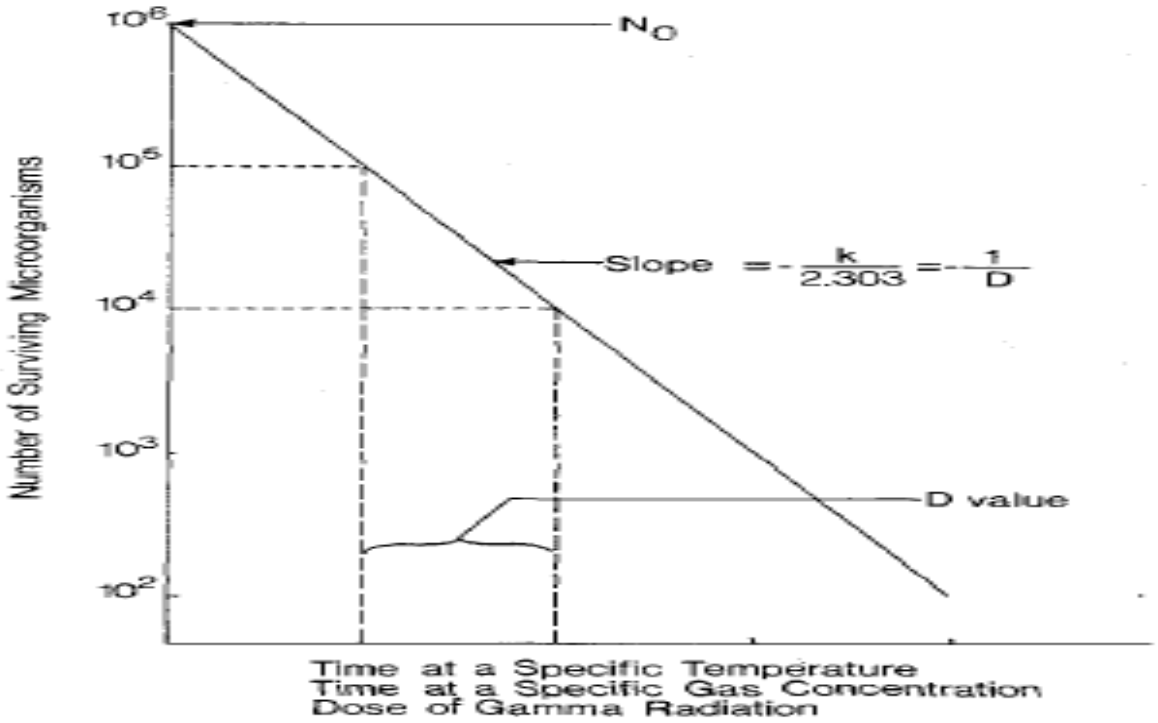

FIG. 21-1. *Graphic representation of the semilogarithmic microbial death rate.*

D values have been defined precisely for various microorganisms contained in certain environments (liquids and solid surfaces) at specific temperatures for heat sterilization,[5] and at direct exposure to cobalt-60 irradiation.[6] D values cannot be defined precisely for microorganisms exposed to such gases as ethylene oxide because of the complex interaction of heat, concentration of gas, and relative humidity.[7] D values are estimated for gas sterilization when it is possible to keep heat and humidity values constant, varying only the concentration of gas.

Other key terms used in the determination of microbial death rates include *microbial load*, or *bioburden*; the *Z value*; the *F value*; the F_0 value; and the probability of nonsterility. These terms are defined in Table 21-2, and Z value plots are shown in Figure 21-2.

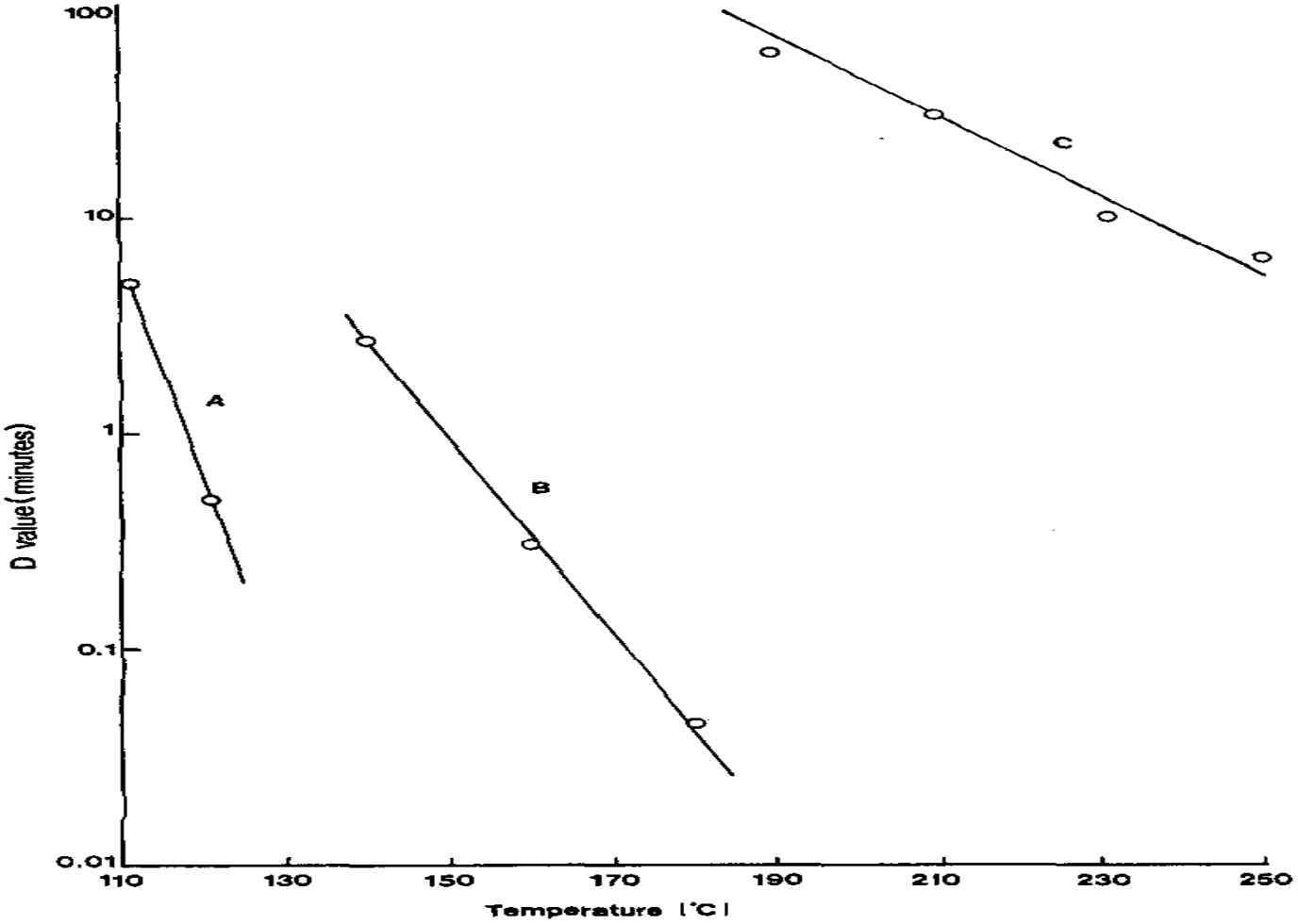

FIG. 21-2. *Z value plots of log D versus temperature.*
Key—A: $Z = 10°C$ *for* B. stearothermophilus *spores exposed to steam sterilization.*
B: $Z = 22°C$ *for* B. subtilis *var* niger *spores exposed to dry heat sterilization.*
C: $Z = 54°C$ *for* E. coli *endotoxin exposed to dry heat sterilization.*

TABLE 21-2. *Definition of Key Terms Employed in Microbial Death Kinetics*

Symbol	*Term*	*Definition*
N_0	Bioburden	The population or number of living microorganisms per defined unit, surface, or system.
Z	Resistance value	The number of degrees (C or F) required for a 1 log reduction in the D value. $Z = -\frac{T_1 - T_2}{\log D_2 - \log D_1}$
F (T,z) or F_T^z	Sterilization process equivalent time	The equivalent time at temperature T delivered to a unit of product calculated using a specified value of z.
F_0	Sterilization process equivalent time	The equivalent time at a temperature of 121°C delivered to a unit of product calculated using a z value of 10°C.
N_u	Probability of nonsterility	The number of nonsterile units per batch or the theoretic or extrapolated number of living microorganisms per defined unit after a given equivalent heating time U at a specific temperature T.

$$N_u = \text{antilog}\left(\log N_0 - \frac{U_T}{D}\right)$$

The F_0 value is a term widely used in sterilization cycle design and validation. Its current application is limited to steam sterilization although an F value can be computed for any thermal method of sterilization. The F_0 value can be defined by the following two equations:

$$F_0 = \Delta t \sum 10^{\frac{T-121}{10}} \qquad (2)$$

where Δt is the time interval between product temperature measurements T.

$$F_0 = D_{121} (\log N_0 - \log N_u) \qquad (3)$$

where N_0 and N_u are those terms defined previously.

The F_o value of equation (2) is obtained by physical measurement of product temperature and substitution of that temperature for T in the exponent. For example, if the product temperature was measured every 5 min from 0 to 30 min

and found to be 25°C, 110°C, 118°C, 120°C, 121°C, and 100°C, the F_o value would be:

$$F_o = 5 \text{ min } (0 + 0.079 + 0.501 + 0.794 + 5.000 + 0.0079)$$
$$F_o = 5 \text{ min } (6.382)$$
$$F_o = 31.91 \text{ min}$$

By definition, when the F_o value is used, the Z value is assumed to be 10°C. This means that for every 10°C increase in product temperature, the D value is decreased by 90%, or 1 log unit.

Equation (3) is the biologic F_o equation because the F_o value is calculated after determining the D_{121} value and the product bioburden, N_o. The probability of nonsterility is whatever level is desired, usually a minimum of 10^{-6}. In general, equation (3) is applied under two circumstances. Given D_{121}, N_o, and N_u, the F_o value is calculated. For example, if $D_{121} = 1$ min, $N_o = 10^2$, and $N_u = 10^{-6}$, then:

$$F_o = 1 \text{ min } (\log 10^2 - \log 10^{-6})$$
$$F_o = 8 \text{ min}$$

Given D_{121}, N_o, and F_o, the achieved level of nonsterility may be calculated. For example, if $D_{121} = 2$ min, $N_o = 10^2$, and $F_o = 8$ min, then:

$$N_u = \text{antilog} \left(\log N_o - \frac{F_o}{D_{121}} \right)$$
$$N_u = \text{antilog} \left(\log 10^2 - \frac{8}{2} \right)$$
$$N_u = 10^{-2}$$

At least three factors affect the F_0 value. They are (1) the container characteristics: size, geometry, and heat transfer coefficient, (2) the product volume and viscosity, and (3) the size and configuration of the batch load in the sterilizer.

Physical Processes of Sterilization

Thermal Methods

The lethal effectiveness of heat on microorganisms depends upon the degree of heat, the exposure period, and the moisture present. Within the range of sterilizing temperatures, the time required to produce a lethal effect is inversely proportional to the temperature employed. For example, sterilization may be accomplished in 1 hour with dry heat at a temperature of 170°C, but may require as much as 3 hours at a temperature of 140°C. While it is common practice to

viously; however, the lethal effect must be computed in terms of the time during which the entire mass of the material is heated. The mechanism by which microorganisms are killed by heat is thought to be the coagulation of the protein of the living cell. The data given in Table 21-3 illustrate this principle, using the effect of varying amounts of water on the temperature required to coagulate egg albumin.[13] The temperature required is inversely related to the moisture present. Further, experience in the laboratory has confirmed that sterilization by thermal methods may be effected at lower temperatures in the presence of moisture.

TABLE 21-3. *Effect of Moisture and Heat on Egg Albumin*

Water (%)	*Temperature (°C)*	*Effect*
50	56	coagulation
25	80	coagulation
6	145	coagulation
0	170	coagulation and oxidation

Thermal methods of sterilization may conveniently be divided into those accomplished by dry heat and those by moist heat.

Dry Heat. Substances that resist degradation at temperatures above approximately 140°C (284°F) may be rendered sterile by means of dry heat. Two hours exposure to a temperature of 180°C (356°F) or 45 min at 260°C (500°F) normally can be expected to kill spores as well as vegetative forms of all microorganisms. This total sterilizing cycle time normally includes a reasonable *lag time* for the substance to reach the sterilizing temperature of the oven chamber, an appropriate hold period to achieve sterilization, and a cooling period for the material to return to room temperature.

Factors in Determining Cycle Time. The cycle time is composed of three parts: (1) the thermal increment time of both the chamber and the load of material to be sterilized, assuming both start at room temperature, (2) the hold period at the maximum temperature, and (3) the cooling time.

The cycle time is most commonly prescribed in terms of the hold time, for example, 2 hours at 180°C dry heat. The hold time may be shown by sensors detecting the temperature of the chamber at its coolest spot; however, a better indication of the actual thermal condition is obtained by sensing, usually with a thermocouple, the coolest spot in the load of the material to be sterilized. When such a location is used, and when this coolest spot is known from previous validation studies, the timing required for sterilization is correctly programmable. It should be remembered that other parts of the load of material may be heated for a longer period, and if it is thermally unstable, degradation could occur. Therefore, the thermal stability of the material to be sterilized must be known and the optimum method of sterilization selected to achieve effective sterilization throughout the entire mass of material while maintaining its stability and integrity.

Sterilizer Types. The ovens used to achieve hot air sterilization are of two types, natural convection and forced convection. Circulation within natural convection ovens depends upon the currents produced by the rise of hot air and fall of cool air. This circulation can be easily blocked with containers, resulting in poor heat distribution efficiency. Differences in tempera-

ture of 20°C or more may be found in different shelf areas of even small laboratory ovens of the natural convection type.[14]

Forced convection ovens provide a blower to circulate the heated air around the objects in the chamber. Efficiency is greatly improved over natural convection. Temperature differences at various locations on the shelves may be reduced to as low as ±1°C. The lag times of the load material therein also are greatly reduced because fresh hot air is circulated rapidly around the objects. The curves shown in Figure 21-3 illustrate the difference in lag time for some of the same containers of corn oil when heated in a natural convection oven as compared with the same oven equipped for forced circulation.[14]

Another type of sterilizer is the tunnel unit with a moving belt, designed to thermally sterilize glass bottles and similar items as they move through the tunnel. The items are cooled with clean air before they exit the tunnel, usually directly into an aseptic room and linked in a continuous line with a filling machine. Such units require careful validation.[15]

Effect on Materials. The elevated temperatures required for effective hot air sterilization in a reasonable length of time have an adverse effect on many substances. Cellulose materials, such as paper and cloth, begin to char at a temperature of about 160°C (320°F). At these temperatures, many chemicals are decomposed, rubber is rapidly oxidized, and thermoplastic materials melt. Therefore, this method of sterilization is reserved largely for glassware, metalware, and anhydrous oils and chemicals that can withstand the elevated temperature ranges without degradation. Expansion of materials is also appreciable, as they are heated from room to sterilizing temperatures. Therefore, glassware must not be wedged tightly in the oven chamber, containers for oils must be large enough to permit expansion of the oil, and provision must be made for the expansion of other substances.

Advantage may be taken of the anhydrous state achieved with this method of sterilization to provide dry glassware and metalware at the end of an adequate heating cycle. Dry equipment and containers are essential in the manufacture of an anhydrous product, but they are also desirable to prevent dilution of an aqueous product. Also, dry equipment can be kept sterile during storage more easily than wet equipment. Further, dry heat effectively destroys pyrogens, usually requiring about twice the hold time for sterilization.

To maintain a sterile condition after sterilization, environmental contamination must be excluded. The openings of equipment must be covered with a barrier material such as aluminum foil. As an alternative, items to be sterilized may be placed in a covered stainless steel box or similar protective container.

Moist Heat. Moist heat is more effective than dry heat for thermal sterilization. It should be remembered, however, that normal moist heat cycles do not destroy pyrogens.

As previously noted, moist heat causes the coagulation of cell protein at a much lower temperature than dry heat. In addition, the thermal capacity of steam is much greater than that of hot air. At the point of condensation (*dew point*), steam liberates thermal energy equal to its heat of vaporization. This amounts to approximately 540 calories per gram at 100°C (212°F) and 524 calories per gram at 121°C (250°F). In contrast, the heat energy liberated by hot dry air is equivalent to approximately only 1 calorie per gram of air for each degree centrigrade of cooling. There-

fore, when saturated steam strikes a cool object and is condensed, it liberates approximately 500 times the amount of heat energy liberated by an equal weight of hot air. Consequently, the object is heated much more rapidly by steam. In addition, when steam under pressure is employed, a rapidly changing fresh supply of heat-laden vapor is applied to the object being heated. This is due both to the pressure under which steam is applied and to the partial vacuum produced at the site where steam is condensed, for it shrinks in volume by about 99% as it condenses.

Air Displacement. The density of steam is lower than that of air. Therefore, steam enters an autoclave chamber and rises to the top, displacing air downward, as illustrated by the gravity displacement autoclave shown in Figure 21-4. Objects must be placed in the chamber with adequate circulation space around each object, and so arranged that air can be displaced downward and out of the exhaust line from the chamber. Any trapped air, e.g., air in containers with continuous sides and bottoms or in tightly wrapped packs, prevents penetration of the steam to these areas and thus prevents sterilization. The air trapped in this manner is heated to the temperature of the steam, but hot air at a temperature of 120°C (248°F) requires a cycle time of 60 hours to ensure a lethal effect on spores.[16] A 20-min exposure at this temperature with hot dry air, therefore, would be entirely inadequate.

Factors Determining Cycle Time. Spores and vegetative forms of bacteria may be effectively destroyed in an autoclave employing steam under pressure during an exposure time of 20 min at 15 pounds pressure (121°C [250°F]) or as little as 3 min at 27 pounds pressure (132°C [270°F]). These time intervals are based on the assumption that the steam has reached the innermost recess of the material to be sterilized, and that the temperature of the material is held for at least one half of that time interval. In the case of bottles of solution, the heat must be conducted through the wall of the container, raise the temperature of the solution to that of its environment, and generate steam within the container from the water therein. Therefore, a significant lag time is involved before the solution reaches the sterilizing temperature.

The determination of lag time and its inclusion in the planned total cycle time is no less important for moist heat sterilization than for hot air sterilization, discussed previously. By way of illustration, it has been found that 1200 ampuls, each containing 5 ml of a solution, can be effectively sterilized in an autoclave at 121°C (250°F) during an exposure time of 20 min. A single bottle containing the same total volume of solution (6 L) required an exposure of 60 min at 121°C (250°F).[17]

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.

Application of Thermal Methods of Sterilization. It is generally accepted that the most reliable thermal method of sterilization is the use of moist heat under pressure. Therefore, this method of sterilization should be employed whenever possible. Aqueous pharmaceutical preparations in hermetically sealed containers that can withstand the temperature of autoclaving can be rendered sterile and remain so indefinitely unless tampering with the seal occurs. Nonaqueous preparations in sealed containers cannot be sterilized in this manner during a normal cycle because no water is present within the container to generate steam and thereby effect sterilization.

Moist heat sterilization is also applicable to

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. In such cases, it