Physical Processes of Sterilization

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.

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

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

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 material lags behind the increasing temperature of the chamber. **The time required for all of the material to "catch up" with the temperature of the chamber is longer with larger quantities of material, poorer thermal conductance properties of the material, and lower heat capacity.**

The relationship of these factors must be carefully determined during validation studies so that effective cycle times can be planned.

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

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**.

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.

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, metal ware, 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 metal ware 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 **aluminium 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 *tem*perature 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 centigrade of cooling.

Therefore, **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. A 20-min exposure at this temperature with hot dry air, therefore, would be entirely inadequate.

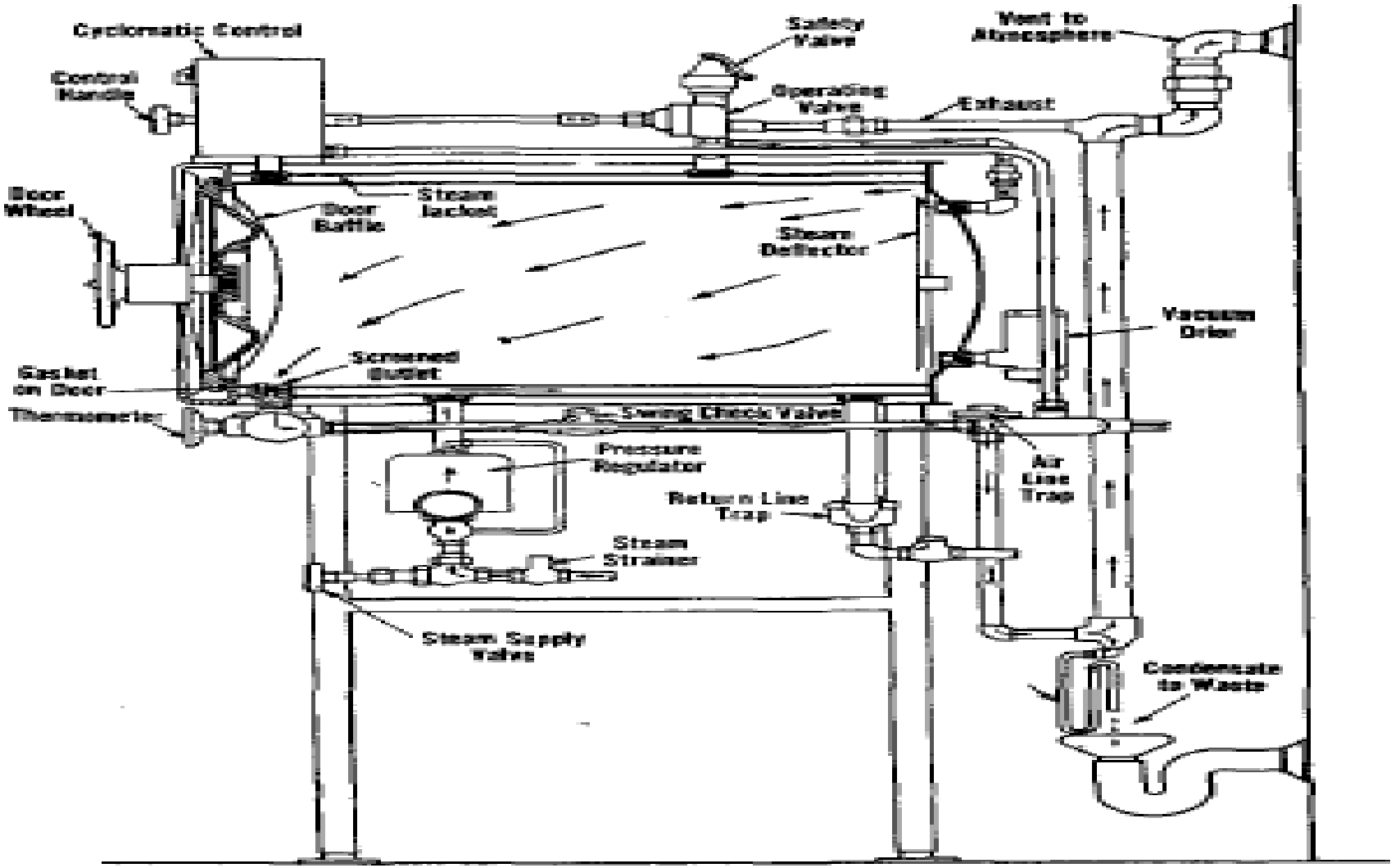

FIG. 21-4. *Cross-sectional diagram of the functional parts of an autoclave. (Courtesy of American Sterilizer Co.)*

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. Byway of illustration, it has been found that 1200 ampules, each containing 5 ml of a solution, can be effectively sterilized *in* an autoclave at 121 ºC (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 ºC (250°F).

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 parenteral (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.

Approaches to Reduction* of *Cycle Time.

Prolonged heating of most objects is detrimental to the material. For example, fabrics and rubber parts deteriorate with loss of tensile strength, solutions may undergo adverse chemical changes, and metal objects may become pitted.

Therefore, the total cycle time should be controlled so that the heating period is not unnecessarily prolonged.

Usually, this is best accomplished by shortening the cooling period. For non-sealed items of equipment or containers that do not contain solutions, the steam may be exhausted *to* the outside rapidly at the end of the sterilizing cycle. Objects are thereby cooled rapidly, particularly if removed from the autoclave chamber. Such a procedure cannot be employed for solutions, whether sealed or unsealed in containers, because the rapid release of chamber pressure would cause violent ebullition of the hot solution, with spattering of the contents of unsealed containers and explosion of sealed containers.

One method for rapid extraction of heat from sealed containers of solutions is to spray the containers with gradually cooling water while the pressure in the chamber is concurrently reduced.

Another **accelerated cooling method** employs short pulses of high pressure steam introduced into the loaded chamber. As the steam expands in the chamber it extracts heat from the containers of *solution*. The *steam is* exhausted from the chamber at a rate that provides for a gradual reduction of the pressure concurrent with the temperature reduction. By these methods, it is sometimes necessary to introduce pulses of air into the chamber to replace all or part of the steam so that the pressure around the containers is not reduced too rapidly. By the spray cooling method, it has been reported that the cooling time for a load of 200 one-liter bottles of solution may be reduced from about 20 hours to about 20 min.

A relatively new approach to a reduction in the total heating cycle time has been the introduction of **a precycle vacuum**. In a specially designed autoclave, a precycle vacuum of at least 20 mm Hg is drawn. More recent studies have shown that a double vacuum drawn in sequence prior to the heating cycle removes, air more effectively from porous materials.

The subsequent introduction of *steam* permits rapid penetration and load heating with complete elimination of air pockets. Since the total heating period is markedly reduced owing to the reduction in the temperature increment time, a higher temperature (usually 135°C [275°F]) may be employed with less deleterious effects on materials. This method is ·particularly suited to operating room packs in hospitals, where the total cycle time for large packs has been reduced from about 78 *min* by the conventional method to about 14 min. Such a method cannot be used for solutions or other objects that cannot withstand the high vacuum employed.

Lower Temperature Sterilization.

Moist heat also is used for lower temperature sterilization procedures. Temperatures of 100°C (212°F) or lower are used for these so-ca1led *marginal*, or *fractional*, methods. The term marginal originates from the questionable reliability of the processes. The term fractional is derived from the fact that these processes are normally ·performed by two or three exposures to moist heat, alternated with intervals during which the material is held at room or incubator temperatures.

Fractional methods of sterilization such as tyndallization, employing a temperature of l00ºC (2l2°F), and inspissation, employing temperatures as low as 60°C (140°F), are relatively effective in reducing the number of vegetative forms of microorganisms, but are unreliable against spores. For certain preparations, the effectiveness of these processes may be improved by the inclusion of a bacteriostatic agent.

These marginal methods of sterilization should be reserved for substances that must be processed by a thermal method but that cannot withstand higher temperatures without degradation. The assurance of sterility is comparatively low, however.

Application of Thermal Methods of Sterilization.

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. Even this process does not usually completely dry the equipment. In some instances, when dry equipment is required and it must be sterilized by autoclaving, the equipment may be dried in a vacuum oven before use.

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 (usually140°C or less) temperatures. In such cases, it must be established that the heating cycle has no deleterious effects on the ingredients and that the cycle time is adequate to achieve sterilization.

They (also must be carefully protected after sterilization until incorporated aseptically in the product to prevent contamination from the environment.

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 253.7 Angstrom units.

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.

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 A 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.

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 supplies also have been sterilized when the limit of penetration has been carefully determined and controlled so that adequate irradiation throughout has been achieved.

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.