

efficiencies and their mechanisms of action differ. In autoclaves, dry saturated steam, i.e. 100% water vapour with no liquid water present, is used at temperatures between 121 and 135 °C, at which it rapidly kills microorganisms. An advantage of using steam is that it possesses a large latent heat of vaporization, which it transfers to any object upon which it condenses. It is essential to use dry saturated steam if maximal autoclaving efficiency is to be achieved. If the steam is wet, i.e. contains liquid water, penetration of vapour-phase steam into dressings may be retarded. If the steam is superheated, i.e. its temperature has been raised while the pressure remains constant, or the pressure fell while the temperature remains constant, it contains less moisture and latent heat than dry saturated steam at the same temperature. In this case the effect is similar to using a steam–air mixture at that temperature. The process by which steam kills cells is hydrolysis of essential proteins (enzymes) and nucleic acids. In contrast, dry heat causes cell death by oxidative processes, although again it is the proteins and nucleic acids that are the vulnerable targets. Dry heat is much less effective at killing microorganisms than steam at the same temperature. Exposures of not less than 2 hours at 160 °C (or an equivalent temperature/time combination) are recommended in the PhEur for sterilization by dry heat methods. The state of hydration of a cell is thus an important factor determining its resistance to heat.

Resistance of microorganisms to moist and dry heat

Numerous factors influence the observed heat resistance of microbial cells and it is difficult to make comparisons between populations unless these factors are controlled. Not surprisingly, marked differences in resistance exist between different genera, species and strains, and between the spore and vegetative cell forms of the same organism. The resistance may be influenced, sometimes extensively, by: the age of the cell, i.e. lag, exponential or stationary phase; its chemical composition, which in turn is influenced by the medium in which the cell is grown; and by the composition and pH of the fluid in which the cell is heated. It is difficult to obtain strictly comparable heat resistance data for grossly dissimilar organisms, but the values quoted in Table 15.2 indicate the relative order of heat resistance of the various microbial groups. Tabulation of *D* values at

a designated temperature is perhaps the most convenient way of comparing resistance but this is only suitable for first-order kinetics. Alternative methods of comparison include the time to achieve a particular percentage kill or the time required to achieve no survivors; the latter is, of course, dependent upon the initial population level.

The most heat-resistant infectious agents (as distinct from microbial cells) are prions, which are proteins rather than living cells and are the cause of spongiform encephalopathies, e.g. Creutzfeldt–Jakob disease (CJD) and bovine spongiform encephalopathy (BSE or ‘mad cow disease’). Prion proteins are so resistant to heat inactivation that an autoclave cycle of 134–138 °C for 18 minutes has been recommended for the decontamination of prion-contaminated materials, and the efficacy of even this extreme heat treatment has been questioned. The World Health Organization recommends that prion-contaminated surgical instruments are autoclaved at 121 °C for one hour in the presence of 1M sodium hydroxide.

Bacterial endospores are invariably found to be the most heat-resistant cell type, and those of certain species may survive boiling water for many hours. The term ‘endospore’ refers to the spores produced by *Bacillus* and *Clostridium* species and is not to be confused with the spores produced by other bacteria, such as actinomycetes, which do not develop within the vegetative cell. The majority of *Bacillus* and *Clostridium* species normally form spores which survive in water for 15–30 minutes at 80 °C without significant damage or loss of viability. Because endospores are more resistant than other cells, they have been the subject of a considerable amount of research in the food and pharmaceutical industries and much of the earlier work has been reviewed by Russell (1999).

Mould spores and those of yeasts and actinomycetes usually exhibit a degree of moist heat resistance intermediate between endospores and vegetative cell forms; *D*-values of the order of 30 minutes at 50 °C would be typical of such organisms, although some species may be substantially more resistant. Bacterial and yeast vegetative cells and mould mycelia all vary significantly in heat resistance: mycobacteria, which possess a high proportion of lipid in their cell wall, tend to be more resistant than others. Protozoa and algae are, by comparison, susceptible to heat and when in the vegetative (uncysted) state they, like mammalian cells, rapidly die at temperatures much in excess of 40 °C.