B. cereus produced at temperatures between 20 and 41 °C showed maximal resistance at 30 °C.

The effects of medium pH, buffering capacity, oxygen availability and the concentrations of culture medium components are often complex and interrelated. An unsuitable pH, inadequate buffer or insufficient aeration may all limit the extent of growth, with the result that the cells that *do* grow each have available to them a higher concentration of nutrients than would be the case if a higher cell density had been achieved. The levels of intracellular storage materials and metal ions may therefore differ and so influence resistance to heat and other lethal agents. Cells existing in, or recently isolated from, their 'natural' environment, e.g. water, soil, dust or pharmaceutical raw materials, have often been reported to have a greater heat resistance than their progeny that have been repeatedly subcultured in the laboratory and then tested under similar conditions.

pH and composition of heating menstruum

It is frequently found that cells survive heating more readily when they are at neutrality (or their optimum pH for growth if this differs from neutrality). The combination of heat and an unfavourable pH may be additive or even synergistic in killing effects; thus *B. stearothermophilus* spores survive better at 110 °C in dilute pH 7.0 phosphate buffer than at 85 °C in pH 4.0 acetate buffer. Differences in heat resistance may also result merely from the presence of the buffer, regardless of the pH it confers. Usually an apparent increase in resistance occurs when cells are heated in buffer rather than in water alone. A similar increase is often found to occur on the addition of other dissolved or suspended solids, particularly those of a colloidal or proteinaceous nature, e.g. milk, nutrient broth and serum.

Because dissolved solids can have such a marked effect on heat resistance, great care must be taken in attempting to use experimental data from simple solutions to predict the likely heat treatment required to kill the same cells in a complex formulated medicine or food material. An extreme case of protection of cells from a lethal agent is the occlusion of cells within crystals. When spores of *B. subtilis* var. *niger* were occluded within crystals of calcium carbonate, their resistances to inactivation were approximately 900 times and nine times higher than for unoccluded spores when subjected to steam and dry heat, respectively; an exposure period of 2.5 hours at 121 °C (moist heat) was required to eliminate survivors within the crystals. It is to minimize the risk of such situations arising that the *Rules and Guidance for Pharmaceutical Manufacturers and Distributors* places such emphasis on hygiene and cleanliness in the manufacture of medicines.

The solute concentrations normally encountered in dilute buffer solutions used as suspending media for heat resistance experiments cause no significant reduction in the vapour pressure of the solution relative to that of pure water, i.e. they do not reduce the water activity, A_w, of the solution (which has a value of 1.0 for water). If high solute concentrations are used, or the cells are heated in a 'semi-dry' state, the A_w is significantly lower and the resistance is increased, e.g. a 1000-fold increase in D value has been reported for *B. megaterium* spores when the water activity was reduced from 1.0 to between 0.2 and 0.4.

Recovery of heat-treated cells

The recovery conditions available to cells after exposure to heat may influence the proportion of cells that produce colonies. A heat-damaged cell may require an incubation time longer than normal to achieve a colony of any given size, and the optimum incubation temperature may be several degrees lower. The composition of the medium may also affect the colony count, with nutritionally rich media giving a greater percentage survival than a 'standard' medium, whereas little or no difference can be detected between the two when unheated cells are used. Adsorbents such as charcoal and starch have been found to have beneficial effects in this context.

Ionizing radiations

Ionizing radiations can be divided into electromagnetic and particulate (corpuscular) types and are of sufficient energy to cause ejection of an electron from an atom or molecule in their path. Electromagnetic radiations include γ-rays and X-rays, whereas particulate radiation includes α and β particles, positrons and neutrons.

Particulate radiation

The nuclear disintegration of radioactive elements results in the production of charged particles.