4 Bacterial growth, physiology and death

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KEY POINTS

- Bacterial growth and multiplication is of practical value in the detection and identification of pathogens, and is generally a necessary component of infection.
- Bacteria divide asexually through a process of *binary fission*, passing through *lag, exponential* and *stationary* phases of *planktonic* growth in broth cultures. Bacterial growth can also be recognized in *sessile* form as *colonies* or *biofilms.* A given bacterial strain may have profoundly different physiological properties in each of these growth states.
- Recovery of pure bacterial cultures was greatly enhanced by the development of solidified agar media. Different medium designs enable *selection, enrichment, identification or defined* growth conditions.
- Different bacteria have evolved to grow and survive in widely differing habitats and these define their potential reservoirs and sources of infection. The growth atmospheres required by different bacteria are an important defining characteristic, and *obligate aerobes, obligate anaerobes, micro-aerophilic* and *facultative* organisms are recognized.
- Bacterial viability is generally recognized and quantified by detecting growth of single cells into colonies in colony-forming unit (cfu) counts. Discrepancies between cfu counts and the number of cells seen by microscopy have led to recognition that many cells in natural samples do not form colonies.
- Bacteria may die through senescence in stationary cultures, through genetically programmed or prophage-induced cell death, or as result of external noxious influences such as antibiotics or the deliberate processes of *sterilization* and *disinfection*.
- Sterilization involves the destruction of all propagating biological entities, whereas disinfection involves a reduction in microbial load to an acceptable level. Both processes can be achieved by application of *moist and dry heat, ionizing radiation, filtration, gaseous chemical agents* and *liquid chemical agents*.

Most of what we know about bacteria derives from their growth. Their ability to propagate may be seen as a supreme achievement that enables them to attain enormous populations at rates that are breathtaking from a human perspective. These properties underpin their capacity for change by mutation and the rapidity with which some infections develop.

Bacterial growth involves both an increase in the size of organisms and an increase in their number. Whatever the balance between these two processes, the net effect is an increase in the total mass (*biomass*) of the culture. Medical microbiologists have traditionally concentrated on the number of individuals in growth studies. Whether this emphasis on cell number is appropriate remains uncertain; none the less, it will be adopted here, as the number of individual bacteria involved is important in the course and outcome of infections and in the measurement of the effects of antibiotics.

Students of medicine may be surprised and even dismayed to hear that organisms as small as bacteria have a physiology. However, the complement of enzymes and the biochemical and biophysical processes occurring in a prokaryotic cell at any one time represent the product of genetic and biochemical control mechanisms that are every bit as sophisticated and tightly regulated as those in eukaryotic cells. Moreover, the recognition and definition of the mechanisms by which bacteria sense and adapt to nutritional and noxious stimuli in their environments have provided insights that are likely to translate into medically significant advances in the foreseeable future.

In some sense asexual organisms such as bacteria appear to be immortal, but bacterial death or loss of viability occurs in many natural settings. This has practical consequences, as only viable bacteria can initiate infections and most microscopic, molecular and immunological detection methods do not differentiate between live and dead organisms. Of course, we often need to assess the lethal effects of antibiotics and processes aimed at *sterilization*, *disinfection* and *antisepsis*. The practical approach to assessing the effects of antibiotics is introduced in Chapter 5, but the principles of sterilization and disinfection are introduced here.

Although this chapter discusses growth and physiology only from a bacteriological perspective, some of the principles are also applicable to fungi, particularly yeasts. The central difference between their growths is that cell division is generally achieved in bacteria by *binary fission* to produce identical offspring that cannot be distinguished as parents and progeny, whereas fungi divide by budding in the case of yeast growth and hyphal septation in the mould form. In contrast, the principles of sterilization and disinfection refer to all infective agents. Their application is considered further in Chapter 68.

BACTERIAL GROWTH

When placed in a suitable nutritious environment and maintained under appropriate physical and chemical conditions, a bacterial cell begins to grow; when it has manufactured approximately twice the amount of component materials that it started with, it divides. The range of specific components that define 'suitable' and 'appropriate' for all known bacteria (and *Archaea*) is so broad that it actually defines the global biosphere (those environments that can sustain life), and includes temperatures and pressures present at the opening of hydrothermal vents on the ocean floor to the outer reaches of the atmosphere. Although these conditions do not regularly occur in man, they serve to illustrate that no part of the body or medical device with which it may come in contact is too difficult for bacteria to colonize and that bacteria may lurk in surprising environmental niches. Conversely, the conditions required for some organisms to grow are so precise that, so far, we have not been able to reproduce them in artificial laboratory media. This applies to some well known organisms such as the agents of leprosy and syphilis, but also to many other potential pathogens about which we are beginning to learn through molecular methods that do not depend on growth. In fact, it is estimated that we have not yet isolated more than 1% of all the bacterial species that exist, and it is almost certain that there are many medically important organisms among the 'as yet uncultivated' micro-organisms.

As the central technique in bacteriology, growth in the laboratory has been used to serve many different purposes. From the clinical perspective, growth is used for detection and identification, and for the assessment of antibiotic effects, whereas scientific and industrial objectives are often served by growth in bulk to obtain sufficient biomass for detailed biochemical analysis and to produce the desirable products of the brewing and biotechnology industries.

Types of growth

In the laboratory, bacterial growth can be seen in three main forms:

1. By the development of *colonies*, the macroscopic product of 20–30 cell divisions of a single cell.
2. By the transformation of a clear broth medium to a turbid suspension of 10^7–10^9 cells per mL.
3. In *biofilm* formation, in which growth is spread thinly (300–400 μm thick) over an inert surface and nutrition obtained from a bathing fluid.

In natural systems only biofilms, such as those that develop on the surfaces of intravascular cannulae, appear to function in a manner comparable to biofilms produced in the laboratory, whereas colonies, the other form of *sessile* growth, rarely reach macroscopic dimensions. Turbid liquid systems caused by *planktonic* growth of a single organism are also a rarity in nature. Single organism infections affecting normally sterile sites in the body are one exception to this, whereas most natural microbial communities are complex assemblies of micro-organisms competing, and in many cases co-operating, to exploit the local resources. However, in spite of these unrepresentative features, pure growth of single organisms in *monocultures* to produce macroscopic colonies or high cell densities in broth offer great practical advantages and remain central techniques.

While much has been learned about the nature of bacteria by studying them in lab cultures, it is clear that they change their properties in different patterns of growth and non-growth, and in response to their environment. Thus, when we try to treat or immunize by targeting properties revealed in standard lab cultures, we often fail because the organism is not expressing those properties. This is particularly the case for bacteria in biofilms and in non-replicating states.

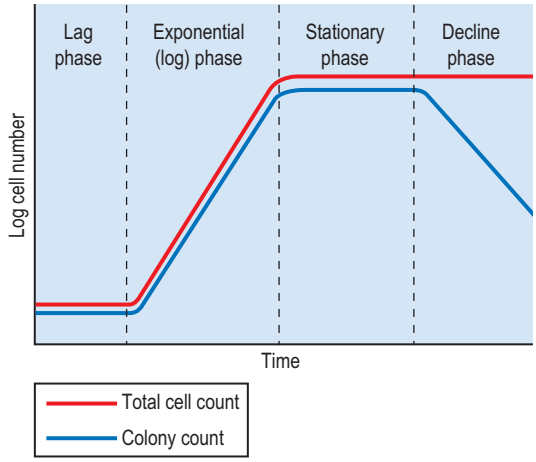

Fig. 4.1 Phases of growth in a broth culture.

Growth phases in broth culture

Bacterial growth in broth has been studied in great detail and has provided a framework within which the growth state or growth phase of any given pure culture of a single organism can be placed; these phases are summarized in the idealized *growth curve* shown in Figure 4.1. When growth is initiated by inoculation into appropriate broth conditions, the number of cells present appears to remain constant for the *lag phase*, during which cells are thought to be preparing for growth. Increase in cell number then becomes detectable, and its rate accelerates rapidly until it is established at the maximum achievable rate for the available conditions. This is known as the *exponential phase*, because the number of cells is increasing exponentially with time. To accommodate the astronomic changes in number, the growth curve is normally displayed on a logarithmic scale, which shows a linear increase in log cell number with time (hence the older term, *log phase*). This log-linear relationship is sufficiently constant for a given bacterial strain under one set of conditions that it can be defined mathematically, and is often quoted as the *doubling time* for that organism. Doubling times have been measured at anything between 13 min for *Vibrio cholerae* and 24 h for *Mycobacterium tuberculosis*. On this basis it is not surprising that cholera is a disease that can kill within 12 h, whereas tuberculosis takes months to develop. A further consequence is that, when specimens are submitted to diagnostic laboratories for the detection of these organisms by culture, a result is usually available for *V. cholerae* the next day, whereas several weeks are required for conventional culture of *M. tuberculosis*.

It is often difficult to grasp fully the scale of exponential microbial growth; the message may be strengthened by considering that the progeny of a lecture theatre containing 150 students would exceed the global population of humanity (6×10^9) within 8.5 h if they were able to breed like *Escherichia coli*!

Exponential growth cannot be sustained indefinitely in a closed (*batch*) system with limited available nutrients. Eventually growth slows down, and the total bacterial cell number reaches a maximum and stabilizes. This is known as the *stationary* or *post-exponential phase*. At this stage it becomes important to know what method has been used to determine the growth curve. If a direct method that assesses the total number of cells present is used then the count remains constant. Such methods include counting cells in a volumetric chamber observed by microscopy, electronic particle counters and measurement of turbidity. If, however, the growth potential of the individual cells present in the culture is assessed by taking regular samples, making tenfold dilutions of these and inoculating them on to agar, the number of *colony-forming units* (cfu) per unit volume can be determined at each sample time. Although such cfu counts closely parallel the results obtained by direct counting methods in the exponential and early stationary phases, a divergence begins to emerge towards the end of the latter; the total cell number remains constant whereas the colony count declines. This marks the beginning of the final, *decline phase*, in the sequence of growth states that can be observed in broth. The discrepancy between the total and cfu counts is conventionally held to represent the death of cells because of nutrient exhaustion and accumulation of detrimental metabolic end-products. However, there is some doubt concerning this interpretation (see below).

As noted above there has been increased interest in the properties of bacteria in non-growing states. While there are many different systems for studying non-replicating bacteria and their separate relevance to infection is argued, one phenomenon is of particular interest as it illustrates their capacity for adaptation based on mutation. The growth advantage in stationary phase (GASP) phenomenon is now well established and illustrates that while total cell numbers in a population may remain constant or decline, multiple genetic variants arise, some of which come to dominate the population. The genes and polymorphisms that lead to the growth advantage have been informative in improving our understanding of survival and competition under nutrient limited conditions.

The study of bacterial growth in broth provides a valuable point of reference to which practical, experimental and routine diagnostic procedures are often

related. For example, the length of the lag phase and rates of exponential growth in different circumstances are used to make predictions and contribute to safety standards for storage in the food industry. An important feature to emerge is that cultures inoculated with cells prepared at different stages in the growth curve yield different results. The exponential phase is the most reproducible and readily identified, and is therefore used most frequently. It can be extended in an open system known as *continuous culture* using a *chemostat* in which cells of a growing culture are harvested continuously and nutrients replenished continuously. Chemostat studies have provided very detailed information on the chemistry of microbial growth and the way in which different organisms convert specific substrates into biomass. The extraordinary efficiency of this process has made natural and genetically manipulated microbes a powerful resource for the biotechnology industry.

In contrast to growth in broth, far less is known about the state of the bacteria in a mature macroscopic colony on an agar plate. Such a colony presents a wide range of environments, from an abundance of oxygen and nutrients at the edge to almost no oxygen or nutrients available to cells in the centre. It is likely that all phases of growth are represented in colonies, depending on the location of a particular cell and the age of the culture. Although in practice colonies can be used reliably to inoculate routine tests of antimicrobial susceptibility in clinical laboratories, they cannot be considered a defined starting point for experimental work because they comprise such a heterogeneous population of cells. In fact, colonies are complex and dynamic communities in which cells at different locations can show startlingly different phenotypes. In spite of its complexity, the capacity for and quality of colonial growth of specific organisms on specialized media is central to the laboratory description of medically important bacteria.

MEDIA FOR BACTERIAL GROWTH

The media used in a medical diagnostic bacteriology laboratory have their origins, for the most part, back in the 'golden age of bacteriology' in the late nineteenth and early twentieth centuries. A vast amount of experience and knowledge has accrued from their use and, apart from better standardization and quality control in their production, little has changed in their basic design. The objectives of early medium design were to grow pathogenic bacteria, separate them from other organisms present in samples and, ultimately, differentiate their phenotypic properties so that they could be identified. A critical development was the introduction of solidifying agents, most particularly the largely indigestible polysaccharide extract of seaweed known as agar. Alternative solidifying agents include gelatine and egg albumen. Before the development of solid media, pure cultures could be achieved only by dilution of inocula so that only one growing cell or clump of cells was present at the initiation of growth, a very laborious and unreliable procedure. In contrast, solid media in Petri dishes provided a growth substrate on to which mixed cultures could be inoculated and, provided the population density could be made low enough to allow development of well separated colonies, the different organisms present could be differentiated and subsequently separated into pure cultures.

Media used for isolation and identification of pathogens

The central features of media in medical bacteriology are:

1. a source of protein or protein hydrolysate, often derived from casein or an infusion of brain, heart or liver obtained from the nearest butcher
2. control of pH in the final product (after sterilization)
3. a defined salt content.

Early media often included blood or serum in an attempt to reproduce nutritional features present in the human body. Growth of some pathogens was found to be dependent on such supplements, and it was recognized that these relatively *fastidious* or *nutritionally exacting* organisms were dependent on *growth factors*. The identity of many of the growth factors is now known (e.g. haemin and several coenzymes), but blood often remains their most convenient source.

Selective and indicator media

Tremendous ingenuity has gone into designing growth media that provide information relevant to patient management as early as possible. There are two main approaches, both of which depend on adding supplements to the basal medium. *Selective media* contain substances such as bile salts or antibiotics that inhibit the growth of some organisms but have little or no effect on the organisms for whose isolation they were designed. They are essential for samples containing a normal microbial flora such as faeces. The inclusion of components or specific reagents that show whether the bacteria possess a particular biochemical property characterizes an *indicator medium*. Such media are

critical to the rapid presumptive identification of isolates. Combinations of selective and indicator supplements in agar media have led to formulations with some remarkably elegant differential properties that effectively colour-code the colonies according to their biochemical properties and restrict growth to a desired range of organisms. Broth indicator media tend to be much simpler, as they generally require a pure inoculum of a single organism and reveal only one property per formulation. Broth media with selective properties are usually referred to as *enrichment media* as they change the balance of organisms inoculated in favour of the desired range of organisms, thereby enriching them.

Media for laboratory studies

Most of the objectives of a clinical diagnostic laboratory can be fulfilled with the range of media outlined above. However, the composition of these media is not defined, and this poses problems for some investigations, including the detailed analysis of antibiotic action. Wherever possible, such investigations are based on a *defined* or *synthetic medium* where every chemical component is carefully regulated. In genetic experiments use is often made of a *minimal medium* in which every component is required for the growth of the organism under investigation, so that if one component is removed growth cannot occur. Minimal media also prevent the growth of mutants that have additional nutritional requirements to those of the parent strain. For some organisms, particularly those that can grow outside the human body, minimal media may comprise as little as an ammonium salt to provide nitrogen, a carbon source, which in some cases can be as simple as methane or carbon monoxide, trace amounts of iron and other essential elements, and pH adjustment to within an appropriate range. Defined and minimal media generally have to be developed for small groups of closely related organisms and should not be used for other organisms.

Relatively well defined media are preferred, even for routine antibiotic tests, because quantitative aspects of bacterial biochemistry, growth and susceptibility to noxious stimuli can be influenced substantially by minor changes in medium composition. The use of fully defined media has underpinned almost all of what we know about bacterial physiology. Rather curiously, however, it is well recognized that defined media are often suboptimal for the recovery of bacteria from environments in which they have been stressed. This may reflect the support provided to injured bacteria by complex media. Defined media can really be optimized only for bacteria in a single physiological state, whereas complex media have greater potential to cope with the diversity of states present in natural samples.

BACTERIAL PHYSIOLOGY

The complement of processes that enable an organism to occupy and thrive in a particular environment places certain requirements on its physiology. Traditional descriptions of bacterial groups emphasize features that place a microbe in particular ecological niches. Thus we have *acidophiles* for organisms such as *Lactobacillus* spp. that grow at lower pH levels than most other organisms, and *halophiles* for organisms that grow at high salt concentrations. The environments that can be colonized by a pathogen are, of course, critical in determining its reservoirs and potential modes of transmission. More recently it has been recognized that individual bacteria are not restricted to a single physiological state. Rather, they respond to environmental stimuli and undergo *adaptive responses* that confer improved capacity for survival in adverse conditions. All of these properties sustain the *viability* of the organism. However, it has become apparent that our ability to measure viability by conventional means may be inadequate.

The specific means by which a particular organism obtains energy and raw materials to sustain its growth (its nutritional type) and the physical conditions it requires reflect its fundamental physiological characteristics. Placing an organism into the groups defined by these characteristics is an important step in its conventional classification.

Nutritional types

Traditionally, all living organisms have been divided into two nutritional groups: *heterotrophs* and *autotrophs*. The former depend on the latter to produce organic molecules by fixing carbon dioxide, predominantly by photosynthesis. Bacterial metabolism is now recognized to be so diverse that it cannot be encompassed by these two terms. Three basic features are used in the present terminology: the *energy source*, the *hydrogen donors* and the *carbon source*.

Energy for adenosine triphosphate (ATP) synthesis may be obtained from light in a *phototrophic* organism and from chemical oxidations in the case of a *chemotrophic* organism. The hydrogen donor type characterizes an organism as an *organotroph* if it requires organic sources of hydrogen and as a *lithotroph* if it can use inorganic sources (e.g. ammonia or hydrogen

sulphide). Finally, the terms autotroph and heterotroph are reserved for the carbon source; the former can fix carbon dioxide directly whereas the latter require an organic source. In general, only the energy and hydrogen donor designations are referred to routinely by combining the two terms. Hence we refer to *chemo-organotrophs* (the vast majority of currently recognized medically important organisms) and *chemolithotrophs* (e.g. some *Pseudomonas* spp.). Surprisingly, there are even some *photolithotrophs* with medical significance; the cyanobacteria are now known to produce many toxins that can affect man.

Physical conditions required for growth

All living organisms use oxidation to transfer energy to compounds that participate in their internal biochemical and biophysical processes. Oxidation of a molecule is equivalent to the removal of hydrogen, and requires another molecule to receive electrons in the process. In *aerobic* respiration the final electron recipient in the oxidation process is molecular oxygen (i.e. O_2), whereas under *anaerobic* conditions (in the absence of oxygen) most medically important organisms use an organic molecule as the final electron recipient, and the oxidative process is referred to as *fermentation*. There are also some forms of anaerobic respiration that use inorganic electron acceptors such as nitrates. Respiration in this context is generally used to denote involvement of a membrane-associated electron transport chain in the oxidation. In the early period of development of life on Earth there was no oxygen in the atmosphere; thus, at this time, all bacteria were *anaerobes*. Subsequently, following the development of photo-autotrophic organisms, atmospheric oxygen became abundant, and organisms capable of using oxygen evolved.

Although aerobic metabolism is a more efficient means of obtaining energy than anaerobiosis, it is not without its cost. Some oxidation–reduction (redox) reactions occurring in the presence of oxygen commonly result in the formation of the reactive superoxide (O_2^-) and hydroxyl (OH^-) radicals as well as hydrogen peroxide (H_2O_2), all of which are highly toxic. To cope with this, aerobic organisms or *aerobes* have developed two enzymes that detoxify these molecules. *Superoxide dismutase* converts superoxide radicals to hydrogen peroxide ($2\ O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$), whereas *catalase* converts hydrogen peroxide to water and oxygen in the reaction $2\ H_2O_2 \rightarrow H_2O + O_2$. Possession or lack of these enzymes has the important consequence of defining the atmosphere necessary for growth and survival of different organisms. Moreover, when produced in large amounts, the enzymes also provide protection for pathogenic organisms against the reactive oxygen intermediates deliberately produced as a defence mechanism by phagocytic cells.

Growth atmosphere

These oxygen-related features underpin the major practical grouping of bacteria according to their atmospheric requirements (Table 4.1). Thus, *strict* or *obligate aerobes* require oxygen, usually at ambient levels (≈20%), and *strict* or *obligate anaerobes* require the complete absence of oxygen. Many organisms exhibit intermediate properties: *facultative anaerobes* generally grow better in oxygen but are still able to grow well in its absence; *micro-aerophilic* organisms require a reduced oxygen level (≈5%); *aerotolerant*

Table 4.1 Key descriptive terms used to categorize bacteria according to their growth requirements

Descriptive term	Property	Example
Growth atmosphere		
Strict (obligate) aerobe	Requires atmospheric oxygen for growth	*Pseudomonas aeruginosa*
Strict (obligate) anaerobe	Will not tolerate oxygen	*Bacteroides fragilis*
Facultative anaerobe	Grows best aerobically, but can grow anaerobically	*Staphylococcus* spp., *E. coli*, etc.
Aerotolerant anaerobe	Anaerobic, but tolerates exposure to oxygen	*Clostridium perfringens*
Micro-aerophilic organism	Requires or prefers reduced oxygen levels	*Campylobacter* spp., *Helicobacter* spp.
Capnophilic organism	Requires or prefers increased carbon dioxide levels	*Neisseria* spp.
Growth temperature		
Psychrophile	Grows best at low temperature (e.g. <10°C)	*Flavobacterium* spp.
Thermophile	Grows best at high temperature (e.g. >60°C)	*Bacillus stearothermophilus*[a]
Mesophile	Grows best between 20–40°C	Most bacterial pathogens

[a]Not a pathogen; its spores are very heat resistant and are used for testing the efficiency of heat sterilization.

anaerobes have a fermentative pattern of metabolism but can tolerate the presence of oxygen because they possess superoxide dismutase. Many medically important organisms are facultative anaerobes. There is a mixture of aerobic and anaerobic micro-environments in the human body, and the capacity to replicate in both is clearly advantageous. For obvious reasons, strict anaerobes are particularly associated with infection of tissues where the blood supply has been interrupted.

Among the various physical requirements for the growth of different bacterial groups, atmosphere assumes particular importance because, in practice, agar cultures from most clinical specimens are set up aerobically and anaerobically. Thus, when growth is first inspected after overnight incubation, the isolates can readily be differentiated into strict aerobes, anaerobes and facultative anaerobes according to the conditions under which they have grown. Various atmospheric conditions can also be obtained in broth media. If the medium is unstirred, strict aerobes tend to grow on the surface, micro-aerophiles just under the surface and anaerobes in the body of the medium away from the surface. Growth of anaerobes is often improved by the addition of a reducing agent such as cysteine or thioglycollate to mop up any free oxygen.

Growth temperature

The other significant physical condition for bacterial growth from the medical perspective is temperature (see Table 4.1). Pathogens that actually replicate on or in the human body must be able to grow within the temperature range of 20–40°C, and are generally referred to as *mesophiles*. Organisms that can grow outside this range are either *psychrophiles* (cold loving) or *thermophiles* (heat loving). The former may be capable of growth in food or pharmaceuticals stored at normal refrigeration temperatures (0–8°C), whereas the latter can be a source of proteins with remarkable thermotolerant properties, such as *taq* polymerase, the key enzyme used in the polymerase chain reaction. Organisms such as the leprosy bacillus that prefer lower growth temperatures are often associated with skin and superficial infections, whereas organisms that grow in the colon (often a few degrees warmer than normal body temperature) can grow well up to 44°C.

Extremophiles

Some bacteria require ostensibly bizarre physical conditions for growth. For example, barophiles isolated from the ocean floor may require enormous pressures before they can replicate. Such organisms are often referred to as *extremophiles*. The properties of these organisms serve to remind us that microbes have the potential to occupy any environmental niche where energy and nutrition are available. It should be noted that most extremophiles actually turn out to belong to the Archaea (see Ch. 2).

Bacterial metabolism

Although some bacteria are able to obtain their resources for growth in ways that seem alien to us, the core of their metabolism is essentially very similar to that of mammalian cells. The basic details of glycolysis, the tricarboxylic acid cycle, oxidative phosphorylation, ATP biosynthesis and amino acid metabolism are constant although some notable minor differences occur. Variations in the pathways that feed into and flow from these core processes are readily detected by what are loosely termed *biochemical tests* in medical laboratories. These detect traits such as the ability to use individual carbohydrate sources to produce acid and the possession of specific enzymes.

The common nature of central catabolic and anabolic pathways in bacteria and multicellular organisms reflects the economy of biology and evolution. Processes that work well cannot be outcompeted and tend to be preserved in the genetic stock. Thus, many of the specific enzymes involved in bacterial metabolism show remarkable levels of conservation in their amino acid sequences across very substantial distances in evolutionary terms. DNA sequencing has enabled the identification of *molecular families* of proteins with a common evolutionary origin. In addition to the metabolic enzymes, it has been recognized that many transport proteins responsible for importing and exporting specific substrates into and out of the bacterial cytoplasm are closely related in their structure and mode of function to those present in mammalian cells. Of course, because bacteria generally have only one cell compartment in which to operate, the location of these proteins is often different; for example, as they have no mitochondria, the cytoplasmic membrane contains the components of the electron transport chain, and the proton gradient across the inner mitochondrial membrane is generated across the cytoplasmic membrane instead. This feature actually means that bacteria can perform some energy-requiring processes at the cell surface, notably flagellar rotation (motility), by directly exploiting the proton gradient rather than consuming ATP.

Aside from its role in identification and intrinsic biological interest, bacterial metabolism has real consequences for humans. In direct terms, the resident

microbiota have consequences in human health and disease. For example, the bacteria in dental plaque produce acid when presented with certain carbohydrate sources, and this acid is responsible for tooth decay; on the positive side, bacteria in the intestines deconjugate bile salts and thereby contribute to the enterohepatic circulation. It seems likely that the importance of such bioconversions will be recognized increasingly in the future. In particular, the role of bacteria in recovering nitrogen excreted into the colon in marginal human nutritional states and metabolic activity leading to the formation of carcinogens or other biologically active molecules are both areas where there is much room for further work. The totality of microbes (and their genomes) within the human body is referred to as the *microbiome.* The application of high throughput nucleotide sequencing to directly determine the range of microbes and microbial genes present in different areas of the body is opening up fascinating new insights into the way in which microbial genes and their expression can interact with humans to produce health or disease. The recent proposal that particular patterns of microbes in the human colon can predispose to obesity is a particularly striking example of this.

Human beings are also indirectly affected by microbial metabolism. At one level, the chemistry of our environment has been shaped extensively by microbes; the original development of oxygen in our atmosphere, the availability of elemental sulphur and the flow of nitrogen are all critically dependent on microbial metabolism. Exploitation of microbial metabolism in industry has, of course, given us ethanol, and many of the other alcohols and acids that result from fermentation have commercial value. Finally, bacteria have been used to combat the deleterious effects of environmental pollution in the process referred to as *bioremediation*.

Adaptive responses in bacteria

The extent to which bacteria respond to environmental stimuli was originally recognized by monitoring gross phenotypic, biochemical and behavioural changes. Much of the genetic basis for how bacteria change their phenotypes was established in the 1960s and 1970s following on from the paradigm established for β-galactosidase regulation in *E. coli* by Jacob and Monod. The scale and rapidity (major changes can be seen in seconds) of bacterial responses became apparent through the 1980s and 1990s as the use of global analytical approaches that attempt to characterize the instantaneous expression of every gene the organism carries became established. At the translational level, the use of two-dimensional gel electrophoresis and, more recently, sophisticated forms of mass spectrometry have underpinned the so-called *proteomic* approach. This technique reveals and separates most of the several hundred proteins that are being synthesized by a pure culture at a particular time. The catalogue of different proteins detected represents those proteins that the organism requires to function in the circumstances from which the sample was drawn. Assays of this type have shown that different sets of proteins are made in the exponential and stationary phases of the growth cycle and, indeed, in response to almost any environmental change. This finding underpins the recognition of just how different the phenotype of a single organism can be in different physiological states and reinforces the need to define the inoculum used in laboratory experiments. More recently the development of DNA arrays has enabled global analysis of responses at the transcriptional level by detecting messenger RNA (mRNA) molecules relating to every gene in the organism in a single analysis. The complement of RNA species present in an organism at a given time is referred to as the *transcriptome*. The basic features of the comparative protein and mRNA analyses are outlined in Figure 4.2.

The comprehensive analyses achieved by transcriptome and proteome analyses followed on from the recognition that global genome analyses and comparisons or *genomics* (see Ch. 6) have the potential to explain many – some would say most – biological and medical phenomena. The complexities linking genotype to phenotype remain overwhelming in most instances; none the less, we have now entered an era where global analyses of mRNA, proteome and metabolic function (recently termed 'metabolomics') are being addressed enthusiastically in an integrative computational approach pooling data from different analyses in what has been termed the 'systems biology' approach.

The effects of specific sublethal but noxious stimuli on gene expression are the subject of intense current study. Each different stimulus leads to an adaptive *stress response*, which is to some extent specific to the stimulus applied. Heat shock (the effects of raising temperature to 45°C and above for a few minutes) has been studied most extensively. The newly synthesized proteins elicited in this response are referred to as *heat shock proteins.* When the amino acid sequences of the principal heat shock proteins were determined, they were found to belong to a molecular family now recognized in all prokaryotic and eukaryotic cells. Apart from their role in improving the ability of bacteria to survive heat shock, these proteins, by virtue of their similarity to analogous host cell antigens, seem to be

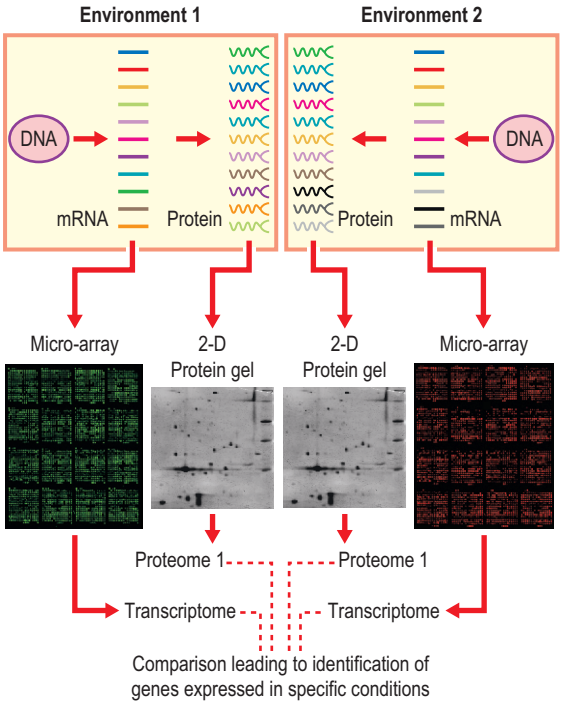

Fig. 4.2 Global strategies for identifying differentially expressed genes. Analysis at both mRNA and protein levels is preferred as there may be differences between the two. Individual spots on the two-dimensional (2-D) gels may be identified as specific gene products by mass spectrometry. Transcriptome analysis requires a representation of all the genes concerned in a DNA array and therefore needs prior knowledge (ideally a complete sequence) of the genome of the organism to be tested. These two global approaches provide a broad picture of the physiology of the organism under study.

involved in initiating autoimmune damage and immune dysfunction. A very important feature of the stress response in bacteria is that many of the stimuli used are prominent aspects of the stresses applied by the human immune system to an invading pathogen. Thus, acid stress is provided by the stomach and the hostile environment of phagolysosomes includes both oxidative and pH stress.

The information built up from studying stress responses has made it possible to identify sets of proteins that are made in response to several different stresses and those that appear exclusive to one stress. Together with other approaches, this has allowed recognition of *global regulatory systems* or *networks* within bacteria that are responsible for differential gene expression under different circumstances. The hierarchy of specific control mechanisms involved has spawned two important new terms, *stimulon* and *regulon*. A stimulon denotes all the genes whose expression is increased or decreased by a specific external stimulus, whereas a regulon refers to all the genes under the influence of a specific regulatory protein. A regulon may affect several operons (see Ch. 6), and there may be many regulons in one stimulon.

Regulatory networks have been identified in almost every area of bacterial physiology. Thus, in addition to the stimuli cited above, osmotic stress, cold shock, nutrient limitation (separate responses for carbon, nitrogen and phosphate), anaerobic and many other stimulons are recognized. These control systems are responsible for making sure the organism synthesizes only those proteins appropriate to its current circumstances. Particularly important medical examples of this are the regulation of proteins concerned with an organism's progress in an infection (virulence factors) and those made in response to sub-lethal levels of antibiotics. Equally important from the scientific perspective is the recognition that chemicals secreted by an organism can themselves act as regulatory stimuli to individuals of the same species in a way analogous to the pheromones released by insects.

Although it is still important to recognize that different organisms are particularly adapted to special environmental niches with descriptive terms such as mesophile, acidophile and halophile, the discovery of adaptive responses in bacteria has pushed us into an uncertain period where much of what has been established about the tolerance of micro-organisms to noxious stresses will have to be re-examined. Furthermore, as the extent to which bacteria modulate their phenotype according to their circumstances is now clear, the need for caution in concluding that any property detected in the laboratory is significant in a natural infection is unavoidably obvious.

Bacterial defence against noxious chemicals

The features outlined above all contribute to the well-being of bacteria. In the natural world micro-organisms encounter many chemicals that could cause their destruction and, in their 3.5 billion years on earth they have evolved numerous protective mechanisms. In clinical practice these are recognized as *biochemical mechanisms of antibiotic resistance*, and four basic categories are recognized:

1. *Preventing access:* achieved by low cell envelope permeability or efflux pumps affecting the chemical concerned.
2. *Destruction:* achieved by enzymes that modify or degrade the chemical.
3. *Lack of target:* many chemicals that damage bacteria work through specific targets. The target

may be absent or be altered by mutation (see Ch. 6).
4. *Bypass of target:* in some cases an alternate or modified pathway can be used.

These mechanisms may be *intrinsic* to the organism concerned or they may be *acquired* through mutation or gene transfer (see Ch. 6).

Not all resistance to noxious molecules is mediated by the mechanisms listed above. In certain non-replicating states and in biofilms, bacteria classified as sensitive to certain agents by standard tests become resistant particularly to the lethal action of the agent concerned. This *phenotypic resistance* or *tolerance* appears to be a significant factor in the recalcitrance of certain chronic infections to antibiotic treatment and the survivors against antibiotic exposures that might reasonably be expected to be effective from conventional susceptibility tests are termed *persisters*. It is important to appreciate that these survivors are no more antibiotic-resistant than their forebears.

Bacterial viability

A central feature of the general and adaptive physiology of bacteria is the capacity to preserve the viability of a particular organism. There is, however, a persistent problem – how do we define viability in practical terms? Traditionally, the operational definition of the capacity of a cell to form a colony on an appropriate agar medium (the colony or cfu count) has been almost universally accepted. It is also often expressed as the proportion of cells within a population that are capable of forming colonies. This has been used extensively in recognizing the *cidal* (lethal) and *static* (growth inhibitory) activities of antibiotics. In the former case, cfu counts decline, and in the latter they remain constant. However, it must be emphasized that viability is not a clearly measurable property. At the individual level it expresses the expectation that, in a suitable environment, a particular cell has the capacity to grow and undergo binary fission and that its progeny will have the same potential. The key assumption is that colony counts provide an accurate measure of viability.

The central problem can be stated as follows: it is self-evident that if a bacterial cell produces a colony it must have been viable, but to what extent is it true that a cell that fails to do this is non-viable or dead? Immediately contradictions to this proposal can be identified. The bacterial pathogens, such as *Mycobacterium leprae* and *Treponema pallidum*, which cannot be induced to form colonies on available agar media, are clearly viable. Similarly, all the 'as yet uncultivated organisms' (possibly as many as 99% of all bacterial species) are clearly able to propagate themselves. They simply have not been sporting enough to do it on our laboratory media. A further exception is the phenomenon of bacterial recovery from injury (e.g. cold or osmotic shock) in which colony counts can be shown to rise in the absence of cell division.

It is possible that some organisms that are readily cultivable may be able to switch to a physiological state in which they cannot be induced to form colonies. A popular terminology for cells in this putative state is *viable but non-culturable* (VBNC).

Epidemiological and laboratory evidence provide some support for the existence of a VBNC state. In particular, the occurrence of several infectious diseases acquired from environmental sources, notably cholera, is at variance with our ability to recover the causal organisms from the implicated source. Environmental studies have demonstrated cells with immunological properties compatible with those of the cholera vibrio while failing to recover the organism in culture, and laboratory studies have indicated that the organism can persist in a non-culturable form. There is also evidence that non-culturable forms may revert to their 'normal' culturable state.

A major attraction of the VBNC hypothesis is that it may resolve a number of important mysteries in medical microbiology. In general, these are situations in which we know the organism must be present but are unable to culture it. This is particularly so with diseases such as tuberculosis that have latent phases.

The most significant problem for the VBNC hypothesis is that it has not been defined in physiological, biochemical or genetic terms. On the face of it, one might expect transition to the VBNC state to result from an adaptive response such as those described in the previous section. Alternatively, transition might be the result of a programme of gene expression such as that observed in spore formation or starvation. From this standpoint the stationary and decline phases in the growth cycle outlined above (when spore formation is induced in sporulating bacteria) may represent the initiation of and transition to a non-culturable phase rather than loss of viability (the traditional view). In spite of their popularity, these ideas must presently be viewed as interesting speculations for which there is circumstantial but no conclusive evidence.

Measurement of viability has been of great practical value in medical microbiology. Colony counts performed to investigate the action of antibiotics and other disruptive influences such as heat, and those performed at different stages during experimental infections in animal models of human infections, have

provided a wealth of valuable information. Moreover, there is no reason to doubt that this approach will continue to be extremely useful.

None the less, it is necessary to maintain a clear view of the limitations of bacterial culture as a measure of the organisms present in a sample and of their viability. Studies often use the term 'viability' when in fact growth on agar or in broth was measured, and confusion would be prevented if the terms 'culturability' and 'colony counts' were used instead.

We are now entering an era in which many diagnostic and investigational techniques may be replaced by molecular detection procedures. The fact that signals based on such techniques may come from culturable, dead and potentially VBNC cells should be recognized. Unravelling these three possibilities presents ample challenge for medical and non-medical microbiologists alike.

Bacterial death

Notwithstanding the problems outlined in the previous section, the ability to recognize and quantify bacterial death is of great practical significance in the practice of medicine. At present, except in highly defined circumstances, the cfu count remains the cornerstone for such measurements. In natural systems where no actively noxious environmental conditions pertain, if bacterial growth ceases, as in the stationary phase described above, after a variable period of time depending on the conditions and the organism concerned, then cfu counts begin to decline. In some cases this may lead to complete loss of viability, whereas in others a stable but lower cfu count is established. For example, *E. coli* appears to survive indefinitely in buffered salt solutions, the constant lysis of dying cells apparently providing for a balancing level of cell replication. Even after adaptation to starvation or other conditions leading to stasis, the rate at which viability is lost seems to follow a well defined pattern. Cells in stasis are clearly getting older and this provides a bacterial correlate of senescence. Although the study of bacterial cell senescence is relatively new, it is emerging that cumulative oxidative damage to cell proteins and other key macromolecules is one critical determinant of survival. This observation fits very well with the observation that one can often recover higher cfu counts of stressed facultative organisms on media containing catalase or other reagents that provide protection against reactive oxygen intermediates or following incubation under micro-aerophilic conditions.

It is not widely appreciated that many (probably all) bacteria carry genes encoding for programmed cell death. While several mechanisms are involved, these are distinct from the process of apoptosis that occurs in eukaryotic cells. These systems were first identified as toxin–antitoxin pairs functioning to maintain particular genes in the bacterial cell. However, it now seems likely that their occurrence cannot be explained solely on this basis and that, over time, they become integrated into the regulatory networks of the cell, particularly in controlling growth rate under certain conditions. It should be noted that the activation of latent prophages (see Ch. 6) constitutes another endogenous mechanism by which bacteria can initiate their own demise.

In addition to killing bacteria with antibiotics, medical practice is frequently concerned with decontaminating locations and materials that have been in contact with infectious patients. Moreover, the safe practice of surgery, parenteral administration of therapy, and the preparation of media and sampling materials for bacteriological studies all require the reduction or complete elimination of bacteria from key locations and devices. Although the methods applied to remove live bacteria may be checked with tests of biological efficacy, the relatively predictable rate of decline achieved with specific methods and target organisms enables safe practice. Because the cfu count is relatively convenient, it has been used in the establishment of most methodologies. However, removal or destruction of all infective agents is necessary to achieve sterility, and tests directed to all of these are required to some extent in establishing safe practice.

STERILIZATION AND DISINFECTION

Key definitions

Sterilization. The inactivation of all self-propagating biological entities (e.g. bacteria, viruses, prions) associated with the materials or areas under consideration.

Disinfection. The reduction of pathogenic organisms to a level at which they no longer constitute a risk.

Antisepsis. Term used to describe disinfection applied to living tissue such as a wound.

Methods used in sterilization and disinfection

In practice, all processes of sterilization have a finite probability of failure. By convention, an article may be regarded as sterile if it can be demonstrated that there is a probability of less than one in a million of

there being viable micro-organisms on it. As will be seen below, the level of microbial killing achieved by applying a particular method is dependent on the intensity with which the method is applied and its duration. Five main approaches are used.

Heat. The only method of sterilization that is both reliable and widely applicable is heating under carefully controlled conditions at temperatures above 100°C to ensure that bacterial spores are killed. There is some concern that even this temperature is insufficient to destroy prions. Shorter applications of lower temperatures, such as in pasteurization can effectively remove specific infection hazards.

Ionizing radiation. Both β-(electrons) and γ-irradiation (photons) are employed industrially for the sterilization of single-use disposable items such as needles and syringes, latex catheters and surgical gloves, and in the food industry to reduce spoilage and remove pathogens. Ultraviolet irradiation can be used to cut down the level of contamination, but is generally too mild to achieve sterility.

Filtration. Filters are used to remove bacteria and all larger micro-organisms from liquids that are liable to be spoiled by heating, for instance blood serum and antibiotic solutions in which contamination with filter-passing viruses is improbable or unimportant. Industrial scale filtration is used widely to reduce bacterial load and remove cysts of protozoa that are not killed by chlorination in the production of drinking water.

Gaseous chemical agents. Ethylene oxide is used mainly by industry for the sterilization of plastics and other thermolabile materials that cannot withstand heating. Formaldehyde in combination with subatmospheric steam is used more commonly in hospitals for reprocessing thermolabile equipment. Both processes carry toxic and other hazards for the user and the patient. Formaldehyde vapour on its own is used widely to decontaminate rooms and laboratory equipment.

Liquid chemical agents. Use of liquids such as glutaraldehyde is generally the least effective and most unreliable method. Such methods should be regarded as 'high-grade disinfection' only, to be applied when no other sterilization method is available, for example for heat-labile fibreoptic instruments such as flexible endoscopes. Various chemicals with antimicrobial properties are used as disinfectants. They are all liable to be inactivated by excessive dilution and contact with organic materials such as dirt or blood, or a variety of other materials. Nevertheless, they may provide a convenient method for environmental disinfection and other specific applications.

Choice of method

The choice of method of sterilization or disinfection depends on:

- the nature of the item to be treated
- the likely microbial contamination
- the risk of transmitting infection to patients or staff in contact with the item.

Choice is based on an assessment of risk according to different categories of patient (e.g. immunocompromised), the equipment involved and its application. The selection of sterilization, disinfection or simple cleaning processes for individual items of equipment and the environment should be agreed as part of the infection control policy of a hospital (see Ch. 69). The preferred option wherever possible, for both sterilization and disinfection, is heat rather than chemicals. This relates not only to the antimicrobial efficacy but to safety considerations, which are more difficult to control in some chemical processes. Wherever chemicals are to be used for disinfection and sterilization, the safety of persons involved directly or indirectly in the procedure must be considered. It should be remembered that all sterilizing and disinfecting agents have some action on human cells. No method should be assumed to be safe unless appropriate precautions are taken.

Measurement of microbial death

Every method used must be validated to demonstrate the required degree of microbial kill. With heat sterilization and irradiation, a biological test may not be required if the physical conditions are sufficiently well defined and controlled.

When micro-organisms are subjected to a lethal process, the number of viable cells decreases exponentially in relationship to the extent of exposure. If the logarithm of the number of survivors is plotted against the lethal dose received (e.g. time of heating at a particular temperature), the resulting curve is described as the *survivor curve*. This is independent of the size of the original population and is approximately linear. The linear survivor curve is an idealized concept and, in practice, minor variations, such as an initial shoulder or final tail, occur (Fig. 4.3).

D value

The *D value* or *decimal reduction value* is the dose required to inactivate 90% of the initial population. From Figure 4.3, it can be seen that the time (dose) required to reduce the population from 10^6 to 10^5 is

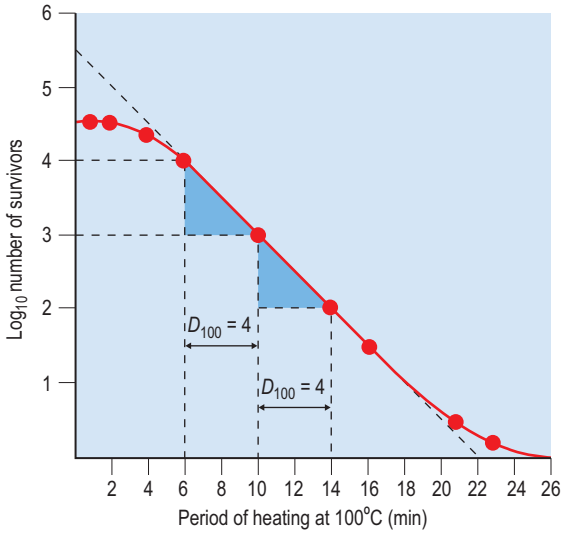

Fig. 4.3 Rate of inactivation of an inoculum of bacterial spores showing the decimal reduction time (*D* value) at 100°C and the nonlinear 'shoulder and tail' effects.

the same as the time (dose) required to reduce the population from 10^5 to 10^4, that is, the *D* value remains constant over the full range of the survivor curve. Extending the treatment beyond the point at which there is one surviving cell does not give rise to fractions of a surviving cell but rather to a statement of the probability of finding one survivor. Thus, by extrapolation from the experimental data, it is possible to determine the lethal dose required to give a probability of less than 10^{-6}, which is required to meet the pharmacopoeial definition of 'sterile'. Note that in preparations intended for mass use, if the probability of a single live organism in a batch from which 10 million doses are to be administered is 10^{-6}, then it is likely that around ten people will receive doses containing live organisms! Another consequence recognizable from Figure 4.3 is that the greater the number of microbes in the material to be sterilized, the longer the required exposure time. Thus, where efficient decontamination is the target, thorough cleansing can reduce the microbial load by several orders of magnitude and dramatically reduce both the time required and the level of certainty that sterility or adequate disinfection has been achieved.

Resistance to sterilization and disinfection

Many common factors affect the ability of microorganisms to withstand the lethal effects of sterilization or disinfection processes. Factors specific to individual processes are considered in the description of those processes. In general, vegetative bacteria and viruses are more susceptible, and bacterial spores the most resistant, to sterilizing and disinfecting agents. However, within different species and strains of species there may be wide variation in intrinsic resistance. For example, within the Enterobacteriaceae, *D* values at 60°C range from a few minutes (*E. coli*) to 1 h (*Salmonella enterica* serotype Senftenberg). The typical *D* value for *Staphylococcus aureus* at 70°C is less than 1 min, compared with 3 min for *Staph. epidermidis*. However, an unusual strain of *Staph. aureus* has been isolated with a *D* value of 14 min at 70°C. Such variations may be attributed to morphological or physiological changes such as alterations in cell proteins or specific targets in the cell envelope affecting permeability.

Inactivation data obtained for one micro-organism should not be extrapolated to another; thus it should not be assumed that bactericidal disinfectants are also potent against viruses. The inactivation data for scrapie, bovine spongiform encephalopathy and Creutzfeldt–Jakob disease (CJD) suggest that prions are highly resistant agents, requiring six times the normal heat sterilization cycle (134°C for 18 min). This has led to requirements for the mandatory use of disposable instruments that are in direct contact with brain or other nervous tissue (including the retina) or tonsils where the risk of exposure to the prion causing CJD is high.

Owing to the adaptive processes described above, the conditions under which the micro-organisms were grown or maintained before exposure to the lethal process have a marked effect on their resistance. Organisms grown under nutrient-limiting conditions are typically more resistant than those grown under nutrient-rich conditions. Resistance usually increases through the late logarithmic phase of growth of vegetative cells and declines erratically during the stationary phase. Finally bacterial endospores, formed principally by *Bacillus* and *Clostridium* species, are relatively resistant to most processes. Similarly, fungal spores are more resistant than the vegetative mycelium, although they are not usually as resistant as bacterial spores. Bacterial spores were used to define the sterilization processes in current use, and preparations of bacterial spores (biological indicators) are used to monitor the efficacy of ethylene oxide sterilization, in which physical monitoring is inadequate. In general, disinfection processes have little or no activity against bacterial spores.

The micro-environment of the organism during exposure to the lethal process has a profound effect on its resistance. Thus, micro-organisms occluded in salt have greatly enhanced resistance to ethylene oxide; the

presence of blood or other organic material will reduce the effectiveness of hypochlorite solution.

Sterilization by moist heat

Moist heat is much more effective than dry heat because hydrated proteins can be denatured with less energy than dehydrated semi-crystalline proteins. Further, where steam is used, its condensation delivers the latent heat of vaporization to the surface concerned. It is therefore necessary that all parts of the load to be sterilized are in direct contact with the water molecules in steam. Sterilization requires, in most cases, exposure to moist heat at 121°C for 15 min.

Moist heat sterilization requires temperatures above that of boiling water. Such conditions are attained under controlled conditions by raising the pressure of steam in a pressure vessel (*autoclave*). At sea level, boiling water at atmospheric pressure (1 bar) produces steam at 98–100°C, whereas raising the pressure to 2.4 bar increases the temperature to 125°C, and at 3.0 bar to 134°C. Conversely, at subatmospheric pressures, including those at higher altitude, water boils at lower temperatures.

Steam is non-toxic and non-corrosive, but for effective sterilization it must be *saturated*, which means that it holds all the water it can in the form of a transparent vapour. It must also be *dry*, which means that it does not contain water droplets. When dry saturated steam meets a cooler surface it condenses into a small volume of water and liberates the latent heat of vaporization. The energy available from this latent heat is considerable; for example, 6 L of steam at a temperature of 134°C (and a corresponding pressure of 3 bar absolute) will condense into 10 mL of water and liberate 2162 J of heat energy. By comparison, less than 100 J of heat energy is released to an article by the sensible heat from air at 134°C.

Steam at a higher temperature than the corresponding pressure would allow is referred to as *super-heated steam,* and behaves in a similar manner to hot air. Conversely, steam that contains suspended droplets of water at the same temperature is referred to as *wet steam* and is less efficient. The presence of air in steam affects the sterilizing efficiency by changing the pressure–temperature relationship.

As can be seen from the foregoing, sterilization by moist heat requires delivery of steam at exactly the right temperature and pressure, and for the right time. This places considerable demands on the engineering and maintenance of autoclaves, and in critical situations such as provision of sterile materials for clinical practice their performance must be monitored continually and precisely. Physical measurements of temperature, pressure and time with thermometers and pressure gauges are recorded for every load, and periodic detailed tests are undertaken with temperature-sensitive probes (thermocouples) inserted into standard test packs. Biological indicators comprising dried spore suspensions of a reference heat-resistant bacterium, *Bacillus stearothermophilus*, are no longer considered appropriate for routine testing, although spore indicators are essential for low-temperature gaseous processes in which the physical measurements are not reliable.

Sterilization by dry heat

Dry heat is believed to kill micro-organisms by causing a destructive oxidation of essential cell constituents. Killing of the most resistant spores by dry heat requires a temperature of 160°C for 2 h. This high temperature causes slight charring of paper, cotton and other organic materials.

Incineration is an efficient method for the sterilization and disposal of contaminated materials at a high temperature. It has a particular application for pathological waste materials, surgical dressings, sharp needles and other clinical waste. Red heat is achieved by holding inoculating wires, loops and points of forceps in the flame of a Bunsen burner until they are red hot.

Hot air sterilizers are used to process materials that can withstand high temperatures for the length of time needed for sterilization by dry heat, but that are likely to be affected by contact with steam. Examples include oils, powders, carbon steel microsurgical instruments and empty laboratory glassware. The overall cycle of heating up and cooling may take several hours.

Disinfection by chemicals

Chemicals used in the environment or on the skin (*disinfectants* or *antiseptics*) cannot be relied on to kill or inhibit all pathogenic micro-organisms. The distinction between disinfectants and antiseptics is not clear-cut; an antiseptic can be regarded as a special kind of disinfectant that is sufficiently free from injurious effects to be applied to the surface of the body, though not suitable for systemic administration. Some would restrict the term *antiseptic* to preparations applied to open wounds or abraded tissue, and prefer the term *skin disinfection* for the removal of organisms from the hands and intact skin surfaces.

The efficacy of a particular method of chemical disinfection is heavily dependent on the concentration and stability of the agent; the number, type and

accessibility of micro-organisms; the temperature and pH; and the presence of organic (especially protein) or other interfering substances.

In general, the rate of inactivation of a susceptible microbial population in the presence of an antimicrobial chemical is dependent on the relative concentration of the two reactants, the micro-organism and the chemical. The optimum concentration required to produce a standardized microbial effect in practice is described as the *in-use* concentration. Care must always be taken in preparing an accurate in-use dilution of concentrated product. Accidental or arbitrary overdilution may result in failure of disinfection.

The velocity of the reaction depends on the number and type of organisms present. In general, Gram-positive bacteria are more sensitive to disinfectants than Gram-negative bacteria; mycobacteria and fungal spores are relatively resistant, and bacterial spores are highly resistant. Enveloped or lipophilic viruses are relatively sensitive, whereas hydrophilic viruses such as poliovirus and other enteroviruses are less susceptible. Although difficult to test in vitro, there is evidence that hepatitis B virus is more resistant than other viruses (including human immunodeficiency virus) and most vegetative bacteria to the action of chemical disinfectants and heat.

Glutaraldehyde is highly active against bacteria, viruses and spores. Other disinfectants, such as hexachlorophane, have a relatively narrow range of activity, predominantly against Gram-positive cocci. Some disinfectants are more active or stable at a particular pH value; although glutaraldehyde is more stable under acidic conditions, use at a higher pH (8.0) improves the antimicrobial effect.

Disinfectants may be inactivated by hard tap water, cork, plastics, blood, urine, soaps and detergents, or another disinfectant. Information should be sought from the manufacturer or from reference authorities to confirm that the disinfectant will remain active in the circumstances of use.

Maintenance of effective disinfection in large healthcare facilities is a major challenge requiring management skills and technical understanding in equal measure. The selection of appropriate disinfectants and maintaining standards in practice is supported by the development of local disinfection policies. These points are considered further in Chapter 68.

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