Fourth edition

Russell, Hugo & Ayliffe's

Principles and Practice of

Disinfection Preservation & Sterilization

Edited by
A P Fraise, P A Lambert and J-Y Maillard

Blackwell Publishing

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Preface to the fourth edition

It has been a privilege to take on the editing of this textbook. The major change that has taken place is that the organization of the chapters has been altered such that Chapters 1–10 deal with the principles of disinfection, preservation and sterilization, and Chapters 11–21 deal with the practice. Although the book has always been aimed at microbiologists, physicians and pharmacists, the content of this fourth edition has been modified to reflect this clinical emphasis more. Consequently, chapters on textile, leather, paint and wood preservation have been removed, whereas sections on biofilms, prions and specific clinical areas such as dentistry have been updated and expanded. All other chapters have been revised, with new material added where appropriate.

Inevitably much of the content of the previous editions is still valid and we are grateful for the efforts of the previous editorial team and authors, without whom it would have been impossible to achieve this fourth edition within the allotted timescale. We are especially grateful to authors of chapters in previous editions, who have allowed their text to be used by new authors in this edition. We also thank all contributors (both old and new) for their hard work in maintaining this text as one of the foremost works on the subject.

A.P.F.
P.A.L.
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Preface to the first edition

Sterilization, disinfection and preservation, all designed to eliminate, prevent or frustrate the growth of microorganisms in a wide variety of products, were incepted empirically from the time of man's emergence and remain a problem today. The fact that this is so is due to the incredible ability of the first inhabitants of the biosphere to survive and adapt to almost any challenge. This ability must in turn have been laid down in their genomes during their long and successful sojourn on this planet.

It is true to say that, of these three processes, sterilization is a surer process than disinfection, which in turn is a surer process than preservation. It is in the last field that we find the greatest interactive play between challenger and challenged. The microbial spoilage of wood, paper, textiles, paints, stonework, stored foodstuffs, to mention only a few categories at constant risk, costs the world many billions of pounds each year, and if it were not for considerable success in the preservative field, this figure would rapidly become astronomical. Disinfection processes do not suffer quite the same failure rate and one is left with the view that failure here is due more to uninformed use and naïve interpretation of biocidal data. Sterilization is an infinitely more secure process and, provided that the procedural protocol is followed, controlled and monitored, it remains the most successful of the three processes.

In the field of communicable bacterial diseases and some virus infections, there is no doubt that these have been considerably reduced, especially in the wealthier industrial societies, by improved hygiene, more extensive immunization and possibly by availability of antibiotics. However, hospital-acquired infection remains an important problem and is often associated with surgical operations or instrumentation of the patient. Although heat sterilization processes at high temperatures are preferred whenever possible, medical equipment is often difficult to clean adequately, and components are sometimes heat-labile. Disposable equipment is useful and is widely used if relatively cheap but is obviously not practicable for the more expensive items. Ethylene oxide is often used in industry for sterilizing heat-labile products but has a limited use for reprocessing medical equipment. Low-temperature steam, with or without formaldehyde, has been developed as a possible alternative to ethylene oxide in the hospital.

Although aseptic methods are still used for surgical techniques, skin disinfection is still necessary and a wider range of non-toxic antiseptic agents suitable for application to tissues is required. Older antibacterial agents have been reintroduced, e.g. silver nitrate for burns, alcohol for hand disinfection in the general wards and less corrosive hypochlorites for disinfection of medical equipment.

Nevertheless, excessive use of disinfectants in the environment is undesirable and may change the hospital flora, selecting naturally antibiotic-resistant organisms, such as *Pseudomonas aeruginosa*, which are potentially dangerous to highly susceptible patients. Chemical disinfection of the hospital environment is therefore reduced to a minimum and is replaced where applicable by good cleaning methods or by physical methods of disinfection or sterilization.

A.D.R.
W.B.H.
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Part 1

Principles

Chapter 1
Historical introduction

Adam P Fraise

1 Early concepts

Throughout history it is remarkable how hygienic concepts have been applied. Examples may be found in ancient literature of the Near and Middle East, which date from when written records first became available. An interesting example of early written codes of hygiene may be found in the Bible, especially in the Book of Leviticus, chapters 11–15.

Disinfection using heat was recorded in the Book of Numbers, in which the passing of metal objects, especially cooking vessels, through fire was declared to cleanse them. It was also noted from early times that water stored in pottery vessels soon acquired a foul odour and taste and Aristotle recommended to Alexander the Great the practice of boiling the water to be drunk by his armies. It may be inferred that there was an awareness that something more than mechanical cleanness was required.

Chemical disinfection of a sort could be seen in the practice recorded at the time of Persian imperial expansion, *c.* 450 BC, of storing water in vessels of copper or silver to keep it potable. Wine, vinegar and honey were used on dressings and as cleansing agents for wounds and it is interesting to note that dilute acetic acid has been recommended comparatively recently for the topical treatment of wounds and surgical lesions infected by *Pseudomonas aeruginosa*.

The art of mummification, which so obsessed the Egyptian civilization (although it owed its success largely to desiccation in the dry atmosphere of the country), also employed a variety of balsams which contained natural preservatives. Natron, a crude native sodium carbonate, was also used to preserve the bodies of human and animal alike.

Not only in hygiene but in the field of food preservation were practical procedures discovered. Thus tribes which had not progressed beyond the status of hunter-gatherers discovered that meat and fish could be preserved by drying, salting or mixing with natural spices. As the great civilizations of the Mediterranean and Near and Middle East receded, so arose the European high cultures and, whether through reading or independent discovery, concepts of empirical hygiene were also developed. There was, of course, a continuum of contact between Europe and the Middle and Near East through the Arab and Ottoman incursions into Europe, but it is difficult to find early European writers acknowledging the heritage of these empires.

An early account of procedures to try and combat the episodic scourge of the plague may be found in the writings of the fourteenth century, where one Joseph of Burgundy recommended the burning of juniper branches in rooms where the plague sufferers had lain. Sulphur, too, was burned in the hope of removing the cause of this terrible disease.

The association of malodour with disease and the belief that matter floating in the air might be responsible for diseases, a Greek concept, led to these procedures. If success was achieved it may be due to the elimination of rats, later to be shown as the bearers of the causal organism. In Renaissance Italy at the turn of the fifteenth century a poet, philosopher and physician, Girolamo Fracastoro, who was professor of logic at the University of Padua, recognized possible causes of disease, mentioning contagion and airborne infection; he thought there must

exist 'seeds of disease', as indeed there did! Robert Boyle, the sceptical chemist, writing in the mid-seventeenth century, wrote of a possible relationship between fermentation and the disease process. In this he foreshadowed the views of Louis Pasteur. There is no evidence in the literature that Pasteur even read the opinions of Robert Boyle or Fracastoro.

The next landmark in this history was the discovery by Antonie van Leeuwenhoek of small living creatures in a variety of habitats, such as tooth scrapings, pond water and vegetable infusions. His drawings, seen under his simple microscopes (× 300), were published in the *Philosophical Transactions of the Royal Society* in 1677 and also in a series of letters to the Society before and after this date. Some of his illustrations are thought to represent bacteria, although the greatest magnification he is said to have achieved was 300 times. When considering Leeuwenhoek's great technical achievement in microscopy and his painstaking application of it to original investigation, it should be borne in mind that bacteria in colony form must have been seen from the beginning of human existence. A very early report of this was given by the Greek historian Siculus, who, writing of the siege of Tyre in 332 BC, states how bread, distributed to the Macedonians, had a bloody look. This was probably attributable to infestation by *Serratia marcescens*; this phenomenon must have been seen, if not recorded, from time immemorial.

Turning back to Europe, it is also possible to find other examples of workers who believed, but could not prove scientifically, that some diseases were caused by invisible living agents, *contagium animatum*. Among these workers were Kircher (1658), Lange (1659), Lancisi (1718) and Marten (1720).

By observation and intuition, therefore, we see that the practice of heat and chemical disinfection, the inhibitory effect of desiccation and the implication of invisible objects with the cause of some diseases were known or inferred from early times.

Before passing to a more rationally supported history it is necessary to report on a remarkable quantification of chemical preservation published in 1775 by Joseph Pringle. Pringle was seeking to evaluate preservation by salting and he added pieces of lean meat to glass jars containing solutions of different salts; these he incubated, and judged his end-point by the presence or absence of smell. He regarded his standard 'salt' as sea salt and expressed the results in terms of the relative efficiency as compared with sea salt; nitre, for example, had a value of 4 by this method. One hundred and fifty-three years later, Rideal and Walker were to use a similar method with phenolic disinfectants and *Salmonella typhi*; their standard was phenol.

Although the concept of bacterial diseases and spoilage was not used before the nineteenth century, very early in history procedures to ensure preservation of water and food were designed and used. It is only more recently (i.e. in the 1960s), that the importance of microorganisms in pharmaceuticals was appreciated (Kallings *et al.*, 1966) and the principles of preservation of medicine introduced.

2 Chemical disinfection

Newer and purer chemical disinfectants began to be used. Mercuric chloride, corrosive sublimate, found use as a wound dressing; it had been used since the Middle Ages and was introduced by Arab physicians. In 1798 bleaching powder was first made and a preparation of it was employed by Alcock in 1827 as a deodorant and disinfectant. Lefevre introduced chlorine water in 1843. In 1839 Davies had suggested iodine as a wound dressing. Semmelweis was to use chlorine water in his work on childbed fever occurring in the obstetrics division of the Vienna General Hospital. He achieved a sensational reduction in the incidence of the infection by insisting that all attending the birth washed their hands in chlorine water; later (in 1847) he substituted chlorinated lime.

Wood and coal tar were used as wound dressings in the early nineteenth century and, in a letter to the *Lancet*, Smith (1836–37) describes the use of creosote (Gr. *kreas* flesh, *soter* saviour) as a wound dressing. In 1850 Le Beuf, a French pharmacist, prepared an extract of coal tar by using the natural saponin of quillaia bark as a dispersing agent. Le Beuf asked a well-known surgeon, Jules Lemair, to evaluate his product. It proved to be highly efficacious. Küchenmeister was to use pure phenol in solution as a wound dressing in 1860 and Joseph

Lister also used phenol in his great studies on antiseptic surgery during the 1860s. It is also of interest to record that a number of chemicals were being used as wood preservatives. Wood tar had been used in the 1700s to preserve the timbers of ships, and mercuric chloride was used for the same purpose in 1705. Copper sulphate was introduced in 1767 and zinc chloride in 1815. Many of these products are still in use today.

Turning back to evaluation, Bucholtz (1875) determined what is called today the minimum inhibitory concentration of phenol, creosote and benzoic and salicylic acids to inhibit the growth of bacteria. Robert Koch made measurements of the inhibitory power of mercuric chloride against anthrax spores but overvalued the products as he failed to neutralize the substance carried over in his tests. This was pointed out by Geppert, who, in 1889, used ammonium sulphide as a neutralizing agent for mercuric chloride and obtained much more realistic values for the antimicrobial powers of mercuric chloride.

It will be apparent that, parallel with these early studies, an important watershed already alluded to in the opening paragraphs of this brief history had been passed. That is the scientific identification of a microbial species with a specific disease. Credit for this should go to an Italian, Agostino Bassi, a lawyer from Lodi (a small town near Milan). Although not a scientist or medical man, he performed exacting scientific experiments to equate a disease of silkworms with a fungus. Bassi identified plague and cholera as being of microbial origin and also experimented with heat and chemicals as antimicrobial agents. His work anticipated the great names of Pasteur and Koch in the implication of microbes with certain diseases, but because it was published locally in Lodi and in Italian it has not found the place it deserves in many textbooks.

Two other chemical disinfectants still in use today were early introductions. Hydrogen peroxide was first examined by Traugott in 1893, and Dakin reported on chlorine-releasing compounds in 1915. Quaternary ammonium compounds were introduced by Jacobs in 1916.

In 1897, Kronig and Paul, with the acknowledged help of the Japanese physical chemist Ikeda, introduced the science of disinfection dynamics; their pioneering publication was to give rise to innumerable studies on the subject lasting through to the present day.

Since then other chemical biocides, which are now widely used in hospital practice, have been introduced, such as chlorhexidine, an important cationic biocide which activity was described in 1958 (Hugo, 1975).

More recently, a better understanding of hygiene concepts has provided the basis for an explosion in the number of products containing chemical biocides. Of those, quaternary ammonium compounds and phenolics are the most important. This rise in biocide-containing products has also sparked a major concern about the improper use of chemical disinfectants and a possible emergence of microbial resistance to these biocides and possible cross-resistance to antibiotics. Among the most widely studied biocides are chlorhexidine and triclosan. The bisphenol triclosan is unique, in the sense that it has recently been shown that at a low concentration, it inhibits selectively an enoyl reductase carrier protein, which is also a target site for antibiotic chemotherapy in some microorganisms. These important aspects in biocide usage will be discussed later.

3 Sterilization

As has been stated above, heat sterilization has been known since early historical times as a cleansing and purifying agent. In 1832 William Henry, a Manchester physician, studied the effect of heat on contagion by placing contaminated material, i.e. clothes worn by sufferers from typhus and scarlet fever, in air heated by water sealed in a pressure vessel. He realized that he could achieve temperatures higher than 100 °C by using a closed vessel fitted with a proper safety valve. He found that garments so treated could be worn with impunity by others, who did not then contract the diseases. Louis Pasteur also used a pressure vessel with safety valve for sterilization.

Sterilization by filtration has been observed from early times. Foul-tasting waters draining from ponds and percolating through soil or gravel were sometimes observed on emerging, spring-like, at a

lower part of the terrain to be clear and potable (drinkable), and artificial filters of pebbles were constructed. Later, deliberately constructed tubes of unglazed porcelain or compressed kieselguhr, the so-called Chamberland or Berkefeld filters, made their appearance in 1884 and 1891 respectively.

Although it was known that sunlight helped wound healing and in checking the spread of disease, it was Downes and Blunt in 1887 who first set up experiments to study the effect of light on bacteria and other organisms. Using *Bacillus subtilis* as test organism, Ward in 1892 attempted to investigate the connection between the wavelength of light and its toxicity; he found that blue light was more toxic than red.

In 1903, using a continuous arc current, Barnard and Morgan demonstrated that the maximum bactericidal effect resided in the range 226–328 nm, i.e. in the ultraviolet light, and this is now a well-established agent for water and air sterilization (see Chapter 12.2).

At the end of the nineteenth century, a wealth of pioneering work was being carried out in subatomic physics. In 1895, the German physicist, Roentgen, discovered X-rays, and 3 years later Rieder found these rays to be toxic to common pathogens. X-rays of a wavelength between 10^{-10} and 10^{-11} nm are one of the radiations emitted by ^{60}Co, now used extensively in sterilization processes (Chapter 12.2).

Another major field of research in the concluding years of the nineteenth century was that of natural radioactivity. In 1879, Becquerel found that, if left near a photographic plate, uranium compounds would cause it to fog. He suggested that rays, later named Becquerel rays, were being emitted. Rutherford, in 1899, showed that when the emission was exposed to a magnetic field three types of radiation (α, β and γ) were given off. The γ-rays were shown to have the same order of wavelength as X-rays. β-Rays were found to be highspeed electrons, and α-rays were helium nuclei. These emissions were demonstrated to be antimicrobial by Mink in 1896 and by Pancinotti and Porchelli 2 years later. High-speed electrons generated by electron accelerators are now used in sterilization processes (Chapter 12.2).

Thus, within 3 years of the discovery of X-rays and natural radiation, their effect on the growth of microorganisms had been investigated and published. Both were found to be lethal. Ultraviolet light was shown in 1893 to be the lethal component of sunlight.

These and other aspects have been discussed by Hugo (1996).

Sterilization can also be achieved by chemicals, although their use for this purpose do not offer the same quality assurance as heat- or radiation-sterilization. The term 'chemosterilizer' was first defined by Borick in 1968. This term has now been replaced by 'liquid chemical sterilants', which defined those chemicals used in hospital for sterilizing reusable medical devices. Among the earliest used 'liquid chemical sterilants' were formaldehyde and ethylene oxide. Another aldehyde, glutaraldehyde has been used for this purpose for almost 40 years (Bruch, 1991). More recently compounds such as peracetic acid and *ortho*-phthalaldehyde (OPA) have been introduced as alternative substitutes for the di-aldehyde.

After this time, the science of sterilization and disinfection followed a more ordered pattern of evolution, culminating in the new technology of radiation sterilization. However, mistakes—often fatal—still occur and the discipline must at all times be accompanied by vigilance and critical monitoring and evaluation.

4 Future developments for chemical biocides

This is a very interesting time for biocides. For the last 50 years, our knowledge of biocides has increased, but also our concerns about their extensive use in hospital and domiciliary environments. One encouraging sign is the apparent willingness of the industry to understand the mechanisms of action of chemical biocides and the mechanisms of microbial resistance to biocides. Although, 'new' biocidal molecules might not be produced in the future, novel 'disinfection/antisepsis' products might concentrate on synergistic effects between biocides or/and the combination of biocide and permeabilizer, or other non-biocide chemicals, so that an increase in antimicrobial activity is achieved. The

ways in which biocides are delivered is also the subject of extensive investigations. For example, the use of polymers for the slow release of biocidal molecules, the use of light-activated biocides and the use of alcoholic gels for antisepsis are all signs of current concerted efforts to adapt laboratory concepts to real life situations.

Although, this might be a 'golden age' for biocidal science, many questions remain unanswered, such as the significance of biocide resistance in bacteria, the fine mechanism of action of biocides and the possibility of primary action sites within target microorganisms, and the effect of biocides on new emerging pathogens and microbial biofilms. Some of these concepts will be discussed further in several chapters.

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