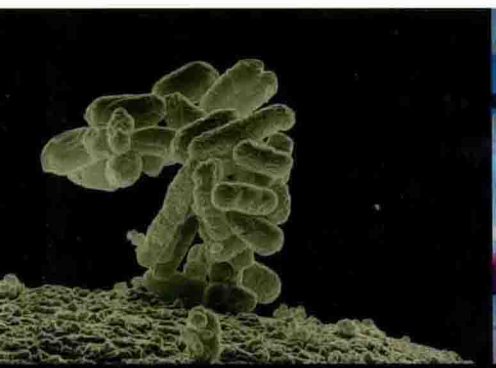
Russell, Hugo & Ayliffe's

Principles and Practice of Disinfection, Preservation and Sterilization

FIFTH EDITION



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Preface to the Fifth Edition

It has been a particular privilege to be editors of this fifth edition, which has been substantially revised. Thirty-six of its 40 chapters are new and those from the previous edition have undergone major revisions/updates. Every attempt also has been made to cover the subject matter in all chapters from a global perspective.

Putting this edition together has been a daunting task in view of the rapidly expanding significance and scope of the subject matter covered while also considering the wide acceptance and utility of its previous editions. We thank the authors for their contributions and the publisher's staff for coordinating all dealings between the contributors and the editors.

We are most grateful to our respective families for allowing us to devote the long hours needed to edit this book.

Adam P. Fraise
Jean-Yves Maillard
Syed A. Sattar
November 2012

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 necesssary 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.

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Contents

1 Historical Introduction

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Early concepts

Disinfection and hygiene are concepts that have been applied by humans for thousands of years. Examples may be found in ancient literature such as the Bible where disinfection using heat was recorded in the Book of Numbers; 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 odor 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 awareness that something more than mechanical cleanness was required.

Chemical disinfection of a sort was practiced at the time of Persian imperial expansion, *c.* 450 BC, when water was stored 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 diluted 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 region), employed a variety of balsams containing natural preservatives. Natron, a crude native sodium carbonate, was also used to preserve the bodies of human and animal alike.

Practical procedures involving chemical agents were also applied in the field of food preservation. Thus tribes who 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, and European cultures arose, so the precepts of empirical hygiene were also developed. There was, of course, ongoing 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 14th century, where Joseph of Burgundy recommended the burning of juniper branches in rooms where plague sufferers had lain. Sulfur, too, was burned in the hope of removing the cause of this disease. The association of malodor 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 have been due to the elimination of rats, later to be shown as the bearers of the causative organism.

In Renaissance Italy at the turn of the 15th century, a poet, philosopher and physician, Girolamo Fracastoro, who was

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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". Robert Boyle, the skeptical chemist, writing in the mid-17th century, wrote of a possible relationship between fermentation and the disease process. In this he foreshadowed the views of Louis Pasteur. There is, however, 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 Transaction of the Royal Society of London* 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. 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 Tire in 332 BC, states how bread, distributed to the Macedonians, had a bloody look. This was probably attributable to contamination by pigmented strains of *Serratia marcescens* and 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 moving on to a more formally scientific period in 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; niter, for example, had a value of 4 by this method. Rideal and Walker, 153 years later, were to use a similar method to measure the activity of phenolic disinfectants against *Salmonella typhi*; their standard was phenol.

Although the concept of bacterial diseases and spoilage was not widespread before the 19th century, procedures to preserve food and drink were used early in history. It is only more recently, that is in the 1960s, that the importance of microorganisms in pharmaceuticals was appreciated [1] and the principles of preservation of medicines introduced.

Chemical disinfection

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

Wood and coal tar were used as wound dressings in the early 19th century and, in a letter to the *Lancet*, Smith described the use of creosote (Gr. *kreas* flesh, *soter* savior) as a wound dressing [2]. 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 sulfate was introduced in 1767 and zinc chloride in 1815. Many of these products are still in use today.

Turning back to evaluation, Bucholtz in 1875 determined what is known today as the minimum inhibitory concentration of phenol, creosote and benzoic and salicylic acids against 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 sulfide 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, in parallel with these early studies, an important watershed 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 physician, 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 [3] was to give rise to innumerable studies on the subject lasting through to the present day.

Since then other chemical microbicides, which are now widely used in hospital practice, have been introduced – such as chlorhexidine, an important cationic microbicide, whose activity was described in 1958 [4].

More recently, a better understanding of hygiene concepts has provided the basis for an explosion in the number of products containing chemicals. In particular, quaternary ammonium compounds are being developed with altered chemistry and improved activity. Peroxygen compounds are gaining popularity due to their good *in vitro* activity (including activity against spores), and mechanisms for preparing compounds that release hypochlorous acid are also being adopted widely in the healthcare, veterinary and food industries. This rise in microbicide-containing products has also sparked a major concern about the improper use of chemical disinfectants and a possible emergence of microbial resistance to these microbicides and possible cross-resistance to antibiotics. Among the most widely studied microbicides are chlorhexidine and triclosan. The bisphenol triclosan is unique, in the sense that it has 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.

Sterilization

Heat has been known as a cleansing and purifying agent for centuries. In 1832, William Henry, a Manchester physician, studied the effect of heat on contaminated material, that is clothes worn by sufferers from typhus and scarlet fever. He placed the material in a pressure vessel and realized that he could achieve temperatures higher than 100°C by using a sealed vessel fitted with a 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 a 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 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 the test organism, Ward, in 1892, attempted to investigate the connection between the wavelength of light and its antimicrobial activity; he found that blue light was more active 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, that is, light in the ultraviolet range. Ultraviolet light is now a well-established agent for water and air decontamination.

At the end of the 19th century, a wealth of pioneering work was being carried out in subatomic physics. In 1895, the German physicist Röntgen discovered X-rays, and 3 years later Rieder found these rays to be harmful to common pathogens. X-rays of a wavelength between 10^{-10} and 10^{-11} are emitted by ^{60}Co and are now used extensively in sterilization processes.

Another major field of research in the concluding years of the 19th century was that of natural radioactivity. In 1879, Becquerel found that, if left near a photographic plate, uranium compounds would cause the plate 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 wavelengths of the same order as X-rays. Beta-rays were found to be 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.

Thus, within 3 years of the discovery of X-rays and natural radiation, their effect on the growth and viability of microorganisms had been investigated and published. Both were found to be lethal. Ultraviolet light was shown in 1993 to be the lethal component of sunlight.

For more information on this aspect of sterilization see Hugo [5].

Sterilization can also be achieved by chemicals, although their use for this purpose does not offer the same quality assurance as heat or radiation sterilization. The term "chemosterilizer" was first defined by Borick in 1968. This has now been replaced by the term "chemical sterilants", which is used to refer to those chemicals used in hospital for sterilizing reusable medical devices. Among the earliest used chemical sterilants were formaldehyde and ethylene oxide. Another aldehyde, glutaraldehyde, has been used for this purpose for almost 40 years [6]. Compounds such as peracetic acid, chlorine dioxide and *ortho*-phthalaldehyde (OPA) have been introduced as substitutes for the dialdehyde and these compounds have been widely adopted for the decontamination of flexible fiberoptic endoscopes.

In the latter half of the 20th century the science of sterilization and disinfection followed a more ordered pattern of evolution, culminating in new technologies such as radiation sterilization

and gas plasma sterilization. However, no method is foolproof and human error will always occur. Therefore, whatever technologies are used, all staff working in the field of sterilization must be vigilant and maintain a critical approach where evaluation of methodologies is an integral part of the process.

Future developments for microbicides

This is a very interesting time for those involved in the use of microbicides. For the last 50 years, our knowledge of microbicides has increased, but so have 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 microbicides and the mechanisms of microbial resistance to microbicides. Although "new" microbicidal molecules might not be produced in the future, novel products might concentrate on synergistic effects between microbicides and the combination of microbicide and permeabilizer or other non-microbicidal chemicals, so that an increase in antimicrobial activity is achieved. The ways microbicides are delivered is also the subject of extensive investigations. For example, the use of polymers for the slow release of microbicidal molecules, the use of light-activated microbicides and the use of alcoholic rubs for antisepsis are all signs of current concerted efforts to adapt laboratory concepts to practical situations.

Although, this might be a "golden age" for microbicidal science, many questions remain unanswered, such as the significance of microbicide resistance, the fine mechanism of action of microbicides, the possibility of primary action sites within target microorganisms, and the effect of microbicides on emerging pathogens and microbial biofilms. Some of these concepts will be discussed further in following chapters.

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2 Types of Microbicidal and Microbistatic Agents

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Introduction

This chapter serves as a source of reference for those interested in developing an initial or general understanding of the chemistry and mode of action of a particular group of microbicidal agents. It is not intended to provide a definitive description of individual agents, but rather to introduce the reader to the general concepts of those agents and to provide key references as a starting point for more thorough investigations. With this in mind, the authors have undertaken a "hard edit" of the previous version of this chapter, including the removal of dated information and updates to the chemical groups discussed. Given this approach, the authors wish to acknowledge those who have nurtured and developed this chapter in previous editions of the book: Barry Hugo and Denver Russell (first, second and third editions) and Suzanne Moore and David Payne (fourth edition).

Phenols

Hugo [1, 2] and Marouchoc [3] showed that phenols and natural product distillates containing phenols shared, with chlorine and iodine, an early place in the armory of antiseptics. Today, they are widely used as general disinfectants and as preservatives for a variety of manufactured products [4], except where there is risk of contamination of foods. As a result of their long history, a vast literature has accumulated dealing with phenol and its analogs and a comprehensive review of these compounds can be found in Goddard and McCue [5]. While many different parameters have been used to express their microbicidal and microbistatic power, the phenol coefficient is perhaps the most widely employed.

A reasonable assessment of the relationship between structure and activity in the phenol series was compiled by Suter [6]. The main conclusions from this survey were:

Russell, Hugo & Ayliffe's: Principles and Practice of Disinfection, Preservation and Sterilization, Fifth Edition. Edited by Adam P. Fraise, Jean-Yves Maillard, and Syed A. Sattar.

1. *Para*-substitutions of an alkyl chain up to six carbon atoms in length increases the bactericidal action of phenols, presumably by increasing the surface activity and ability to orientate at an interface. Activity falls off after this due to decreased water solubility. Straight chain *para*-substituents confer greater activity than branched-chain substituents containing the same number of carbon atoms.
2. Halogenation increases the bactericidal activity of phenols. The combination of alkyl and halogen substitution, which confers the greatest bactericidal activity, is that where the alkyl group is *ortho*- to the phenolic group and the halogen *para*- to the phenolic group.
3. Nitration, while increasing the toxicity of phenols towards bacteria, also increases the systemic toxicity and confers specific biological properties on the molecule, enabling it to interfere with oxidative phosphorylation. This has now been shown to be due to the ability of nitrophenols to act as uncoupling agents. Studies [7] have shown that the nitro group is not a prerequisite for uncoupling, as ethylphenol is an uncoupler. Nitrophenols have now been largely superseded as plant protection chemicals, whereas at one time they were in vogue, although 4-nitrophenol is still used as a preservative in the leather industry.
4. In the bisphenol series, activity is found with a direct bond between the two C_6H_5 groups or if they are separated by $-CH_2-$, –S– or –O–. If a –CO–, –SO– or –CH(OH)– group separates the phenyl groups, activity is low. In addition, maximum activity is found with the hydroxyl group at the 2,2′- position of the bisphenol. Halogenation of the bisphenols confers additional microbicidal activity.

Chemistry of phenols

The phenol parent compound C_6H_5OH (Figure 2.1) is a white crystalline solid (melting point (m.p.) 39–40°C), which becomes pink and finally black on long standing. It is soluble in water 1 : 13 and is a weak acid, pK_a 10. Its biological activity resides in the undissociated molecule. Phenol is effective against both Gram-positive and Gram-negative vegetative bacteria, but is only slowly effective against bacterial spores and acid-fast bacteria.

Phenols are the reference standard for the Rideal–Walker (RW) and Chick–Martin tests for disinfectant evaluations. They find limited application in medicine today, but are used as preservatives in such products as animal glues. Although first obtained from coal tar, they are now obtained largely by synthetic processes, which include the hydrolysis of chlorobenzene of the high-temperature interaction of benzene sulfonic acid and alkali.

Mode of action

At low concentrations, phenols interact with bacterial enzymes needed for cell wall synthesis, resulting in cell lysis. High concentrations of phenols cause general coagulation of the cytoplasm and act as general protoplasmic poisons. In addition, phenols can affect the cytoplasmic membrane [8, 9] resulting in leakage of potassium ions first, then the cytosol. Hexachlorophene was found to have additional activity as an inhibitor of the electron transport chain, thus inhibiting the metabolic activities in bacteria [10].

Sources of phenols: the coal-tar industry

Most of the phenols used to make disinfectants are a by-product of the destructive distillation of coal. Coal is heated in the absence of air and the volatile products, one of which is tar, are condensed. The tar is fractionated to yield a group of products that include phenols (called tar acids), organic bases and neutral products, such as alkyl naphthalenes, which are known in the industry as neutral oils.

The cresols consist of a mixture of 2-, 3- and 4-cresol. The "xylenols" consist of the six isomeric dimethylphenols plus ethylphenols. The combined fraction, cresols and xylenols, is also available as a commercial product known as cresylic acid. High-boiling tar acids consist of higher alkyl homologs of phenols: for example the diethylphenols, tetramethylphenols and methylethylphenols, together with methylindanols, naphthols and methylresorcinols, the latter being known as dihydrics. There may be traces of 2-phenylphenol. The chemical constituents of some of the phenolic components are shown in Figure 2.1.

Properties of phenolic fractions

The passage from phenol (boiling point (b.p.) 182°C) to the higher-boiling phenols (b.p. up to 310°C) is accompanied by a well-defined gradation in properties, as follows: water solubility decreases, tissue trauma decreases, bactericidal activity increases, inactivation by organic matter increases. However, the ratio of activity against Gram-negative to activity against Gram-positive organisms remains fairly constant, although in the case of pseudomonads, activity tends to decrease with decreasing water solubility (Table 2.1).

Formulation of coal-tar disinfectants

It is seen from the above data that the progressive increase in desirable biological properties of the coal-tar phenols with increasing boiling point is accompanied by a decrease in water solubility. This presents formulation problems and part of the story

Table 2.1 Phenol coefficients of coal-tar products against *Salmonella typhi* and *Staphylococcus aureus*.

Product and m.p. range	Phenol coefficient		Water solubility (g/100 ml)
	S. typhi	*S. aureus*	
Phenol (182°C)	1	1	6.6
Cresols (190–203°C)	2.5	2.0	2.0
4-Ethylphenol (195°C)	6	6	Slightly
Xylenols (210–230°C)	5	4.5	Slightly
High-boiling tar acids (230–270°C)	40	25	Insoluble
High-boiling tar acids (250–275°C)	60	40	Insoluble

m.p., melting point.