Disinfection, Sterilization, and Preservation

Fourth Edition

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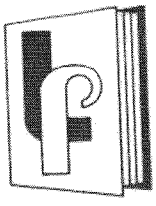

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CHAPTER 50

PRESERVATION OF MEDICINES AGAINST MICROBIAL CONTAMINATION

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Most drug delivery systems are associated with undesirable side effects. These are largely inherent in the active component of the formulation and thus difficult to avoid. However, side effects such as infection and pyrexia caused by microbiologically contaminated dosage forms are easily avoided. In recent years reports of clinical complications arising from microbial contamination of oral and topical products in several European countries and the United States has resulted in product recalls. Tightened regulatory and compendia limits have reemphasized the need for the drug formulator to carefully and thoroughly consider all aspects of formulation design.

The formulation of an elegant, efficacious medicine that is both stable and acceptable may necessitate the use of a wide variety of ingredients in a complex physical state. This could create conditions conducive to the survival, and even extensive replication, of contaminant microorganisms that might enter the product either during its manufacture or with use by the patient or medical staff.

Microbial contamination of pharmaceuticals has been studied extensively and various methods have been discussed whereby the microbial content of dosage forms may be limited, and their susceptibility to microbial spoilage reduced (Ringertz and Ringertz, 1982; Spooner, 1985). In addition Good Manufacturing Codes of Practice (GMPs) have been widely adopted. Many drug preparations for parenteral administration, and preparations likely to contact broken skin or internal organs must be sterile in order to avoid the possibility of infection arising from their use. Injections, ophthalmic preparations, irrigation fluids, dialysis solutions, sutures, implants, surgical dressings, and instruments necessary for their administration are presented in a sterile condition and should remain sterile throughout the period of use.

The quality of non-sterile pharmaceuticals has so improved, that today the majority contain only minimal microbial populations. In spite of this, the incidence of product contamination with unacceptable levels and types of contaminant has accumulated over the past 25 years (Crompton, 1973; Kuehne and Ahearn, 1971; Noble and Savin, 1966; Beveridge, 1975; Kallings et al., 1966; Pederson and Ulrich, 1968).

Many of the ingredients used in drug formulations become suitable substrates for microorganisms when conditions of water availability, pH, and temperature are favorable for growth. Pharmaceutical preparations may be contaminated by molds, yeasts, or bacteria, with the latter generally favoring a slightly alkaline medium and the others an acid medium. Although few microorganisms can grow outside the pH range 4 to 9, most pharmaceutical preparations are within the vulnerable pH range and must be protected against microbial growth.

Several studies have illustrated the wide range of raw materials (colors, dyes, pigments, talcs, starches, clays, fillers, natural gums, and thickening agents) and finished products that may contain viable microorganisms. Although the United States Pharmacopeia (USP) XXI has procedures for determining the microbial content of raw materials and finished products, limits on the number and types of microorganisms have not been officially specified. However, all materials must be free of the "harmful microorganisms" listed in the USP XX. Most of the manufacturers have set up their own microbiologic specifications suitable to their raw materials and finished products. In addition to the use of contaminated raw materials, contamination may occur during manufacture (contaminated equipment, water, operators, air, and packaging materials) and, after manufacture, by the user (Baird, 1987; Denyer, 1987; Fassihi and Parker, 1987; Parker and Fassihi, 1988). Living organisms, by virtue of their growth potential, metabolic versatility, and ability to act in concert, propagate at the expense of their environment, leading to product spoilage and subsequent loss of drug efficacy. If this is uninhibited by any

restraining influence, it often causes rapid and profound changes in the chemical environment. In the synthesis of new protoplasm, many complex chemical reactions are accomplished within a remarkably short period.

Substances added to medicines to prevent microbial spoilage, retard deterioration, and restrain organismic growth to low levels are known as preservatives. Governmental regulatory agencies require the use of a preservative whenever a formulation will be used more than once from the same container. They must be nontoxic because they are included in oral, topical, and some parenteral injections. They are used primarily in multiple-dose containers to inhibit the growth of microorganisms that may be introduced during or subsequent to the manufacturing process. Antimicrobial agents should not be used solely to reduce the viable microbial count as a substitute for GMPs. All useful preservative agents are toxic. For maximum protection of the consumer, the concentration of the preservative shown to be effective in the final packaged product should be considerably below the toxic level for humans. It should also be recognized that the presence of living microorganisms or fragments of dead microorganisms may produce pyrogenic reactions or may cause adverse reactions in sensitized persons.

It has been known for many years that microbial contaminants may effect the spoilage of pharmaceutical products through chemical, physical, or aesthetic changes, thereby rendering them unfit for use. Active drug constituents may be metabolized to less-potent or chemically inactive forms. Therapeutic agents as diverse as morphine, barbiturates, aspirin, paracetamol, thalidomide, atropine, steroids, and mandelic acid, and pharmaceutical ingredients such as surfactants, polymers, fats, oils, sweetening agents, flavoring agents, and coloring agents can be metabolized by microorganisms and serve as substrates for microbial growth (Marshall and Wiley, 1982; Grant et al., 1970; Grant, 1971; Bucherer, 1965; Brookes et al., 1981). Physical changes commonly seen are due to breakdown of emulsions, visible surface growth on solids, or the formation of slimes, pellicles, or sediments in liquids. These changes are sometimes accompanied by the production of such things as gas, odors, or unwanted flavors. Chemical reactions are catalyzed by a wide variety of enzymes produced by microorganisms, and include hydrolysis, dehydration, oxidation, reduction, decarboxylation, deamination, phosphorylation, and dephosphorylation (Wedderburn, 1964). The metabolic pathways employed by bacteria differ between different genera and species and depend on the environmental conditions. For example, *Aerobacter aerogenes* will attack pyruvic acid at pH 8 to give acetic and formic acids.

$$CH_3COCOOH + H_2O \rightleftharpoons CH_3COOH + HCOOH$$

At pH 6 the same organism will decarboxylate pyruvic acid.

$$2CH_3COCOOH \rightleftharpoons CH_3COCHOH + 2CO_2$$

By decomposing constituents of the environment many of these metabolic processes yield end-products that have a growth-limiting effect on the organisms (Wedderburn, 1964). A further consequence of the establishment of primary invaders in a pharmaceutical preparation is a reduction in the efficiency of the preservative system. A variety of genera can cause breakdown of preservatives (Beveridge and Hugo, 1964; Hugo and Foster, 1964; Dagley, 1971; Beveridge, 1975). The growth of microorganisms in a product may produce obvious spoilage and may influence the stability and even the release patterns of the pharmaceutical preparations. More important from the clinical point of view is that microbial growth may be associated with the production of toxins, resulting in food poisoning from an orally administered preparation. The microorganisms capable of producing toxins, including certain strains of *Bacillus cereus* and *Staphylococcus aureus* and mycotoxin-producing fungi, constitute a potential hazard.

FORMULATION DESIGN AND PRESERVATION

Pharmaceutical preparations are manufactured in a clean environment by following the GMP recommendations. Most ophthalmic and injectable preparations are sterilized by physical methods during their manufacture. To enable multidose drug preparations to cope adequately with contaminants gaining access during repeated withdrawal of doses, presence of a potent antimicrobial preservative is essential. It must be noted that only the undissociated fraction or molecular form of a preservative is active, because the ionized portion is incapable of penetrating the microorganism. Thus the preservative selected must be largely undissociated at the pH of the formulation. Consequently, the preservation of medicines involves formulation (including preservative agents), quality control of ingredients and packaging, good manufacturing practice, and storage under appropriate conditions.

Many compatible combinations of preservative agents and other formulation components have been reported in recent years. Conversely, incompatibilities that result in preservative inactivation involve macromolecules such as various cellulose derivatives, polyvinylpyrrolidone, polyethylene glycols, Tween 80, Myrj 52, and sucrose mono-oleate (Kazmi and Mitchell, 1976; Parker and Barnes, 1967). Product storage temperature, as well as interaction with surfactants or other active substances, may change the concentration of unbound preservative in the aqueous phase.

Plastic containers, metal ointment tubes, rubber or plastic caps, or liners may absorb the preservative, thereby decreasing the quantity available for antimicrobial activity.

In almost every drug formulation there are factors predisposing it to or inhibiting microbial growth—e.g., pH, osmotic effects, and the presence of toxic molecules. The balance among these factors, including the presence of a preservative, will determine the microbial growth or

bactericidal rate. As noted, the efficiency of the preservative is itself subject to favorable and unfavorable influences of the formulation components.

Appreciable chemical or physicochemical spoilage probably requires significant growth of microbial contaminants within the formulations. Early indicative signs will often be organoleptic, with the release of unpleasant tasting or smelling metabolites such as "sour" fatty acids, ketones, "fishy" amines, "rotten eggs" (H_2S), ammonia, and bitter, sickly, or alcoholic tastes and smells. Products frequently develop unappealing discoloration because of various microbial pigments, and even polythene may exhibit a pink discoloration when colonized by fungi (Beveridge, 1987). Depolymerization of thickening agents or polymerization of sugar or surfactant molecules will result in a rapid loss of stabilizing efficacy; the accumulation of acidic or basic metabolites can produce marked shifts in product pH.

An effectively designed preservative system must retain its antimicrobial activity for the shelf-life of the product (Sykes, 1971). To ensure compliance with this requirement, the preservative characteristics of the product in its final form must be studied as a function of time.

Most aqueous preparations, especially emulsions, suspensions, and some creams, are excellent media for microbes. Syrups containing approximately 85% sugar resist bacterial growth by virtue of their exosmotic effect on microorganisms. Syrups containing less than 85% sucrose, but sufficient polyol (e.g., sorbitol, glycerin, propylene glycol) to be hypertonic similarly resist bacterial growth. Hydroalcoholic and alcoholic preparations containing more than 15% alcohol may not require the addition of a chemical preservative; hence, elixirs, spirits, and tinctures are self-sterilizing.

MICROBIAL ORGANISMS ASSOCIATED WITH PHARMACEUTICAL PRODUCTS

To determine whether a specific organism is hazardous in given circumstances, one must consider the nature of the product, its dose, the probable state of health of the user, and clinical reports on the frequency and severity of infections caused by the microorganism in question. The FDA recognizes the categories "harmful," "objectionable," and "opportunistic" in respect of microorganisms. "Harmful" refers to those microbial organisms or their toxins responsible for human disease or infection. Examples of organisms that must not be present in a product are given, e.g., *Salmonella* species, *Escherichia coli*, certain species of *Pseudomonas*, including *P. aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. An "objectionable" organism can cause disease, inactivate the drug, or lead to the deterioration of the product. The following are objectionable organisms and should not be present in a pharmaceutical or cosmetic product: *Pseudomonas putida*, *P. multivorans*, *P. maltophilia*, *Proteus mirabilis*, *Serratia marcescens*, *Klebsiella spp.*, *Acinetobacter anitratus*, and *Candida spp.* (Bruch, 1972). Organisms are defined as "opportunistic" if they produce disease in immunocompromised patients, such as the newborn, patients with AIDS, and patients undergoing immunosuppressive therapy.

A medicine may be considered microbiologically spoiled if low levels of acutely pathogenic microorganisms or higher levels of opportunist pathogens are present, or if toxic microbial metabolites persist even after death of the original contaminants, or if microbial growth has initiated significant physical or chemical deterioration of the product. Such spoilage may well have serious financial consequences for the manufacturer, either in the immediate loss of product or in the increasingly expensive process of litigation should the spoilage cause harm to the user.

Contaminants isolated from products range from true pathogens, such as *Clostridium tetani*, to opportunistic pathogens, such as *Pseudomonas aeruginosa* and many other free-living organisms. Some of the microorganisms more frequently isolated from medicinal products are shown in Table 50–1.

The main problem with these organisms is that their simple nutritional requirements enable them to survive in a wide range of pharmaceuticals. They tend to be present in high numbers, sometimes in excess of 10^6 cfu g^{-1} or ml^{-1}. In spite of this the product may itself show no visible sign of contamination.

CONTAMINATED MEDICINES AND CLINICAL IMPLICATIONS

Although isolated outbreaks of drug-related infections have been reported for many decades, it is only in the past 20 years that the significance of these infections has been properly understood. The infection dose of microorganisms is not only largely unknown but variable; furthermore, it varies not only between and within species but also between individual patients. The symptoms and outcome of a medicament-borne infection may be diverse. Clinical reactions may range from local infections of wounds or broken skin following contact with a contaminated cream, to serious systemic infections such as bacteriemia or septicaemia from contaminated parenteral products. Gastrointestinal infections can follow the ingestion of contaminated oral products. The most serious outbreaks of infection have been seen in the past where contaminated products have been injected into the bloodstream of patients whose immunity is already compromised by their underlying disease or therapy.

During the 1970s there were reports of septicaemia following infusion of contaminated solutions (Phillips et al., 1972; Felts et al., 1972; Meers et al., 1973). The contaminating microorganisms were mainly of gram-negative bacilli that grow readily in water, such as *Pseudomonas* and *Enterobacter spp.*, as well as fungi (Robertson, 1970; Goldmann and Maki, 1973). Various antimicrobial agents, usually quaternary ammonium compounds, contaminated with *Pseudomonas*, have repeatedly caused septicaemia when used as skin cleanser

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