# CHAPTER I

## INTRODUCTION

The microbiology of cosmetic products has a relatively short history. The outbreak of tetanus in babies has been attributed to the use of talcum powder contaminated with *Clostridium tetani* in New Zealand in 1946 (Tremewan, 1946; Hills, 1946). In 1947 the death of three babies in Australia was reported to have been traced to the use of tetanus spore-infected talcum (Anonymous, 1947).

Dr. Lars Kallings (1965), in his landmark report, published his findings of the microbiological content of nonsterile pharmaceuticals, some special cosmetics and toiletries - baby powders and baby lotions (Kallings, *et al.*, 1965).

Noble and Savin (1966), Morse, *et al.*, (1967), Morse and Schonbeck (1968) reported about staphylococcal infections in hospitals in the United States and abroad from the use of contaminated hand creams and hand lotions.

These reports stimulated the Food and Drug Administration of the United States (F.D.A.) to look more closely into the potential of cosmetics to support the growth of microorganisms. A highly significant event occured in 1967, when a group of microbiologists working in the cosmetic industry formed a Scientific Committee under the aegis of the

Toilet Goods Association (TGA). That was the time that the cosmetic industry was rapidly becoming aware of the microbiologist's vital role in the development and production of cosmetics and toiletries (Yablonski and Goldman, 1975).

In 1968 Dunnigan and Bruch set out to discuss the regulatory consequences of microbial contamination of cosmetics; and even microbiologists from the cosmetic industry began to publish papers on microbiological problems. These events made the cosmetic industry re-assess its attitude towards microbiology. The demand for microbiologist rose sharply and cosmetic microbiology become a major topic of scientific meetings, conferences, seminars and in the cosmetic literature. The F.D.A. was involved in 25 recalls of contaminated cosmetic during 1966, 1967 and the early part of 1968 (Dunnigan, 1968).

A survey in 1969 found that 61 of 250 marketed cosmetics (almost 25 per cent) were contaminated with various microorganisms. Some products contained more than one type of organism, and 16 were contaminated with Pseudomonads (Wolven and Levenslein, 1969).

In January 1969, the F.D.A. initiated a survey through its then 17 district laboratories to determine the microbiological quality of cosmetics and topical drugs. A report in 1970, that of 169 samples assayed, 33 had microbial contaminants, 17 contained gram negative bacteria and three fungi (Dunnigan and Evans, 1970).

Shortly after that publication, another survey reported that

none of 57 samples of new eye cosmetics contained fungi, but that 12 per cent (53 of 428) of used cosmetics had fungal contaminants. As many as five species were found in some samples, the most common molds being *Penicillium* and *Cladosporium* species; the most common yeast *Rhodotorula rubra* and *Candida parapsilosis*. Cultures from eyelids and conjunctivae from 9 of 29 women who use contaminated mascara yielded the same species as obtained from their respective cosmetics. In one instance, mascara of a women with a corneal ulcer caused by *Fusarium solani* yielded the same fungus. It was found that some of the isolated yeasts could metabolize cosmetic components such as paraffin oil and isopropyl myristate. This study demonstrated that fungi can contaminate eye make-up and present a potential hazard of mycotic keratitis to the user (Kuehne, *et al.*, 1971).

Since then, cosmetic chemists and microbiologists have made great studies towards the manufacturer of better-preserved, safer cosmetic products. The survey in 1972, found that only 8 contaminated samples among the 223 that were tested (Wolven and Levenstein, 1972).

For many years, scientists have recognized that topically-applied products contaminated with pathogenic microorganisms may be harmful to human health. Further confirmation of the health risk associated with the use of poorly preserved, contaminated cosmetics has been provided by some reports in 1975-1976, with their investigations of the clinical consequences of contaminated eye cosmetics. The initial studies involved identification of microorganisms in new and used

mascaras and on the eyelids and fingers of consumers. Microbial contamination of new mascaras was found to be rare, and the microbial population levels of contaminated mascaras usually were low. Used mascaras, however, were frequently contaminated, and the microbial spectrum was broad. The most widely encountered organisms were *Staphylococcus epidermidis* and *Corynebacterium* species. Others were bacilli, micrococci, coliforms and molds. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were also encountered, though rarely. In most instances, the organisms isolated from used samples of mascaras were also those found on the user's outer eye, fingers or saliva. In some instances, the microbial level increased significantly during the storage period. On the other hand the contaminated products which contained, in addition to parabens, an organic mercurial preservative showed a relatively rapid decrease in microbial population levels after withdrawal from use. About ten per cent of the patients with eye infections were found to be using eye cosmetic contaminated with *Staphylococcus epidermidis* or *Fusarium* species, that caused the blepharo-conjunctival infections. All resulted from the use of pseudomonas-contaminated mascaras and the inadvertent abrasion of the patient's cornea with the applicator wand. The ulcers occured within 24 hours of the abrasion. In all cases, useful vision was lost in the ulcerated eye (Ahearn and Wilson, 1975; Wilson, *et al.*, 1975).

Although the healthy eye is known to be a quite resistant to microbial infection, these cases of temporary and permanent eye injury confirm that the cornea and outer eye nevertheless can become infected,

particularly on traumatized eye. A heavily contaminated eye or facial make-up, therefore, constituted a potentially serious health hazard. Of course, equally hazardous to the eye could be shampoos or other hair products that were corrosive to the cornea and contained pathogenic microorganisms.

In 1975, the F.D.A. of the U.S. conducts a survey involving 400 domestic cosmetic products and found only 17, mostly facial make-up and creams, contaminated with microorganisms. During the fiscal years 1974 and 1975, the F.D.A. had only 10 products recalls and one seizure involving microbial contamination this in spite of a sizable expansion of the cosmetic industry during the intervening years (Eiermann, 1976).

## The Important Pathogenic Microorganisms

### *Escherichia coli*

The genus is classified in Family Enterobacteriaceae. Strains of *Escherichia coli* and related coliform bacteria predominate among the aerobic commensal flora present in the gut of men and animals and are widely distributed in the environment. *Escherichia coli* is incriminated as a pathogen outside the gut and particularly in the urinary tract and in the wounds where the infections may be endogenous from the patient's own intestine or acquired from an exogenous sources. Being excreted in very large numbers in faeces, it comes to contaminate the environment including the soil very widely and the bacilli may survive without growth for several days to a few weeks

outside the body. When it is found in a water supply, it is considered to indicate that the supply has recently been subjected to contamination with human or animal faeces.

*Escherichia coli* is a Gram-negative, motile, non-sporing bacillus, morphologically identical with *Salmonellae* and on ordinary culture media their colonies are also similar; however, on MacConkey medium *Escherichia coli* strains yield rose-pink colonies since they ferment the lactose in the medium.

*Escherichia coli,* as a pathogen, is associated with two main clinical syndromes; (1) acute gastroenteritis in infants up to 2 years of age rarely in adults with possibly some lowered resistance, and (2) infections of the urinary tract, particularly in married women but also in girls and in elderly men with prostatic enlargement, *Escherichia coli* may also be the causal organism in appendicular abscess, peritonitis, cholecystitis, wound infections, etc. (Davis, *et al.,* 1973 and Cruickshank, *et al.,* 1973).

*Pseudomonas*

The genus is in Family Pseudomonadaceae, some 30 species are found, for the most part, in water, soil, and wherever organic matter is decomposing. The best known species of *Pseudomonas* and the only one that is pathogenic for man, is *Pseudomonas aeruginosa,* which is also commonly known as *Pseudomonas pyocyanea*. The blue or blue-green strains that sometimes appear upon surgical dressing long ago attracted attention, and even before the cause of the phenomenon had

been discovered. Forclos studied the pigment in 1860. Gessard found, in 1882, that the pigment was the product of a specific microorganisms, *Pseudomonas aeruginosa,* which he isolated in pure culture.

The cells of *Pseudomonas aeruginosa* vary in size and proportion but appear usually as, small, slender rods, 1.5 to 3 micron long and 0.5 micron broad, frequently united in pairs and short chains. There are one to three polar flagella, and is actively motile. Neither capsules nor spores are formed. The colonies are large and spreading, the edges are irregular, the consistency butyrous and are gram-negative.

*Pseudomonas aeruginosa* grows readily on all ordinary culture mediums and most rapidly at a temperature of 30 C to 37 C. Aerobic conditions are required, although there is some growth under anaerobic conditions. One of the most distinctive characteristic of *Pseudomonas aeruginosa* is its production of a bluish green, soluble pigment which does not color the colonies or other mass of growth but instead diffuses into the medium. There are actually two pigments formed. The one, pyocyanin, is a deep blue in color and can be extracted from aqueous solution by chloroform, and the other, fluorescin, a yellowish green fluorescent pigment, is soluble in water but not in chloroform. Pyocyanin is formed only by *Pseudomonas aeruginosa,* but the fluorescent pigment is formed by several other species of *Pseudomonas*.

*Pseudomonas aeruginosa* can produce an enzyme which destroys the cornea by degradation of the corneal collagen (Fisher and Allen, 1958; Marzulli, *et al.,* 1972). It is found in pure culture in abscesses

in different parts of the body, especially in the middle ear. Cases of endocarditis, pneumonia, and meningitis, though rare, also occur in which *Pseudomonas aeruginosa* appears to be the sole responsible microorganism. Infection with *Pseudomonas aeruginosa* and other *Pseudomonas* species are difficult to treat because they do not respond well or uniformly to antibacterial drugs. In general, polymyxin is the most consistently active, while streptomycin and tetracycline are less active and give variable results. They are resisted to penicillin, erythromycin and other antibiotics affecting the gram-positive bacteria.

*Salmonella*

This genus is classified in Family Enterobacteriaceae. These bacilli are gram-negative rods closely resembling and indistinguishable from the coliform bacteria. They are stained readily with the usual dyes such as methylene blue and carbolfuchsin. No particular arrangement of the cells is apparent on microscopic examination. All species except *Salmonella pullorum* and *Salmonella gallinarum* are actively motile by means of peritrichous flagella. No capsules are apparent and spores are not formed.

The bacteria of this group have simple nutritional requirements, growing readily on the usual nutrient media. The optimum temperature is 37 C, but growth occurs at a reasonable rate at room temperature. They are facultative anaerobes, growing equally well under either aerobic or anaerobic conditions. The group is characterized biochemically by failure to ferment lactose or salicin and inability to liquefy gelatin

or produce indol (Burrows, *et al.*,1968).

Infection is by ingestion; from the small intestine the organisms pass via the lymphatics to the mesenteric glands, whence after a period of multiplication they invade the blood stream via the thoracic duct, the liver, gall-bladder, spleen, kidney and bone-marrow become infected during this bacteraemic phase in the first seven to ten days of the disease. From the gall-bladder a further invasion of the intestine results and lymphoid tissue-Peyer's patches and lymphoid follicles-are particularly involved in an acute inflammatory reaction and infiltration with mononuclear cells, followed by necrosis, and the formation of characteristic typhoid ulcers. Haemorrhage of varying degree may occur and less frequently, perforation through a necrotic Peyer's patches may complicate the illness.

*Salmonella typhi* is present in large numbers in the inflamed tissue in the ulcers and is found in the intestinal contents. It may localise in the kidney and appear in the urine sometimes producing a marked bacilluria. The bacillus is found in other lesions occuring as complications on sequelae of typhoid fever such as acute suppurative periosteitis and osteitis abscess of the kidney, acute cholecystitis, bronchopneumonia, empyema and ulcerative endocarditis (Cruickshank, 1965).

*Staphylococcus*

This genus is classified in Family Micrococcaceae, most commonly found in boils, abscesses, carbuncles and similar suppurative processes in man, belongs to the group of staphylococci. The presence of staphylococci in pus was first shown by Pasteur in 1880 and later by Ogston in 1881.

The spherical cells are generally aggregated in loose, irregular masses which have been likened to clusters of grapes, and have given the generic name to this organism. The staphylococci do not form spores, and motile varieties are very rarely observed. The ordinary aniline dyes stain the cells readily, and the great majority of staphylococci are gram-positive. The dimensions of the individual cocci vary within narrow limits, the diameter of the cells ranging between 0.7 and 0.9 micron. The growth on agar media is abundant, opaque and glistening, and the individual colonies are circular with entire edges. Pigment may be formed, a golden yellow in the case of *Staphylococcus aureus* and lemon yellow in the case of *Staphylococcus citreus,* but it is absent and the growth appears white in the case of *Staphylococcus albus* and other less common species.

The optimun temperature is 28 C to 37 C. The staphylococci in general are not fastidious in their nutritive requirements and grow radily on the ordinary nutrient media. The pigments produced by the chromogenic staphylococci are probably lipochromes.

A variety of toxic substances are produced by staphylococci, including hemolysins, leucocidin, coagulase, fibrinolysin, spreading factor, skin-necrotizing substance, a lethal factor and enterotoxin. In general, toxin production is a property of the pathogenic staphylococci, usually of the aureus variety.

The staphylococci have power under certain circumstances to penetrate the skin. A momentary weakness on the part of the tissues in almost any locality may lead to a rapid local invasion, followed by the production of a simple boil or carbuncle condition. Septicemia and pyemia sometimes result through the introduction of staphylococci into the lymphatics or the blood stream from a local abscess. Staphylococcal infection of the lung sometimes occurs, and the resulting bronchopneumonia is often fatal. Out of about 800 patients with pneumonia treated at the Hospital of the Rockefeller Institute in New York City from 1913 to 1918 were infected with staphylococci, and 10 of the 13 died. Under certain conditions, as during the 1918 influenza epidemic at Camp Jackson, *Staphylococcus aureus* may play an important part in the pneumonia complicating primary infections. Chickering and Park in 1919, found that in 49 per cent of 312 postmortem lung cultures this microorganism was present either alone (92 cases) or in association with other bacteria. Similarly, Gaspar found in 1941 that of 144 fatal cases of pneumonia cultured at autopsy, 38 were caused by staphylococci (Burrows, *et al.*,1950).

## Other Microorganisms Concerned

### *Bacillus*

The genus *Bacillus* is composed of large gram positive rods that form spores and grow best under aerobic conditions. Most species are saprophytic and are found on vegetation and in soil, water and air. The only species that is highly pathogenic for man is *Bacillus anthracis,* which causes anthrax, a disease primarily of domestic livestock. Human anthrax is rare in the United States. Although the disease occasionally has been contracted by farmers, veterinarians, and slaughterhouse workers who come into contact with infected livestock (agricultural anthrax). Human infection in the United States, occurs almost exclusively in workers at plants processing imported goat hair, wool, or hides (industrial anthrax).

It should be noted that certain species of Bacillus can be involved, even though infrequently, in human disease processes. *Bacillus cereus, Bacillus circulans, Bacillus pumilus, Bacillus sphaericus,* and *Bacillus subtilis* have been variously incriminated in cases of meningitis, pneumonia, and septicemia. One fatal case of pneumonia with a bacteremia due to *Bacillus cereus* in a patient with subacute lymphocytic leukemia. *Bacillus subtilis* may occasionally cause human eye infections and it may become an opportunistic invader in the presence of a foreign prosthetic material in the human body (Davis, *et al.*, 1973; Bailey and Scott, 1974).

*Aspergillus*

The common blue-green mold seen on damp bread, bacon or most any organic material is usually a member of the genus *Aspergillus*. With every breath we inhale some spores of one or more species. Most of the time these spores are disposed of without injury to the host. In some individuals and under certain conditions, these organisms may provoke injury to man in one of two ways: allergic response to the presence of the spores or, much more rarely, invasion of pulmonary or other tissue. Human cases of aspergillosis are most frequently infections of the external ear (otomycosis). *Aspergillus fumigatus* is the most common invader, *Aspergillus nidulans, Aspergillus flavus* and *Aspergillus niger* are sometimes found (Burrows, *et al.*, 1968).

*Curvularia*

*Curvularia geniculata* and *Curvularia lunata* have been isolated from black grain mycetoma. Mycetoma is a localized swollen lesion, usually on a foot or hand, less often on shoulders, buttocks, head, or any site which is subject to trauma. It involves skin, subcutaneous tissue, fascia and bone. The lesion contains granulomas and abscesses which suppurate and drain through sinus tracts. The pus contains granules which vary from microscopic in size than 2 mm in diameter. Size, color, shape and texture of the granule, and the dimensions of the hyphae vary with the species of actinomycetes on fungus and sometimes suggest the specific etiology. The mycetomas are caused by any one of at least 6 actinomycetes and 16 species of true fungi (Conant, *et al.*, 1971; Emmons, *et al.*, 1977).

*Paecilomyces*

Species of *Paecilomyces* resemble those of *Penicillium* under a microscope but *Paecilomyces* never produces greenish blue colonies and the margin of the colony often appears shredded rather than entire. Conidial structures are much more irregular than those of *Penicillium*. *Paecilomyces varioti* has been isolated from a few cases of endocarditis following cardiac surgery in man, and from a case of secondary infection in dog (Emmons, *et al.*, 1977). George, *et al.* (1962) reported mycoses in tortoises due to *Paecilomyces fumoso-roseus*.

*Penicillium*

*Penicillium* is the common blue-green mold like *Aspergillus*, but is less commonly associated with infection than *Aspergillus*. The ubiquity of species of *Penicillium* and their constant contamination of instruments, wounds, urine, sputum, etc, make the establishment of this diagnosis very difficult. Substantiated infection of the cornea and the external ear, a few mycetomas and a very rare pulmonary infection are recorded for a variety of *Penicillium* species. The pathological picture is similar to aspergillosis (Burrows, *et al.*, 1968).

Practical Aspects : Procedure in the Factory

1. Control of raw materials

All materials used in cosmetics should be checked for quality visually, chemically and microbiologically. Specifications to buyers should include microbiological standards where necessary. It may be possible to blend, improve or "top up" the chemical or functional quality of a crude ingredients, but it is often not possible or practicable to improve the microbiological quality without damaging the product. A defect of this nature can persist right through the processing and packaging to retail sale and use.

2. Formulation and preservation

In general it is always better to prevent microbiological growth by formulation rather than by relying on preservatives. No organism can grow without nutrients but almost anything organic can act as a nutrient for some organisms. Physical factors are highly specific for particular types of organism, e.g. acidity for most bacteria but not for yeasts or molds, absence of oxygen for obligate aerobes and aerobic conditions for anaerobes. A useful measure of control can be exercised by designing formulation, processing and cleansing methods with particular reference to the type of infection to which the product is most vulnerable.

3. Temperature control

Temperature is in practice the most important factor controlling the growth of bacteria and so the safety and keeping quality of the products,

assuming satisfactory hygiene. Two of the biggest mistakes made in factory practice are to assume that dial thermometers and recorders are always accurate, and that calculations made in respect of heat transfer under ordinary conditions also apply in hot weather. Both these fallacious assumptions have led to major catastrophes in more than one industry.

All working (dial) thermometers should be checked against a known accurate thermometer in the laboratory. Mercury in glass thermometers should never be used in the factory.

All cooling systems should be calculated allowing for an atmospheric temperature of 27 C and a mains water temperature of 20 C, or alternatively provision made for additional cooling capacity in hot weather.

4. Design of equipment

The equipment in the cosmetic factory should well designed and hygienically constructed.

5. Sterilization of equipment by heat

Heat is usually applied as hot water, steam at atmospheric pressure or under pressure (e.g. in an autoclave) usually at 15 lb/sq in.

Hot air requires 160 C. for 3 hours or 170 C for 2 hours to ensure sterility of equipment.

6. Sterilization by chemicals

6.1 Cleaning by a detergent (e.g. alkali) and then

sterilization by a sterilant (e.g. hypochlorite) or a quaternary ammonium compound.

6.2 Cleaning by a strong concentration of a detergent sterilant followed by sterilization by a weaker concentration (e.g. iodophors)

6.3 Cleaning and sterilizing by a detergent sterilant (e.g. quaternary ammonium compound + alkali) followed by a 'sterile rinse' (e.g. quaternary ammonium compound or hypochlorite).

6.4 Using a single substance which has powerful cleaning and sterilizing properties (e.g. sodium hydroxide or nitric acid) followed by a sterile rinse.

7. Bactericidal action of detergent

Many detergents have marked germicidal properties although they are used primarily as detergents, and this may be the only claim made by manufacturers of proprietary products. Hot water at 60-80 C will kill most or all vegetative cells but few spores. A detergent will always enhance the killing effect of heat. This effect is especially valuable against spores in those industrial applications, e.g. bottle-washing followed by cold filling, where excessive temperatures have to be avoided.

8. Factory water supplies

The bacteriological purity of water is generally judged on the basis of the ministry of Health Memo NO. 71 (Davis, 1972) which assesses potability by the presumptive and faecal coli tests, supported by total colony count at 22 C and 37 C. Many years experience has

proved the validity of this method, but potability is not the same as quality for a particular industrial purpose. Defects in cosmetics may be caused by *Pseudomonas* and similar gram-negative bacteria, yeasts, molds and other types of no public health significance. The hazards for water, which is drunk, and for cosmetics, which are applied to and remain on the skin, are quite different. The latter include *Staphylococcus aureus, Pseudomonas aeruginosa,* and various skin pathogens which are usually completely ignored in public health water bacteriology. A further fallacy is the assumption that water as used in the factory is as pure as water supplied to the factory. Storage in tanks and passage through pipelines, pumps, filters, softeners, etc. may easily result in gross contamination. Unless constant testing shows that the water is of adequate purity, mild chlorination (2-5 ppm) is recommended.

9. Hands as a source of infection

Apart from obviously bad air conditions, contamination or infection of a product is always caused by contact. Of all the ways in which this can occur, there is little doubt that in practice the hands are the commonest means whereby a product becomes contaminated by a pathogenic organism. Skin is impossible to sterilize and the bacterial load may vary from a few thousands to a few millions, Staphylococci and enterobacteria are nearly always found.

10. Walls, roofs and air in factories

These problems are inter-related because whatever organisms may occur on one will be found on the others. Clearly the factory air cannot be free from contamination which occurs on walls and roofs, or is present

in air outside the factory. An air conditioning system which controls temperature, humidity and removes microorganisms by filtration and/or electrostatic means is by far the best, but is quite expensive. Aerosol of quaternary ammonium compound solution appear to be both effective and cheap. The cleaning solution for walls and roofs is a penetractive, non-foaming, alkaline detergent and may be incorported with bactericide and fungicide.

Microbial Contamination During Manufacturing Process

In order to accomplish the production of a microbially free cosmetic, it is necessary to have microbially free materials and manufacturing facilities with no contamination during processing.

Places contaminated and causes of contamination

1. Contamination caused by air borne microorganisms due to air supply.

2. Cooling: contamination caused by residual liquid after machine washing.

3. Storage: a) Contamination caused by air borne microorganisms.
   b) Contamination caused by insufficient tank cleansing.

4. Filling: a) Contamination caused by air borne microorganisms.
   b) Contamination caused by filling machine.
   c) Contamination caused by the container.
   d) Contamination caused by the unskilled and careless workers (Davis, 1972).