Microbiology and Aging
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Clinical Manifestations
I would like to dedicate this book to Carol, Alex and Tom and my Mum and Dad. ‘The cycle of life is a journey of microbiological intrigue’
Preface

The world’s population is estimated to reach 8.9 billion by 2050 with 370 million people of 80 years of age or older. Ageing is an incurable disease and defined as the ‘deregulation of biochemical processes important for life’, but for the purpose of this book, ageing is better defined as the biological process of growing older. Ageing is part of natural human development.

As you will see throughout this book, the microbiological burden on the host is enormous and clinically significant, and will undoubtedly have a role to play in the ageing process. As humans are living longer, there is a greater propensity to infection. This risk is substantially heightened in elderly individuals who are predisposed to infection. While the process of ageing and its effects on the host’s microbiology are poorly documented and researched, data obtained from gut studies have shown that microbiological changes take place over time suggesting significance to the host. Do the microbiological changes that occur within and upon the host influence the process of ageing or is it the biological changes of the host that affects the microbiology? Does this therefore affect our propensity to disease? As the host’s microbiology changes with ageing, is this significantly beneficial or severely detrimental to the host? Are there ways of enhancing life expectancy by reducing certain bacteria from proliferating or conversely by enhancing the survival of beneficial bacteria?

This book considers the microbiology of the host in different regions of the body and how these vary in the different age groups. Chapter 1 of the book focuses on ageing theories with Chap. 2 considering the human indigenous flora and how this is affected during ageing. Chapter 3 highlights the main infections associated with an elderly population, while Chap. 4 reviews the process of skin ageing and its associated microbiology. Chapter 5 reviews the ageing lung and Chap. 6 reviews influenza in the elderly. Chapter 7 highlights the changes that occur in the oral microflora and host defences with advanced age with Chap. 8 reviewing the influence of the gut microbiota with ageing. Chapter 8 focuses on the gut and its associated immunity. The remaining four chapters of the book consider clostridium and the ageing gut, *Helicobacter pylori* and the hygiene hypothesis and the benefits of probiotics. The microbiology theory of autism in children is reviewed in
Chap. 13. The final chapter of the book examines how the beneficial microbiology of the host leads to human decomposition.

This book encompasses a collection of reviews that highlight the significance of and the crucial role that microorganisms play in the human life cycle.

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Chapter 1
Ageing Theories, Diseases and Microorganisms

Steven L. Percival

Biology of Ageing

Ageing is a ‘progressive decline or a gradual deterioration of physiological function including a decrease in fecundity or irreversible process of loss of viability and increase in vulnerability’.\textsuperscript{1,2} It is often referred to as the deregulation of biochemical processes important for life.\textsuperscript{1,3,4} However, for the purpose of this book and chapter, ageing is best described as the biological process of growing older. The ageing process is associated with physiological changes, an increased susceptibility to certain diseases, an increase in mortality, a decrease in metabolic rate, a reduction in height and reaction times, a fluctuation in weight, menopause in woman, reduction in olfaction and vision, and, in particular, a decrease in the immune response.\textsuperscript{5–7}

Factors known to cause and influence ageing in humans remain diverse, and scientifically problems reside in distinguishing causes from effects.\textsuperscript{8} However, there are strong evolutionary and genetic influences known to effect the ageing process and life expectancy.\textsuperscript{9–11} The physiological changes that do occur during human ageing increase an individual’s susceptibility to various diseases and therefore increase the likelihood of death.

Mechanistic Theories on Ageing

Many ageing theories have been proposed, and scientifically scrutinized. To date, the reasons why humans age are still extensively being researched, but still no overall general consensus regarding the cause of the ageing exists. Nevertheless, what can be agreed upon is the fact that ageing is an ‘inescapable biological reality’.\textsuperscript{12} However, many scientists hypothesize that ageing is ‘a disease that can be cured, or at least postponed’.\textsuperscript{13}
The first ageing theory, founded on genetic principles, can be traced back to the work of Weismann at the end of the nineteenth century. Weismann postulated that evolution introduced ageing to avoid parents, and their more adapted offspring, competing for the same resources. This theory of ‘programmed death’ was severely criticized, as it seemed very unlikely that such a programme could ever be implemented in natural conditions where practically no individual dies of old age. An alternative theory of ageing, proposed by Peter Medawar, stated that ageing resulted from an accumulation of mutations. Such mutations, known to be detrimental to the host, were considered free to accumulate resulting in death. More recently, in 1957, George Williams proposed a complementary ageing theory known as the ‘antagonistic pleiotropy theory’. Agonistic pleiotropy means that a single gene may have an effect on several traits (pleiotropy), and that these effects may affect fitness in opposite ways (antagonistic). This theory states that evolution favours mutations that improve fitness at an earlier age, even at the expense of a reduced fitness later on in life. According to this ageing theory, senescence is a by-product of good adaptation early in life. Even today, this remains a well-respected ageing theory. Evolutionary ageing theories are constantly being challenged by the generation of new findings as a result of long-lived mutant animal models. Data obtained from these studies have resulted in suggestions that Weismann’s programmed death theory should be reconsidered specifically, as all evolutionary theories still need experimental confirmation and should not be considered mutually exclusive.

Within the animal kingdom clear differences in the rates of ageing are evident. Many theories exist to suggest reasons for this. Such theories, however, lie outside the nature of this book. While many theories have been proposed to help explain human ageing, there still remains a lack of adequate ‘ageing’ models. Consequently, this makes hypothesis-testing difficult. Testing the many ageing theories is difficult, expensive and time consuming, and discriminating between causes and effects is presently impossible.

To date, the two most respected human ageing theories include the ‘programmed theories of ageing’ and the ‘damage-based theories of ageing’. Programmed ageing theories suggest that ageing is not a random process but driven by genetically regulated processes, as opposed to the damage-based ageing theories which suggest that ageing is a result of damage accumulation products produced by the normal cellular processes of the body as a result of poor repair systems in the body. The damage-based theories imply that ageing is predominately a result of interactions with the environment, as opposed to the programmed theories which are predetermined. However, there does seem to be a large amount of overlap between these theories. Each of the two theories will be considered in turn.

**Programmed Theories of Ageing**

The programmed theories of ageing defend the idea that ageing is genetically determined: i.e. a programmed and predetermined process. This suggests that the process of ageing is ‘genetically regulated’. The significance of this is apparent
when we consider certain hormones, such as growth hormone, which are known to decline with age and whose target is the insulin-like growth factor 1 (IGF-1). However, it has been found that by restoring or altering hormonal levels in the younger generation, ageing has not been deterred. In fact, hormone replacement has been shown to prematurely accelerate ageing. Nevertheless, there have been numerous research findings that have shown that the endocrine system is able to influence the ageing process.

Only one single gene has been shown to have a possible direct effect on human longevity. This gene is the apoE gene. The apoE gene codes for a protein in the body that is involved in fat transport. Centenarians have been found to have a high prevalence of the apoE e2 variant (or allele) compared to the e4 variant. The e4 variant is known to cause susceptibility to elevated blood cholesterol, coronary artery disease, and Alzheimer disease (the function of this protein in the brain is not yet known).

In animal studies, several genes have been isolated that have been shown to influence ageing and life span. For example, in the nematode worm, *Caenorhabditis elegans*, the inactivation or loss of a few genes increases its life span fivefold, generally (but not always) at the expense of a reduced fertility. Life extension in worms has been linked with the worms’ ability to enter a phase called Dauer larva, during which the animal seals its mouth and does not feed. Some mutant worms (with non-functional or missing DAF-2 gene) show an extended life span, and enter this phase whatever the environmental conditions. Similar life extension by genetic means has been observed in other animal models. Examples include the fruit fly, *Drosophila melanogaster*, and the mouse, where longevity is often connected with dwarfish.

The pattern of genes involved in longevity suggests that a common biochemical pathway may be involved in the regulation of ageing of some organisms. It appears that in humans some genes associated with ageing are involved in molecular pathways homologous to that responding to insulin and (IGF-I) in mammals, a pathway regulating glucose intake and its conversion into fat. It is no surprise then that life span is considered to be affected by dietary restriction. It is possible that genes regulating food consumption, and food intake itself, may affect life span.

Most recently, De Magalhaes has suggested a number of genes thought to directly influence mammalian ageing and known to alter the rate of both ageing and age-related debilitation. These genes have been grouped into three broad pathways: namely, the genes involved with DNA metabolism (CKN1, lamin A, WRN, XPD, Terc, PASG, ATM, and p53), and the genes involved with energy metabolism and the growth hormone/IGF-1 axis and oxidative stress (p66shc, MSRA, and Thdx1). The significance of all these genes having a role in human ageing is currently being investigated further.

**Damage-Based Theories**

The damage-based theories of ageing suggest that aging results from a continuous process of damage accumulation, originating from by-products produced by normal metabolic processes of the body. The damage-based theories are predominantly a
result of interactions with the environment,\textsuperscript{21} whereas programmed ageing, as mentioned previously, seems to be predetermined and occurs on a fixed schedule. Despite this, ageing could be a result of extrinsic or intrinsic factors that cause an accumulation of damage\textsuperscript{27} or a result of changes in gene expression that are either programmed or derived from DNA structural changes.\textsuperscript{28}

Ageing in animals has been quoted to be multi-factorial, with the changes of ageing considered to be under the guidance of biological clocks.\textsuperscript{29} As humans exhibit a gradual ageing process compared to animals, this suggests that the mechanisms of ageing, in particular the existence of biological clocks, may be different in humans when compared to animals.

**Energy Consumption Hypothesis**

In 1908 Max Rubner\textsuperscript{30} suggested a relationship between body size, metabolic rate, and longevity/ageing. Essentially, this theory suggested that bigger animals live longer than smaller animals because they spent fewer calories per gram of body mass. The energy consumption theory suggested that animals are born with a limited amount of potential energy and the faster they use this stored energy the quicker they die. However, the rate of living theory evolved as a continuation of the energy consumption hypothesis, which essentially hypothesized that the faster the metabolic rate, the faster the biochemical activity and, therefore, the faster an animal would age.

In 1935 McCay\textsuperscript{31} first recognized that dietary restriction extended longevity. The theory suggested that a decrease in calories possibly has an effect on the metabolic rate. This theory was investigated and shown to extend the life of rodents. In addition, dietary restrictions have been shown to result in a significant retardation and decline in immunological competence and decline in tumour development in rodents. Irrespective of these and other findings, the dietary restriction theory is at present flawed with many unanswered questions still remaining.

**Free Radical Theory**

In 1954 Rebeca Gerschman and colleagues\textsuperscript{32} first proposed the concept that free radicals were toxic. Such free radicals are referred to as reactive oxygen species (ROS), which originate from exogenous sources such as ionizing and ultraviolet radiation, and from endogenous processes caused by cellular activity, i.e. waste products of metabolism. In 1956 Harman proposed the ‘free radicals theory’ which described that ROS were the source of damage which accumulates in cells.\textsuperscript{33} This theory simply argues that ageing results from the damage generated by ROS. Because there are numerous enzymes to restrict the damage inflicted by the ROS, this suggests a strong reason why these ROS must be of some biological
However, much of the evidence for this theory has come from research in transgenic fruit flies and not humans.

ROS encompass many chemical species, mostly superoxide anions, hydroxyl radicals, and hydrogen peroxide (H$_2$O$_2$). These small molecules (compared to proteins and nucleic acids) are chemically very active, and can therefore cause a great deal of damage and disruption to cells. Reactive radicals of nitrogen (nitric oxide and derivatives) and of oxygen (superoxide anion, hydrogen peroxide, hydroxyl radical) can inflict considerable damage on macromolecules (protein, nucleic acids, complex lipids), which give rise to carcinogens (e.g. nitrosamines) and trigger (or sometimes prevent) apoptotic death of cells, i.e. macrophages and vascular epithelial cells. Unless mechanisms for scavenging these reactive species are effective, the damage inflicted by free radicals is known to substantially increase cell death.

ROS are by-products produced in the mitochondria (the main source of ROS). The efficiency of the mitochondrial electron transport and energy-generating processes deteriorate with age, resulting in increased levels of oxidizing free radicals and ultimately leading to cell death or deterioration. The control of ROS levels and production is fundamentally important in the human body, but it is interesting to note that ROS may not just be causing random damage but may also be used as signalling molecules in various cellular processes of the body. The significance of this warrants further investigation. To further complicate the picture, it has been shown that the pathways that control ROS levels are ageing themselves. These pathways have been found to be less efficient at an older age. In addition to this, Weindruch has found that some animals are known to age more slowly because they produce less ROS.

The human body has evolved mechanisms to control the detrimental effects of harmful chemicals and ROS. One mechanism involves the use of superoxide dismutase (SOD), which has no function other than disposing of superoxide anions. Another enzyme known to be very beneficial in suppressing the effects caused by ROS is methionine sulfoxide reductase A (MSRA). This enzyme is able to catalyse the repair of protein-bound methionine residues known to be oxidized by ROS. Presence of this enzyme suggests that ROS are biologically significant. Interestingly, overexpression of MSRA in Drosophila has been shown to increase longevity.

It is universally accepted that ROS have a role to play in pathologies of the body but the exact influence ROS have on mammalian ageing is as yet undetermined and inconclusive.

**DNA-Damage Theory**

The DNA-damage theory was first proposed by Leo Szilard in 1959, and suggests that damage to DNA causes ageing. However, it is doubtful whether DNA damage accumulation alone is able to drive the ageing process. Nevertheless, as with a
number of the ageing theories proposed, any changes that do occur in DNA may have a role to play in ageing. To date, the essence of these changes and the mechanisms that are involved are as yet undetermined.

Interestingly, accelerated ageing has been noted in humans because of certain genetic mutations. Two rare diseases, namely, Werner’s syndrome (WS) and Hutchinson–Gilford’s syndrome, produce conditions which are similar to an accelerated ageing process due to DNA damage. In addition to this, ROS have also been shown to damage DNA.

**Microorganisms and Ageing**

Ageing/senescence has been documented in a number of microorganisms. For example, by measuring ageing by reproductive output, ageing has been shown to exist in *Caulobacter crescentus*. In addition to this, ageing has been reported in *Escherichia coli* cells, following nutrient depletion. In this example, *E. coli* has been shown to lose its ability to reproduce and recover from injury. Furthermore, it has been shown that when *E. coli* divides, one of the newly formed colonies inherits the oldest end, or pole, of the ‘mother’. This newly formed cell has been shown to have a diminished growth rate, decreased offspring production, and an increased incidence of death, which are all characteristics of ageing.

**Ageing and Disease/Infection**

The world’s population by the year 2050 is estimated to become 8.9 billion (as published by the Department of Economic and Social Affairs). With this increase comes an increase in the number of ageing and aged individuals. For example, in 1998, 66 million people in the world were 80 years of age or older. This figure is projected to become 370 million by the year 2050.

By the year 2030, in the US alone, it is estimated that one in five people will be expected to be 65 years and older. Changes within the immune functions are known to occur during the ageing process which pre-disposes the elderly population, when compared to the younger population, to many types of infectious agents. As well as a change in the types of microorganisms associated with infections comes a greater diversity of pathogens associated with an elderly population compared to that of a younger one. This will have effects on recovery and clinical outcome.

A number of pathologies associated with human ageing are highlighted in Fig. 1.1 and the leading causes of death in humans can been seen in Table 1.1. Specific conditions highlighted in the table include diabetes, heart disease, cancer, arthritis, and kidney disease, with heart disease the number one cause of death in people aged 85 (Fig. 1.2) followed then by cancer, cerebrovascular diseases,
Parkinson’s and Alzheimer’s diseases, pneumonia, and chronic lower respiratory diseases. Infections in the elderly population are commonly due to pyogenic bacteria. Conditions that are highly prevalent in this age group include pneumonia, urinary tract infections, endocarditis, bacteraemia, and skin and soft tissue infections. Certain conditions such as meningitis are rare in the very old but are more significant in a younger population. In addition to this, viral infections in the older population, when compared to the younger population, are infrequent occurrences.
Exceptions to this, however, include herpes zoster reactivation (shingles), influenza, and gastroenteritis.

If we work from present figures, within the next 50 years there will be 20 million elderly persons hospitalized with pneumonia, septicemia, and urinary tract infections. Also, because of the increased usage of prosthetic devices by the ageing population, infections associated with these devices are increasing exponentially. The statistics quoted for diseases and infections in the US are approximately the same within most developed countries. In developing countries, statistics are significantly different, with most infections and deaths attributed to tuberculosis, leishmaniasis, malaria, and the effects of enteric bacteria. In addition to this, the prevalence of nosocomial infections has been shown to increase substantially with age because of the increased risk of infection per day of hospitalization. Most infections in the elderly have a poorer outcome when compared to younger adults. This is due to a number of factors including late diagnosis of therapy, increased frequency of co-morbidities, and poor tolerance to drugs, to name but a few. To help improve this situation, vaccinations are necessary, i.e. an annual influenza vaccination and a pneumococcal vaccination. However, vaccinations are much less effective in the sick and also institutionalized elderly people.

Fig. 1.2  Death by underlying or multiple cause, expressed in rates per 100,000 people, as a function of age for the 2001 US population aged 85 and older (Source: http://www.cdc.gov/nchs/nhcs.htm. National Center for Health Statistics Data Warehouse on Trends in Health and Aging. Courtesy and permission from Dr João Pedro de Magalhães. http://www.senescence.info/definitions.html) (See Color Plates)
Increased Sensitivity of Infection in the Elderly

The elderly population is much more sensitive to infection when compared to the younger population for a number of reasons. One reason is due to immunosenescence, another is due to malnutrition, and others are a result of anatomic and also physiological changes. As mentioned previously, the immune system becomes less effective in the elderly, which enhances an individual’s susceptibility to infection. Malnutrition in the elderly is known to be major cause in decreasing immune function.\textsuperscript{56,57}

Both anatomical and physiological changes in humans are characteristics of the ageing process. For example, the body has many non-immune host defensive mechanisms that prevent infections. As we age, these defence mechanisms, e.g. mucociliary clearance or rapid urine flow, are affected, which leads to efficient removal of bacteria from the human body. Within the lungs, for example, frequent infections, particularly in the elderly, are highly prevalent when compared to the younger population. Colitis and gastroenteritis are also common conditions of the elderly. This is principally due to the fact that ageing is associated with a decrease in gastric acidity and changes in the intestinal flora and intestinal mucus,\textsuperscript{58} with increase in the usage of antibiotics, which in turn increase problems associated with \textit{Clostridium difficile}.

Does Infection Contribute to Ageing?

Ageing, as mentioned previously, is a risk factor for infection but it would seem, on the basis of the evidence to date, that infection, due to exogenous or indigenous microorganisms, may contribute to the ageing phenomenon. This hypothesis is considered conceivable, as pathogens are known to cause tissue and cellular destruction. In addition, the immune response of the body clearly has an effect on invading pathogens but at the same time does cause damage to the host itself.\textsuperscript{59} Furthermore, latent or chronic infections contribute to the ageing process. For example, latent infections may periodically be reactivated, and those microorganisms known to avoid the immune system may contribute to the ageing process.

Inflammation, due to bacteria, is documented in many diseases and associated with the ageing process. \textit{Chlamydia pneumoniae}, \textit{Helicobacter pylori}, cytomegalovirus, herpes simplex virus, and also those responsible for periodontitis\textsuperscript{60–64} are considered significant to atherosclerosis, but it is probable that viruses and bacteria have an aggravating influence on an individual with a genetic susceptibility to disease.\textsuperscript{65}

Conclusion

Ageing is part of natural human development. This suggests that both ageing and human development are regulated by the same genetic processes.\textsuperscript{66–68} In many
animal species it has been suggested that ageing is a product of evolution and that ageing may be an unintended result of evolution. This theory does seem unlikely in humans, however. Evolution does not favour the concept of a long life but favours and optimizes reproductive mechanisms. Many ageing theories do overlap, and how the microbiology of the host affects the ageing process has not been reported. As you will see throughout this book, the microbiological burden on the host is enormous and clinically significant and undoubtedly will have a role to play in the ageing process.

As humans are living longer, there is a greater propensity to infection. This risk is substantially higher in elderly individuals who are predisposed to infection. While the process of ageing and the effects ageing has on the hosts’ microbiology, and vice versa, are poorly documented and researched, data obtained from gut studies suggest that microbiological changes take place in the human host over time suggesting significance to the host. Do the microbiological changes that occur within and upon the host influence the process of ageing, or are they the biological changes of the host that affect the microbiology? Does this therefore affect our propensity to disease? As the host’s microbiology changes with ageing, is this significantly beneficial or severely detrimental to the host? Are there ways of enhancing life expectancy by reducing certain bacteria from proliferating or, conversely, by enhancing the survival and proliferation of beneficial bacteria delay ageing? As you will see throughout this book, the whole area of ageing is complex and to date ageing remains an incurable disease.

The role microorganisms may play in influencing ageing is unquestionably significant to human life, longevity, and disease. Microbiological changes that do occur, specifically in the gut during ageing, may provide some useful research findings that may shed some light on the effects microorganisms have on human development and ageing.

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Chapter 2
Indigenous Microbiota and Association with the Host

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Human Development, Microorganisms, and ‘Normal Flora’

The association between man and microorganism started during the early stages of human evolution. It is therefore of no surprise that the concept of bacteria influencing human development is of significance. In spite of this, evidence that bacteria may influence a specific host’s development has largely been derived from research findings obtained from invertebrate models. Nevertheless, the initial findings generated from these invertebrate models may have some relevance to both animal and human development. For example, research has shown that bacteria, such as *Vibrio fischeri*, are capable of affecting morphological changes in the marine squid *Euprymna scolopes*. The ‘commensal’ relationship between *V. fischeri* and *E. scolopes* occurs when the squid hatches from its eggs. As the eggs hatch, within a few hours, *V. fischeri*, located in the sea water, colonize the newly formed squid. *V. fischeri* are then thought to induce morphological changes in the developing light organ of the squid, brought about by diffusible communication signals released by the bacteria. Although the communication between bacteria and the mammalian cells was first studied in the ‘vibrio–squid model’, the theory and science behind this is thought to have direct relevance to infections and diseases in humans. This would therefore indicate some degree of organization and co-ordination at the molecular level which may be applicable to human development. As bacteria do play a role in human development, it is probable that bacteria contribute significantly to human ageing. In fact, evidence for this has been documented in germ-free mice, wherein the mice had been infected with *Bacteroides thetaiotaomicron*. *Bacteroides* were found to affect the expression of various host genes known to have an influence on things such as nutrient uptake, metabolism, angiogenesis, mucosal barrier function,

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and the development of the enteric nervous system. In addition to this study, it has been found that the commensal bacteria associated with the host may be able to influence the normal development and function of the mucosal and gut immune system. Consequently, on the basis of these and other similar studies, it is plausible to hypothesize that bacteria–host interactions have a role to play in the morphogenesis of mammals and therefore a link to the ageing phenomena.

**Indigenous Microbiota (Normal Flora)**

During early development (in the mother’s womb), ‘man’ is generally not exposed to microbes. It is not until entry into the outside world that microorganisms become significant. These significant microorganisms, which are able to colonize the host, often become companions for life. Such ‘companion’ microorganisms have been referred to as the ‘normal’ microflora which are considered to exist in a symbiotic relationship with ‘man’. However, when we consider any form of symbiotic relationship, the microorganisms found on and within the body are in a mutualistic relationship with the host. This mutualistic relationship occurs when both human and bacteria benefit. However, in some instances this mutualistic relationship becomes one of paratism, when the microbe or human suffers at the expense of the other, or commensalism, where either the human or microbe benefits and the other remains unaffected. Historically the human–microbe relationship is considered to be one of commensalism. Nevertheless, it would appear that the microflora and animal/human relationship is not one of commensalism. This is because each partner has the ability to influence the other. Therefore, the commensal flora of the body is often better described as the ‘indigenous microbiota’, as suggested by Dubos.

The healthy human contains approximately 10 times more bacteria than normal human cells. The bacterial load on or within the host constitutes more than 2,000 different phylotypes. Despite this number, only 8 of 55 main divisions of bacteria have been shown to be present on or in the human host, suggesting some degree of host selection. The selected group of microbes associated with the host has been referred to as the ‘microbiome’. These ‘selected microbes’ are considered to use the human host merely as ‘an advanced fermentor to maximise the productivity of the microbiome’. The capacity of the indigenous microbiota to cause harm and disease to the host is severely limited by the host’s immune system which helps to maintain a microbe–host homeostasis, which in turn helps to prevent the generation of significant microbial disturbances. However, if disturbances to the indigenous microbiota do occur, such as after surgical procedures or with chronic wounds, a person’s host defences are often compromised, predisposing them to changes in their microflora and initiation of infections and diseases.

A number of indigenous bacteria are considered to be pathogenic, particularly those which have inherent multi-drug resistance. Such indigenous bacteria reside in the gut, the skin, and oropharynx but generally do not cause disease.
In the human mouth, some 500–700 different microbial taxa, considered to be part of the indigenous microbiota, have been identified compared to 500–1,000 in the colon. Despite these numbers, it is very difficult to define the true community of microbes in different regions of the body. This is principally due to the complexities of the communities in the human body and the variations in the indigenous microbiota that exist between age, sex, and hygiene. In addition, the different sampling methods that are often used in studies, combined with the changes that take place with microbial nomenclature, affect the correct interpretation of the findings.

**Microbiota Differences in Children and Adults**

The indigenous microbiota of children and adults at different anatomical sites is considered significant. Initially this is due to the fact that young children have immature immune systems, no teeth, and different diets and are not exposed to the same environmental microbes as adults. For example, the initial microbiota colonising the gut of small children are predominately *Bifidobacterium* spp. compared to adults guts which are predominantly colonized by *Bacteroides*. In the elderly population, a decline in the effectiveness of the immune system in conjunction with poor health, diet, and hygiene leads to changes in the ‘normal’ microbiota. Specific microbiological changes that have been observed in the elderly population include a decrease in *Veillonella* and *Bifidobacteria* species in the gut; increased levels of Clostridia, enterobacteria, and lactobacilli; an increase in urinary tract infections (UTI); an increase colonization in the oropharynx by *Candida albicans* and *Klebsiella*; increased levels of enetrobacteria and streptococci on the skin; and increased levels of Gram-negative bacteria in the eye and oral cavity.

**The Development of the Indigenous Microbiota**

The development of the indigenous microbiota begins soon after birth. Upon and within the neonate, colonization of pioneering bacteria occurs within 24 h. In many regions of the body, the microbes generally begin to proliferate in a heterogeneous manner. Autogenic and allogenic factors then prevail, leading to the adhesion and removal of certain microbial populations. Such changes are referred to as microbial succession, and are known to occur in areas where microbial communities exist. Over time, the microbiology diversity and density in certain regions of the body become more stable (see Table 2.1) and less dynamic, eventually culminating in the formation of ‘climax communities’. Consequently, regions of the body begin to develop a defined microflora. Such a defined microflora will be found on the skin or cutaneous regions of the body, the upper respiratory tract, oral cavity, gastrointestinal tract, and genital tract. This microflora, however, is subject to change specifically when modifications in the diet and the host immune system occur. The lack of
research into the microbiological changes that occur with advancing age prevents us from gaining a better understanding of the benefits of the microflora and therefore the therapeutic procedures that could be implemented. In fact, there is mounting evidence that probiotics (discussed further in the book), and their significance to human health, is growing in acceptance for human well-being.

To date, the microbiology of the human microbiota is as yet incomplete despite more than a century of culture-based investigations. Many species of microbes have yet to be recovered from the host, and molecular techniques have been used only occasionally in a number of these environments.

Each anatomical site known to have its own microbiota will be considered in turn.

**Skin**

In the mother’s uterus the unborn baby’s skin is sterile. Following birth, colonization of the baby’s skin occurs. As the skin is a barrier to many pathogenic bacteria, it restricts microorganisms attaching. While the skin is a highly effective organ in preventing the growth and invasion of pathogens, it is well documented that skin infections do occur – a frequent occurrence during the later years of life.

The outermost layer of the epidermis of the skin provides a very good barrier to the effects of environmental pressures. In fact, the keratinocytes, which are dead in the outermost layer of the skin, are continually being sloughed off and as such this prevents exogenous bacteria from colonizing. As the moisture content of the skin is very low, the bacterial growth on the skin is limited. The acidic pH of the skin also helps to suppress bacterial growth.

Indigenous bacteria that are known to colonize the skin are able to produce antimicrobials such as bacteriocin and toxins that inhibit pathogens from attaching. For example, bacteria such as *Staphylococcus epidermidis* are able to bind to keratinocyte receptors on the skin and once attached are known to prevent *S. aureus* from attaching.

The microbes that make up the largest proportion of normal skin flora include the Gram-positive bacteria such as Staphylococcus, Coryneform bacteria, and Micrococcii (see Table 2.2). Gram-negative bacteria occur rarely as part of the normal skin flora. However, *Acinetobacter* spp. have been found routinely on the perineum and

<table>
<thead>
<tr>
<th>Location</th>
<th>Population size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>$10^3–10^6 \text{cm}^{-1}$</td>
</tr>
<tr>
<td>Saliva</td>
<td>$10^4 \text{ml}^{-1}$</td>
</tr>
<tr>
<td>Dental plaque</td>
<td>$10^{12} \text{g}^{-1}$</td>
</tr>
<tr>
<td>Ileal contents</td>
<td>$10^8 \text{ml}^{-1}$</td>
</tr>
<tr>
<td>Colon (faeces)</td>
<td>$10^{10} \text{g}^{-1}$</td>
</tr>
<tr>
<td>Vagina</td>
<td>$10^8 \text{ml}^{-1}$</td>
</tr>
</tbody>
</table>
axilla. Staphylococci found on the skin are generally coagulase-negative and have included S. epidermidis, S. haemolyticus, and S. hominis. In the main, these coagulase negative staphylococcus (CNS) constitute a non-pathogenic group of bacteria that colonize the skin. However, CNS have been known to cause nosocomial infections, particularly in patients with intravascular catheters.

Micrococci, predominately Micrococcus luteus, are found in abundance on the skin surface. Most species of micrococcus are non-pathogenic; however, M. sedentarius has been known to cause pitted keratolysis. Coryneformes are also found on the skin.

The number of bacteria detected on the skin surface is about $10^{12}$, which is equivalent to $10^4$ cm$^{-2}$, which equates to about one bacterium per 100 μm$^2$. This indicates therefore that the bacteria are not evenly distributed but exist in micro-colonies on the skin surface often growing as biofilms.

### Mouth

In 1683, Antonie van Leeuwenhoek first observed the presence of microorganisms in the mouth, but it was not until 1890 that oral bacteria were considered to have a role to play in disease. Today we know that the oral microbiota is beneficial to the host, as indicated by their ability to suppress the effects of exogenous microorganisms. However, imbalances and certain bacteria associated with the normal microbiota in the mouth can lead to oral diseases and cause soft-tissue infections and abscesses. Microorganisms in the mouth and throat are present in high numbers, with levels present in saliva at $10^8$ or $10^9$ ml$^{-1}$.

Colonization of the human mouth by bacteria occurs within the first 6–10 h after birth. After 8–10 h, the bacteria, often only transient, that have been identified in a baby’s mouth have included staphylococcus, streptococcus, coliforms, and enterococci, to name but a few. From 0 to 2 months, the most common bacteria isolated from the oral microbiota of the infant have included mainly the viridans streptococci such as Streptococcus salivarius, S. oralis, S. mitis biovar 1 together with Neisseria spp., Staphylococcus spp., and Prevotella spp. As the infant gets older (6–12 months), other microorganisms begin to appear in the mouth. These have

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter</td>
<td>A. johnsonii, A. baumannii</td>
</tr>
<tr>
<td>Malassazia</td>
<td>M. furfur</td>
</tr>
<tr>
<td><strong>Micrococcus</strong></td>
<td>M. luteus, M. lylae, M. kristinae, M. sedentarius, M. roseus, M. varian</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>C. jeikeium, C. urealyticum, C. minutissimum</td>
</tr>
<tr>
<td>Propionibacterium</td>
<td>P. acnes, P. avidum, P. granulosum</td>
</tr>
</tbody>
</table>
included the ones mentioned above and *S. sanguis*, *Actinomyces* spp., *Fusobacterium* spp. (specifically *F. nucleatum*), and *Capnocytophaga*. At 12 months, in addition to the microbes mentioned above, other microbes such as *S. mutans*, *Clostridium* spp., *Peptostreptococcus* spp., *Prevotella intermedia* and *pallens* together with *Porphyromonas gingivalis* begin to colonize the oral microbiota of the infant’s mouth. Many transient microorganisms appear in the newborn’s mouth, which eventually become part of the resident microbiota or go on to colonize other regions of the body. It is during the teething and the weaning processes that newborns become more significantly exposed to bacteria.

*S. salivarius* has been isolated frequently on the first day of a baby’s life, whereas during the teething process *S. sanguis* is documented to be the first colonizer of teeth. *S. mutans* are also early colonizers of the teeth but these bacteria have been shown to take a longer time to colonize the teeth surface. Over time, as biofilms (plaque) begin to form on the tooth surface, the microbiota becomes more complex and heterogeneous. The microbiota found in plaque isolated from children (4–7 years old) seems to be similar to that of adults. Major microbiota changes in the mouth occur up to age of 19 as a result of the ageing process, possibly brought about by changes in hormones. *Bacteroides* and *Spirochaetes* have been documented in the mouth of children. Levels have been shown to increase around puberty. Contrary to this, levels of *Capnocytophaga* spp. and *A. naeslundii* have been shown to decrease with advancing age. At adolescence, black pigmented anaerobes and *Spirochaetes* begin to increase in numbers in the mouth and form a significant component of the oral microbiota.

Over 45 years ago, the number of bacteria that had been identified to be present in the adult mouth was as low as 30. Today, because of the advancement in microbial identification procedures, this number is documented to be much higher, with some 700 different species of bacteria now having been recognized. In Table 2.3 the commonly encountered and cultured bacteria can be seen, but this is no definitive and complete list of oral bacterial genera, as this table constitutes only a select list of frequently isolated microbes. While the microbiota in the mouth is very heterogenous, principally due to the fact that the oral microbiota is under a constant flux from the shedding of epithelial cells, saliva flow, diet, and the effects of the innate immune response, the dental plaque does show a very high degree of homeostasis.

A large number of microorganisms have been detected in the adult mouth that do not reside there for long periods. These are transient oral microbiota bacteria derived from food or from the other regions of the body. These bacteria are considered insignificant but are capable of causing opportunistic infections in debilitated individuals. The bacteria known to cause diseases such as dental caries and periodontal diseases are caused by the resident microflora found colonizing the teeth. Oral pathogens such as *S. mutans* have been linked to caries, as have *Actinobacillus actinomycetemcomitans*. Treponema has been linked with periodontal disease. These bacteria, however, are considered to constitute part of the indigenous microbiota of the mouth.
On the palate, lips, cheeks, and floor of the mouth, the microbiota is considered sparse.

The tongue generally harbours a polymicrobial microbiota. A study conducted in 1966\textsuperscript{25} has shown that the predominant bacteria found on the human tongue are Streptococci (35%), Gram-positive rods (16%), Veillonella (16%), Gram-negative rods (6%), non-pigmenting Bacteroides (5%), and Peptostreptococcus spp. (4%), and the rest were Gram-positive cocci. Recent studies using 16s rRNA have shown that the tongue contains a very diverse microbiota and these may act as a reservoir for bacteria associated with periodontal diseases.

### Upper Gastrointestinal Tract

The dominant population of bacteria residing in the upper gastrointestinal (GI) tract of a healthy adult human has included *Pseudomonas* spp., *Micrococcus* spp., *Bacillus* spp. as well as lactobacilli, bifidobacteria, and streptococci.\textsuperscript{26} However, the microbial composition of the indigenous microbiota of the GI tract is affected by sex, age, and diet, which significantly alter the microflora. However, depending